Immunity priming uncouples the growth-defense tradeoff in tomato

Running title: Uncoupling growth-defense tradeoffs

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Summary statement

Growth-defense tradeoffs in plants result in loss of yield. Here, we demonstrate that immunity priming in different pathways uncouples this tradeoff and allows for disease resistant plants with robust growth.

Abstract

Lacking an adaptive immune system, plants have developed an array of mechanisms to protect themselves against pathogen invasion. The deployment of defense mechanisms is imperative for plant survival, however, defense activation can come at the expense of plant growth, leading to the "growth-defense trade-off" phenomenon. Following pathogen exposure, plants can develop resistance to further attack. This is known as induced resistance, or priming. Here, we investigated the growth-defense trade-off, examining how defense priming affects tomato development and growth. We found that defense priming can promote, rather than inhibit, plant development. Using defense pathway mutants, we found that defense priming and growth tradeoffs can be uncoupled, with growth and defense being positively correlated. We found that cytokinin response is activated during induced resistance, and is required for the observed growth and disease resistance resulting from resistance activation. Taken together, our results suggest that growth promotion and induced resistance can be co-dependent, and that defense priming can, within a certain developmental window, not necessitate a trade-off on growth, but rather, encourage and drive developmental processes and promote plant yield.

Introduction

Plants, like most organisms on earth, often face various abiotic and biotic stresses such as pathogen infection and climate and ecological changes. These stresses induce physiological, biochemical, and molecular changes, which can be reflected in lowered growth and productivity. Plant stress tolerance is controlled by a network of signals and responses occurring at the cellular and molecular levels, leading to an effective activation of defense mechanisms. Plants lack mobile immune cells and an adaptive immune system. Therefore, they have developed a complex array of mechanisms in order to protect themselves against pathogen invasion. In the interaction between the plant and the attacker, elicitor molecules are released. These elicitors may originate from the plant or the attacker, or both, and include carbohydrate polymers, peptides or fragments of plant proteins, lipids, glycoproteins, and fatty acid–amino acid conjugates (Mishra et al., 2012). Perception of these elicitor molecules by the host plant results in activation of a signaling pathway, leading in turn to activation of various defense mechanisms (Walters and Heil, 2007).

Following pathogen infection, plants can develop an enhanced resistance to further attack. This is known as induced resistance. Induced resistance to microbial pathogens can be split broadly into systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Walters and Heil, 2007). SAR, commonly triggered by local infection, can provide long-term resistance throughout the plant to subsequent infection by different pathogens including viruses, bacteria and fungi (Klessig et al., 2018). The SAR response correlates with the activation of pathogenesis-related (PR) genes, many of which encode PR proteins with antimicrobial activity. These proteins include chitinases and glucanases. Among the best-characterized PR genes is *PR-1*, which is often used as a marker for SAR (Pieterse et al., 2014). SAR generally relies on salicylic acid (SA), which has been shown to increase in both infected and uninfected tissues during pathogenesis, accumulating in the phloem sap (Shoresh et al., 2005). SAR signaling downstream of SA is controlled by the redox-regulated protein NPR1, which upon activation by SA acts as a transcriptional co-activator of a large set of PR genes (Pieterse et al., 2014).

Unlike SAR, which is usually activated during pathogen infection, the defense pathway known as induced systemic resistance- ISR, has usually been described as being initiated by beneficial

soilborne microorganisms such as plant growth-promoting rhizobacteria and fungi. ISR is generally associated with a physiological state in which plants can react more efficiently to pathogen attack (Meller Harel et al., 2014; Shoresh et al., 2005), known as immunity "priming". ISR is commonly regulated by jasmonic acid (JA) and ethylene (ET) dependent signaling pathways, and is typically not associated with a direct activation of PR genes, although recent studies demonstrated that the well-known PR gene *NPR1* is also important to enhance the ISR resistance and that, in some cases, *PR-1* is accumulated during priming (Gupta and Bar, 2020; Meller Harel et al., 2014). ISR activated plants are primed for accelerated JA and ET dependent gene expression (Pieterse et al., 2009). Past research has suggested that biochemical changes characteristic of ISR become obvious only in response to further infection, and only in plant parts where an effective resistance is required. Priming effects can be elicited by chemical ISR inducers, beneficial microbes, and fungi. As a result of priming, upon secondary infection, defense responses such as synthesis of antimicrobial compounds, ROS (reactive oxygen species), or cell wall lignification occur more rapidly and more strongly than during the primary infection, thus enabling a more effective response to the new infection (Heil and Bostock, 2002; Mehari et al., 2015).

While the deployment of defense mechanisms is imperative for plant survival, defense activation has been reported to come at the expense of plant growth. The activation of defense mechanisms at the expense of growth is known as the “growth-defense trade-off” phenomenon, and is the result of a balance between growth and defense or adaptation responses (Figueroa-Macías et al., 2021). Growth–defense tradeoffs have an important role in plant survival (Huot et al., 2014). In nature, there are many examples of this growth and defense trade-off phenomenon. In general, mature organs and tissues are no longer growing and, as a result, can be more prepared for defense as they possess more energy and resources that can be mobilized. In plants, an early example of growth-defense tradeoff was observed in lettuce, where stronger repression of reproductive development under low nutrient conditions was found in pathogen resistant lettuce genotypes (Bergelson, 1994), suggesting that the nutrient balance influences the growth-defense relationship. In another study, induction of plant immunity in Arabidopsis thaliana resulted in significantly reduced growth rates, depending on resource competition from neighboring plants, suggesting that competition for resources, or plant-plant signaling, shape the apparent costs of defense induction (Karasov et

al., 2017). Growth-defense trade-offs in plants have been increasingly investigated of late (Belkhadir et al., 2014; Dwivedi et al., 2021; Ke et al., 2020; Margalha et al., 2019; Monson et al., 2022; Saini and Nandi, 2022; Smakowska et al., 2016). Although some works have suggested that growth and defense are antagonistic, the extent of the "development/ defense tradeoff" and the mechanisms by which it affects plant growth and agricultural yield are largely unknown.

For centuries, the main goal of agricultural practices has been to maximize the growth and yields of crops. Due to climate change and other difficulties that adversely affect the production of agricultural crops worldwide, growth–defense tradeoffs have acquired an increasing ecological, agricultural, and economic importance. SA and JA, which were classically defined as "defense/ stress" hormones, can regulate many different plant processes, including developmental processes (Ghorbel et al., 2021; Rivas-San Vicente and Plasencia, 2011; van Butselaar and Van den Ackerveken, 2020; Wasternack et al., 2013). This comes in addition to "defensive" roles being uncovered for developmental hormones (Bari and Jones, 2009), and suggests that growth and defense may not be completely antagonistic, but rather, have a more complex balance (Koo et al., 2020). Rather than looking at growth and defense as a binary switch, a more holistic view suggests that plant fitness is optimized when growth and defense are appropriately prioritized in response to both environmental and developmental cues. Researching the molecular mechanisms used by plants to balance growth and defense can lead to the possibility of manipulating these mechanisms, with the goal of increasing plant productivity.

In this work, we aimed to investigate the growth-defense trade-off in tomato, by attempting to understand how priming for defense affects tomato development and agricultural qualities. We used two different immunity elicitors, ASM (Acibenzolar-S-Methyl), also known as BTH (benzo (1,2,3) thiadiazole-7-carboxylic acid) and marketed under the trade name "bion" (Oostendorp et al., 2001), a SAR elicitor, and *Trichoderma harzianum*, a microbial ISR elicitor, to investigate differences and commonalities between SAR and ISR pathways, and examine their effect on tomato development. We found that both SAR and ISR affect plant development, with ISR generating a more pronounced positive effect on agricultural qualities. Work with genetic mutants suggests that defense priming and growth tradeoffs can be uncoupled, with growth

and defense being positively correlated- as mutants that lost the ability to activate defenses, also lost the growth effects. In addition, we found that cytokinin response is activated in response to ISR, and is required for the observed growth and disease resistance resulting from ISR activation. Taken together, our results suggest that growth promotion and induced resistance can be co-dependent, and that defense priming can, within a certain developmental window, not necessitate a trade-off on growth, but rather, encourage and drive developmental processes.

Results

Activation of SAR and ISR both promote resistance to B. cinerea and increase plant immunity

The elicitors *Trichoderma harzianum* T39, a *Trichoderma* sp. fungus that induces immunity via the JA/ET pathway (Meller Harel et al., 2014), and ASM (Acibenzolar-S-Methyl), a SA analog that primes the SA pathway (Huot et al., 2014), were used to compare SAR and ISR type induced resistance to pathogen infection. Details on immunity priming methodology are provided in the methods section. We conducted *Botrytis cinerea* infection assays on the primed *S. lycopersicum* M82 plants. We found that pre-treatment with both elicitors reduced disease levels (**Fig. 1**). ASM caused a smaller disease reduction (**Fig. 1B**).

To examine induction of plant immunity using a SAR and ISR elicitor, we conducted plant defense response assays, measuring ethylene and ROS (reactive oxygen species) production. These assays are indices of activation of plant immune system (Gupta and Bar, 2020; Leibman-Markus et al., 2017). We found that pre-treatment with both elicitor types amplified wounding ethylene production and EIX-induced ethylene production in *S. lycopersicum* M82 compared to the mock plants (**Fig. 2A-B**).

Pre-treatment with both elicitors also amplified ROS production elicited with the fungal MAMP (microbe associated molecular pattern) EIX (ethylene inducing xylanase) (**Fig. 2C**) and the bacterial PAMP (pathogen associated molecular pattern) flg22 (**Fig. 2D**).

Activation of SAR or ISR differentially promote plant development and productivity 163

To examine possible growth-defense tradeoffs, we treated plants separately with both elicitors, 164
inducing immunity via both pathways. Plants were treated once a week for 4 weeks, and grown 165
to harvest, and agricultural parameters were sampled. Both T39 and ASM promoted plant 166
growth (**Fig. 3A**), however, only T39 was able to significantly promote yield and Harvest index 167
(**Fig. 3B, C**). ASM significantly reduced harvest index. Brix was unaffected (**Fig. 3D**). 168

After observing significant effects of SAR and ISR elicitation on plant growth and agricultural 169
productivity, we next examined whether immunity elicitation could affect seedling 170
development (**Fig. 4**). 10 day old seedlings were treated with elicitors once and harvested after 171
a week for growth and development parameter quantification, or given two treatments one 172
week apart, and harvested on day 24, one week after the second treatment. Both T39 and ASM 173
affected seedling height (**Fig. 4A, B**), though ASM was only able to do so after two treatments 174
(**Fig. 4B**), while T39 treatment increased plant height even after 1 treatment (**Fig. 4A**). T39 also 175
increased seedling fresh weight (**Fig. 4C, D**) and the number of leaves produced by the seedling 176
(**Fig. 4E, F**), indicating that T39 also affected the plant developmental program. ASM did not 177
affect leaf numbers. Since T39 appeared to have a more extensive effect on plant development, 178
we analyzed additional developmental parameters following T39 treatment (**Fig. S1**). In 179
addition to the parameters described in Figure 4, we found that T39 treatment also increased 180
the number of unfurled leaves (**Fig. S1B, G**), the developmental plastochron (**Fig. S1C, H**) and 181
the meristematic differentiation to flowering of the SAM (shoot apical meristem) (**Fig. S1D, I**). 182
Focusing on leaf development in more depth (**Fig. S2**), we observed that T39 promotes leaf 183
development, as expressed by the number of leaves produced (**Fig. S2A**), and leaf complexity 184
(**Fig. S2B-D**), as early as 2-4 days after the first treatment (**Fig. S2A, B**). Not surprisingly, T39 185
does not alter the developmental program of leaves 1-2, which are known to be relatively 186
simple and have a short developmental window in tomato (Shleizer-Burko et al., 2011) (**Fig.** 187
S2C). ASM had no effect on leaf development (**Figs. 4, S1-S2**). 188

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Cytokinin is involved in altered development in response to immunity elicitation 190

We next wished to examine possible routes through which ASM and T39 are promoting growth 191
and development. As we observed that the leaf developmental window was extended upon 192

T39 treatment, with additional organs being produced (**Figs. 4, S1-S2**), we hypothesized that changes in cytokinin response might be involved. To examine this, we used tomato plants expressing the cytokinin response marker TCS (two component sensor) driving VENUS fluorescent protein expression (Steiner et al., 2020). 10 day old TCS-expressing seedlings received one elicitor treatment, and were imaged 24 h after the treatment. Both ASM and T39 were found to increase the CK-response signal (**Fig. 5**). While ASM increased local signal intensity (**Fig. 5B**) but did not affect total signal levels (**Fig. 5C**), T39 increased both the local signal intensity, to a significantly higher level than ASM, and the total signal measured (**Fig. 5B-C**). This indicates that upon T39 treatment, CK response is both stronger and expanded to additional tissues.

To confirm this result and examine additional possible developmental mechanisms affected by elicitor treatment, we examined gene expression following elicitor treatments as described above (**Fig. 6**). 10 day old seedlings were treated with elicitors once and harvested after a week for RNA preparation, or given two treatments one week apart, and harvested on day 24, one week after the second treatment, for RNA preparation. We examined the effect of one (**Fig. S3**) or two (**Fig. 6**) elicitor treatments on the expression of CK biosynthesis and degradation genes (**Fig.s 6A, S3A**), CK response regulator signaling genes (**Fig.s 6B, S3B**), gibberellin biosynthesis and degradation genes (**Fig.s 6C, S3C**), meristem maintenance genes (**Fig.s 6D, S3D**) and, as a control, SA-sensitive and JA-sensitive defense genes were also examined (**Fig.s 6E, S3E**). In general, the response was stronger after two treatments (**Fig. 6**) as compared with one treatment (**Fig. S3**). Both elicitors activated CK degradation (**Fig.s 6A, S3A**). CK response TRRs were primarily activated by T39 (**Fig.s 6B, S3B**). Likewise, both elicitors activated GA degradation, while only T39 also promoted GA biosynthesis regulation (**Fig.s 6C**), which was attenuated by both elicitors after one treatment (**Fig. S3C**). T39 alone promoted meristem maintenance and inhibited differentiation, though both elicitors activated organ determination (**Fig.s 6D, S3D**). Interestingly, both elicitors activated the expression of *PR1a* and *LoxD*, though ASM activated *PR1a* to a much higher degree (**Fig.s 6E, S3E**).

Since these results indicated that cytokinin is required to transduce the developmental signal generated by immunity induction with elicitors, we examined the response to T39 and ASM in the altered CK transgenes *pBLS>>IPT*, which has high CK levels, and *pFIL>>CKX*, which has low CK levels. These lines were previously characterized (Shani et al., 2010), and we used promoter

driver lines that were previously reported to have normal development and minimal pleotropic effects (Shani et al., 2010). We found that ASM was still able to promote disease resistance and plant growth irrespective of cytokinin content (**Fig. 7A, E, I, D, H, L**). However, T39 was no longer able to promote disease resistance, plant growth, or leaf development in the absence of CK (**Fig. 7I, J, K, L**). The increased cytokinin *pBLS>>IPT* behaved similarly to the M82 background line (**Fig. 7E, F, G, H**).

Blocking SAR or ISR in tomato mutants leads to loss of disease resistance

To further examine the correlative effects we observed between development and defense in WT tomato plants (**Figs. 1-4**), we used defense mutants. Tomato mutant compromised in jasmonate and ethylene pathways have been previously shown not to respond to *Trichoderma* with disease reduction in certain cases (Jogaiah et al., 2017; Korolev et al., 2008), while lines compromised in the salicylic acid pathway are expected not to respond to ASM (Oostendorp et al., 2001). Thus, we used the SA-lacking transgene NahG and its background line Moneymaker (MM) (Brading et al., 2000), the reduced JA mutant *spr-2* and its background line Casterlmart (Cm) (Li et al., 2003), and the reduced ethylene sensitivity mutant *neveripe* (*nr*) and its background line Pearson (Pn) (Lanahan et al., 1994), and examined their disease response to elicitation with T39 and ASM. As can be seen in **Fig. 8**, all 3 background lines responded to both elicitors with reduction in *B. cinerea* incited disease, as was observed for M82 (**Fig. 1**). However, NahG did not respond to ASM (**Fig. 8A**), while *spr-2* and *nr* did not respond to T39 (**Fig. 8B-C**).

Blocking SAR or ISR in tomato results in loss of developmental promotion and yield increases

Given that mutating defense pathways prevented disease reduction by T39 and ASM treatment, we examined whether the developmental effects of these elicitors would also be abolished in the same mutants. Plants of the different mutant genotypes and their background lines were treated once a week for 4 weeks, and grown to harvest (**Fig. 9**). In line with our observations concerning disease resistance, all 3 background lines responded to both elicitors with increases in height, and also responded to T39 with increased yield, as was observed for M82 (**Fig. 3**). However, NahG did not respond to ASM (**Fig. 9A, D**), while *spr-2* and *nr* did not respond to T39 (**Fig. 9B-C, E-F**).

Since we observed that CK response was increased by both elicitors (**Fig.s 5-6**), we examined additional developmental parameters that are known to be CK dependent, side shoot production and leaf complexity (Mller and Leyser, 2011; Shani et al., 2010; Werner et al., 2001). ASM did not affect either of these parameters in WT lines, but was included as a control in case it might have effects on the mutant lines. T39 was found to increase side shoot production and leaf complexity in all background lines (**Fig. 10**). While NahG responded similarly to its background line Moneymaker in response to T39 (**Fig. 10A, D**), *spr-2* and *nr* did not respond to T39 (**Fig. 10B-C, E-F**). ASM had not effect on these parameters in either WT lines or defense mutants (**Fig. 10**).

We also examined early seedling development in defense mutant genotypes (**Fig. 11**). 10 day old seedlings of the SA-lacking transgene NahG and its background line Moneymaker (MM), the reduced JA mutant *spr-2* and its background line Casterlmart (Cm), and the reduced ethylene sensitivity mutant *neveripe* (*nr*) and its background line Pearson (Pn), were treated with elicitors twice, one week apart, and harvested on day 24, one week after the second treatment. Both T39 and ASM affected seedling height in the background lines (**Fig. 11A, D, G**). As observed with M82 (**Fig.s 4,7**), T39 increased the number of leaves produced by the seedling (**Fig. 11B, E, H)**, as well as leaf complexity (**Fig. 11C, F, I**). As observed with the mature plants (**Fig. 9**), NahG did not respond to ASM (**Fig 11A-C**), while *spr-2* and *nr* responded to ASM, but did not respond to T39 (**Fig. 11D-I**).

Thus, the competence to respond to each elicitor in increased immunity, is the same as the competence to respond in altered development and increased growth and yield.

Discussion

Activating plant defense does not always generate a growth tradeoff

The concept of growth-defense tradeoffs is based on the notion that limited resources are available to an organism, and must therefore be prioritized according to internal and ambient conditions being experienced by the organism in a particular moment. In plants, which are sedentary and lack circulatory immunity, the role of ambient conditions such as abiotic and

biotic stresses becomes more prominent, because plants have limited ability to regulate their environment. Early research of growth defense tradeoffs was in part prompted by observations that plants with highly active defense pathways or imbalances in defense hormones can be highly resistant to pathogens, but have severely impaired growth (Huot et al., 2014; Vos et al., 2013). However, many of these reports investigated severely pleiotropic mutants, and dramatic changes to hormonal pathways and hormone levels can have severe impacts on development, particularly considering that most defense hormones also have significant developmental roles (De Vleesschauwer et al., 2014). Cross-talk between different hormones is one of the key tenets at the basis of growth-defense tradeoffs, with classical defense hormones being reported to have negative crosstalk with growth hormones in several cases (Huot et al., 2014; Vos et al., 2015).

Constitutively active plant defense can result in growth inhibition (Gómez-Gómez et al., 1999; Zipfel et al., 2006), although genetically increasing plant immunity in several cases did not have a negative effect on plant growth or development (Cao et al., 1998; Heidel et al., 2004; Leibman-Markus et al., 2021; Pizarro et al., 2020). This suggests that the mode of constitutive defense activation, whether by external or genetic means, can affect the level of the "tradeoff" with growth and development, opening the possibility for activating defense without significantly impairing plant development, a highly sought after agricultural advantage.

Priming agents can have positive growth effects

Previous reports have shown that immunity priming agents can affect plant growth. The underlying hypothesis of the present work was that certain priming agents can in fact improve plant growth, breaking the growth defense tradeoff and potentially generating added agricultural value. To examine this, we selected two known defense priming agents in tomato, the SA analog ASM, also known as BTH, a molecular priming agent known to activate SAR (Ishii et al., 2019), and the fungus *T. harzianum* T39, a biological priming agent known to activate ISR (Gupta and Bar, 2020).

ASM/ BTH promotes disease resistance and has been reported to have various growth effects. However, ASM can be phytotoxic (Ishii et al., 2019), similar to effects observed in plant with abnormally high SA levels or constitutively active SA signaling (Genot et al., 2017; Pokotylo et al., 2022). BTH was reported to induce host resistance against a wide range of pathogens, in

correlation with increased gene expression of resistance-related genes, including the pathogenesis-related protein 1 gene (PR1), a well-known marker of SA-induced SAR (Kobayashi et al., 2020). BTH application led to induction of systemic resistance to Gray Mold incited by *B. cinerea* in tomato, and increased the expression of defense related genes upon gray mold infection (Meller Harel et al., 2014). Inducing resistance against a wide array of pathogens in many hosts, BTH also suppressed *Pepper golden mosaic virus* symptoms observed in pepper (Trejo-Saavedra et al., 2013). In connection with growth, BTH promoted both morphogenesis and differentiation in cell cultures of ornamental plants from the *Ornithogalum* species, with increases observed in formation of meristematic centers, shoot formation, and differentiation of cell organelles (Tun et al., 2013). In wheat, application of BTH was observed to negatively impact fitness in the absence of pathogens, but increased fitness under powdery mildew stress (Stadnik and Buchenauer, 1999). In addition, over expression of NPR1 was shown to enhance resistance to biotrophic and hemi-biotrophic pathogens without adversely affecting growth or fitness (Cao et al., 1998; Heidel et al., 2004).

Likewise, *Trichoderma* spp. are known to promote disease resistance and affect plant growth. *T. harzianum* has been reported to enhance disease resistance against several pathogens, by inducing ISR in a JA/ET dependent manner. Like BTH, *T. harzianum* T39 suppressed the severity of Gray Mold in tomato (Meller Harel et al., 2014), and *Trichoderma* spp. promoted resistance against a wide array of pathogens in different plant hosts (Gupta and Bar, 2020). *Trichoderma* spp. positive growth effects are numerous. In tomato, seedling height, crown diameter, shoot fresh and dry weight, as well as leaf number and total area of leaves, were increased significantly by applying *T. harzianum* (Azarmi et al., 2011). *Trichoderma* strains stimulated early stages of growth in bean (Hoyos-Carvajal et al., 2009). In cotton, increases in shoot and root length, fresh and dry weight, and plant vigor, were observed upon *Trichoderma* treatment (Shanmugaiah et al., 2009). Many additional positive growth effects on plant have been observed with *Trichoderma* spp. treatments (Gupta et al., 2016; Nesler et al., 2015; Salas-Marina et al., 2011).

Defense priming can evade negative tradeoffs and result in growth promotion

Here, we demonstrate that defense priming can result in both induced pathogen resistance, and plant growth (**Figs. 1-4**). Both ASM and T39 induced resistance against *B. cinerea*. We selected *B. cinerea* as a model pathogen, though this was also reported for both inducers with additional pathogens (Gupta and Bar, 2020; Marolleau et al., 2017; Oostendorp et al., 2001; Soylu et al., 2003). ASM also promoted vertical growth, while T39 had a more dramatic growth promoting effect, and improved several agricultural traits (**Fig. 3**). Both inducers were effective in promoting plant growth and development in mature plants in an agricultural-like nethouse setting (**Fig. 3**), as well as in seedlings (**Fig.s 4, S1-S2**). Though ASM did not significantly positively impact most agricultural growth parameters, it also did not have a negative effect on them in the applied concentration, which could be significant agriculturally under a high disease burden. ASM and T39 were found to affect the expression of several assayed developmental genes (**Figs. 6, S3**), suggesting that priming with these inducers also leads to transcriptional reprogramming that can effect changes in plant developmental programs, and result in increased growth. Of note is that treatments with *T. harzianum T39* were previously found to activate resistance to downy mildew in grapevine, without negative effects on plant growth (Palmieri et al., 2012).

Roles for CK in growth-defense tradeoffs

In recent years, CK has been established as a priming agent, and its role as a defense hormone is increasingly recognized (Argueso et al., 2012; Choi et al., 2010; Cortleven et al., 2019; Gupta et al., 2020a). In the context of disease resistance, most reports support the idea that CK promotes immunity and disease resistance by activating SA-dependent processes (Argueso et al., 2012; Choi et al., 2010; Gupta et al., 2020), though roles in JA-mediated defense cannot be ruled out. CK has also previously been suggested to be involved in growth-defense tradeoffs (Cortleven et al., 2019). As a central growth hormone, it is perhaps expected that CK should be involved in regulating growth-defense trade-offs. CK is known to have crosstalk with different defense hormone pathways. Stress hormones are known to negatively regulate CK signaling (Albrecht and Argueso, 2017; Naseem et al., 2015), and in the context of abiotic stress, reduction in CK signaling can increase abiotic stress resistance (Nishiyama et al., 2011). In the context of biotic stress, CK can act synergistically with SA (Argueso et al., 2012; Choi et al., 2010; Cortleven et al., 2019; Gupta et al., 2020a; Gupta et al., 2020b). CK signaling through

response regulators was shown several times to be regulated following pathogen infections (Argueso et al., 2012; Gupta et al., 2020a; Gupta et al., 2020b; Igari et al., 2008).

In some instances, CK was suggested to either promote (Choi et al., 2010) – or inhibit (Argueso et al., 2012; Choi et al., 2010) - growth and defense simultaneously, rather than effecting a tradeoff, although experimental evidence of this has thus far been limited. Here, we observed that priming immunity and growth with T39 or ASM also induced CK signaling (**Figs. 5-6**). CK signaling was induced to a greater extent with T39, with the two-component sensor being strongly activated (**Fig. 5**), and the expression of response regulators and CKX enzymes being significantly induced (**Fig. 6A-B**). GA signaling, which is often the result of a balance between CK and GA (Shwartz et al., 2016), was also affected by both elicitors (**Fig. 6C**). Meristem maintenance was promoted, and differentiation was reduced, with T39 alone, though both elicitors promoted organ determination (**Fig. 6D**). Interestingly, ASM was able to continue to promote plant height and induce disease resistance under CK attenuation or increase, while T39 was not able to promote increases in plant height under CK attenuation (**Fig. 7**). Growth parameters that are only promoted by T39, such as increased numbers of initiated leaves and increased leaf complexity, could no longer be promoted in *pFIL>>CKX* overexpressing plants (**Fig. 7H-I**), indicating that CK is required for these growth promotion activities. Thus, in the case of T39, uncoupling of the growth-defense tradeoff can be achieved in a CK-dependent manner. Interestingly, though CK requires SA to induce immunity and promote disease resistance (Argueso et al., 2012; Choi et al., 2010; Cortleven et al., 2019; Gupta et al., 2020a; Gupta et al., 2020b), the relationship between SA and CK appears not to be reciprocal, as SA did not appear to require CK in order to induce immunity and promote vertical growth.

Defense priming competence and plant growth promotion competence have overlapping pathways

In this work, we chose to activate SAR and ISR, and examine both the plants' growth response and induced immunity against *B. cinerea*. SAR is purported to depend on SA signaling, while ISR relies on the JA/ET circuit. While SA was shown to be antagonistic to the JA signaling pathway (Pieterse et al., 2009), other works have demonstrated that there are cases where SA and JA are necessary for each other's activities. In most cases, ISR is reportedly regulated by JA/ET signaling, but dependence on SA signaling has been reported as well (Martínez-Medina et al., 2013). Distinctions between ISR and SAR are not clear cut in tomato, and overlap between the

two was reported (Betsuyaku et al., 2018; Liu et al., 2016). Interestingly, though *Trichoderma* spp. are known to potentiate increases in defense via the JA/ET pathway, T39 activated the SA-dependent PR1a (**Fig. 6**, (Meller Harel et al., 2014). Furthermore, *B. cinerea* itself, a necrotrophic fungal pathogen against which the plant host required intact JA signaling to defend itself (AbuQamar et al., 2008), can also activate the SA pathway (Meller Harel et al., 2014). To examine whether the same pathways required for defense in response to priming agents are required for plant growth promotion, we used the SA deficient transgenic line NahG (Brading et al., 2000), the reduced JA mutant *spr-2* (Li et al., 2003), and the reduced ET sensitivity mutant *neveripe* (*nr*, (Lanahan et al., 1994)). *spr-2* is known to have increased susceptibility to *B. cinerea* infection (AbuQamar et al., 2008), while *nr* has similar susceptibility to the background line (Mehari et al., 2015), and NahG has reduced susceptibility or similar susceptibility to the background line (Angulo et al., 2015; Audenaert et al., 2002; Martínez-Hidalgo et al., 2015; Mehari et al., 2015), likely depending on the level of transgene expression. As expected, ASM was not able to induce resistance in NahG, but remained able to do so in *nr* and *spr-2*, while T39 induced resistance in NahG, but not in *spr-2* or *nr* (**Fig. 8**). Investigating the growth promoting effects in parallel, we observed similar results: T39 promoted growth and increased agricultural and developmental parameters in NahG, but was not able to do so in *spr-2* or *nr,* while ASM increased plant height in *spr-2* and *nr*, but not in NahG (**Fig.s 9-11**). The background lines behaved as expected. These results support the notion that development-defense tradeoffs can be uncoupled, with priming for defense resistance also serving to promote plant growth, suggesting that components of these two pathways may overlap.

Defense and developmental pathways partially overlap genetically

In previous work (Israeli et al., 2021) we generated a dataset of genes that promote morphogenesis. Our approach, which was validated in that work, relied on published data (Ichihashi et al., 2014) that includes transcriptomics of three *Solanum* species at four developmental stages. The set of "morphogenetic" genes was generated by selecting genes that showed successive downregulation over successive developmental stages, in the M82 background. Many classical morphogenesis genes were identified in the dataset based on this rule, validating the approach (Israeli et al., 2021). KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis was conducted to identify significantly differential pathways within the

morphogenetic dataset. The "plant-pathogen interaction" pathway was found to be significantly enriched in the morphogenetic dataset (Fisher's exact test, $p<0.05$). Genes of the plant-pathogen interaction pathway found to be significantly enriched in the morphogenetic group are provided in **Supplemental Table 1**. **Fig. 12** provides a cartoon model depicting plant immune responses in the cell, with all the gene groups found to be morphogenetically enriched coded in a purple color. From this model, it emerges that almost all of the immune pathway is represented in the morphogenesis group, providing a framework for the overlap between defense and development we observed here. This suggests that the "development-defense" tradeoff concept may need to be updated to include the notion that within a defined window, defense priming may actually serve to support developmental processes, rather than inhibit them. Pending future research, this could potentially be exploited agriculturally in certain cases.

While plants in nature tend to be uniquely adapted to their particular habitat, and can develop and complete their life cycle in the face of environmental and pathogenic stressors, changing climate and intense cropping practices have in some cases rendered such adaptations ineffective. Our work provides basis for required further research towards the prevention of negative effects that increased immunity can have on plant development and growth.

Materials and methods

Plant materials and growth conditions

Tomato cultivars and mutant and transgenic lines were used throughout the study as detailed in Table 1. Plants were grown from seeds in soil (Green Mix; Even-Ari, Ashdod, Israel) in a growth chamber, under long day conditions (16 hr:8 hr, light: dark) at 24°C.

Table 1: Plant genotypes used in this study

Genotype name	Source	References
S. lycopersicum cultivar M82		
S. lycopersicum cultivar Moneymaker (MM)		
S. lycopersicum cultivar Castelmart (Cm)		
S. lycopersicum cultivar Pearson (Pn)		

pTCSv2::3×VENUS Overexpression of VENUS driven by the synthetic two-component signaling sensor pTCSv2. M82 background line.	Bar lab	(Bar et al., 2016; Steiner et al., 2020)
Suppressor of the prosystemin-mediated response 2 "*spr-2*" - reduced JA production. Castlemart background line.	Prof. Bettina Hause	(Goetz et al., 2012; Montero-Vargas et al., 2018).
Decreased SA transgene "NahG", Moneymaker background line.	Prof. Yigal Elad	(Mehari et al., 2015).
Decreased ethylene sensitivity mutant *neverripe* (*nr*). Pearson background line.	Prof. Yigal Elad	(Mehari et al., 2015).
"pBLS>>IPT7" - elevated endogenous levels of CK. M82 background line.	Prof. Naomi Ori	(Bar et al., 2016; Shani et al., 2010).
"*pFIL>>CKX3"*- decreased endogenous CK content. M82 background line.	Prof. Naomi Ori	(Bar et al., 2016; Shani et al., 2010).

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Elicitor treatments 463

For activation of SAR we used ASM (Marolleau et al., 2017), and for activation of ISR we used *T. harzianum* isolate T39 (Elad, 2000). For mature plant assays (agricultural parameters, disease and immunity assays), elicitors were applied to plants by soil drench, using 5 mL / pot of the indicated concentrations. For developmental analyses, elicitors were sprayed to drip on seedlings of the indicated ages. 464 465 466 467 468

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ASM (Acibenzolar-S-Methyl), also known as BTH (Benzothiadiazole), a synthetic analog of SA, is used commercially to enhance disease resistance (and marketed under the trade name "Bion" (Marolleau et al., 2017; Oostendorp et al., 2001). Previously, application of BTH reduced plant biomass and was correlated with induction of SA-mediated defense responses (Huot et al., 2014). SA analogues have been found to effectively reduce gray mold (*Botrytis cinerea*) disease in tomato (Meller Harel et al., 2014). ASM was shown to be processed in vivo by an SA 470 471 472 473 474 475

processing enzyme (Tripathi et al., 2010). ASM was applied to plants at a concentration of 0.001% v/v.

Trichoderma spp. are established plant growth promoting fungi that enhance disease resistance by inducing ISR in a JA/ET dependent manner (Meller Harel et al., 2014). *Trichoderma* spp. have been shown to promote plant growth and activate ISR against a broad spectrum of pathogens. They are ubiquitous filamentous fungi that colonize the rhizosphere and phyllosphere, promote plant growth, and antagonize numerous foliar and root pathogens (Gupta and Bar, 2020). *T. harzianum* isolate T39 was cultured on PDA plates (Difco lab) at 25°C in natural light. Conidia were harvested in water and applied to plants at a concentration of 10^7 conidia/ ml.

Measuring growth and agricultural parameters

Plants were treated with T39 or ASM once a week by soil drench for 4 weeks, starting from when the plants reached three unfurled leaves, typically 10-14 days after germination. The first 3 treatments were given at a volume of 5 ml, and at last treatment was given at a volume of 10 ml. Growth parameters were measured once a week: shoot length was measured using a hand ruler from root crown to shoot apical meristem. The number of side shoots was counted taking into account only shoots of at least 5 cm in length. For leaf complexity, we counted the primary, secondary and intercalary leaflets on the indicated leaf. Primary leaflets are separated by a rachis, and some of them develop secondary and tertiary leaflets. Intercalary leaflets are lateral leaflets that develop from the rachis later than the primary leaflets and between them, and typically have a very short petiolule.

The following parameters were measured for yield: weight of fruits, weight of shoot, calculated harvest index (yield weight/total plant weight (shoot + fruit weight), and fruit soluble sugar content brix%, which was measured using a ref-85 Refractometer (mrc).

Developmental analysis of seedlings

Seedlings were sprayed to drench with elicitors ten days after germination, and 17 days after germination. Growth and development parameters were analyzed one week after each treatment. Parameters analyzed include height, number of unfurled leaves, leaf developmental stage (No. of the leaf that is Plastochron 1), formation of floral meristem, and leaf complexity.

Shoots were dissected with a surgical blade and No.5 forceps. Analyses following dissection were conducted using a Nikon SMZ-25 binocular stereomicroscope or a Dino-Lite hand held microscope.

B. cinerea infection and disease monitoring

Pathogenesis assays were conducted on detached leaves. We have previously demonstrated that in most cases in tomato, relative disease levels are similar on detached leaves and whole plants, though the disease develops faster in detached leaves (Gupta et al., 2020a). *B. cinerea* isolate *Bc*I16 cultures were maintained on potato dextrose agar (PDA) (Difco Lab) plates and incubated at 22 °C for 5-7 days. 0.4 cm diameter discs were pierced from 5 day old mycelia plates and used for inoculation. The leaves were incubated in a humid chamber. The area of the necrotic lesions was measured five days post inoculation using ImageJ software.

Ethylene measurement

Leaf discs 0.9 cm in diameter were harvested from plants treated as indicated, and average weight was measured for each plant. Discs were washed in water for 1-2 h. Every six discs were sealed with rubber septa in 10-mL Erlenmeyer flasks, containing 1 ml assay buffer (10 mM MES pH 6.0, 250 mM sorbitol) with or without the fungal elicitor ethylene inducing xylanase (EIX), 1 µg/mL, overnight at room temperature. Disposable syringes (5 mL) with 21 gage needles were used to pump out the gaseous sample, and rubber stoppers were used to hold the sample until it was injected into the Gas chromatograph. Ethylene content was measured by gas chromatography (Varian 3350, Varian, California, USA).

Measurement of ROS generation

ROS was measured as previously described (Leibman-Markus et al., 2017). Leaf discs 0.5 cm in diameter were harvested from indicated genotypes. Discs were floated in a white 96-well plate (SPL Life Sciences, Korea) containing 250µl distilled water for 24 h at room temperature. After incubation, water was removed, and 50 µL ddH2O water were immediately added to each well to prevent the tissue from drying up. ROS measurement reaction containing: Luminol 150 µM, HRP 15 µg/mL and either 1 µM flg22 or 1µg/ mL EIX. Light emission was measured immediately and over the indicated time using a microplate reader luminometer (Spark, Tecan, Switzerland).

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Imaging of the CK-response synthetic promoter pTCSv2::3×VENUS 539

Stable transgenic M82 tomato pTCSv2::3 × VENUS plants that express VENUS driven by the synthetic two-component signaling sensor pTCSv2 (Bar et al., 2016; Steiner et al., 2020) were sprayed with elicitors ten days after germination. VENUS expression was analyzed 24 h after treatment using a Nikon SMZ-25 stereomicroscope equipped with a Nikon-D2 camera and NIS Elements v. 5.11 software. ImageJ software was used for analysis and quantification of captured images. 540 541 542 543 544 545

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RNA extraction and qRT-PCR: 547

Plants were sprayed with elicitors ten days after germination, and, optionally, a second treatment was given 1 week later. Plants tissue was collected 48 hours after each treatment. In both cases, ~70 mg of young shots (typically consisting of the meristem and 4 youngest leaf primordia, m+4) were collected (5-7 shoots). Total RNA was extracted from tomato shoots using Tri-reagent (Sigma-Aldrich) according to the manufacturer's instructions. RNA (3µg) was converted to first strand cDNA using reverse transcriptase (Promega, United States) and oligodT. qRT-PCR was performed according to the Power SYBR Green Master Mix protocol (Life Technologies, Thermo Fisher, United States), using a Rotor-Gene Q machine (Qiagen). Relative expression was quantified by dividing the expression of the relevant gene by the geometric mean of the expression of three normalizers: ribosomal protein *RPL8* (Solyc10g006580), Cyclophillin *CYP* (Solyc01g111170) and EXPRESSED *EXP* (Solyc07g025390). All primers pairs had efficiencies in the range of 0.97-1.03. Primers used for qRT-PCR are detailed in **Supplemental Table 2**. 548 549 550 551 552 553 554 555 556 557 558 559 560

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Supplemental Materials 562

Figure S1: Treatment with T39 affects seedling growth and development.

Figure S2: Treatment with T39 accelerates leaf development.

Figure S3: Treatment with T39 and ASM affects developmental gene expression after one treatment.

Supplemental Table 1: Genes recognized as "plant-pathogen interaction" by KEGG that are defined as morphogenetic**.**

Supplemental Table 2: qPCR primers used in this work.

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Competing interests

The authors declare no competing interests.

Author contributions

M.B. conceived and designed the study. A.S., M.L-M., R.G., I.M., D.R-D., Y.E. and M.B. formulated the methodology and carried out the experiments. A.S., M.L-M., D.R-D., R.G., Y.E. and M.B. analyzed the data. All authors contributed to the writing of the manuscript.

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the paper and its Supporting Information files. Raw data is available from the corresponding author upon reasonable request.

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Figures and legends

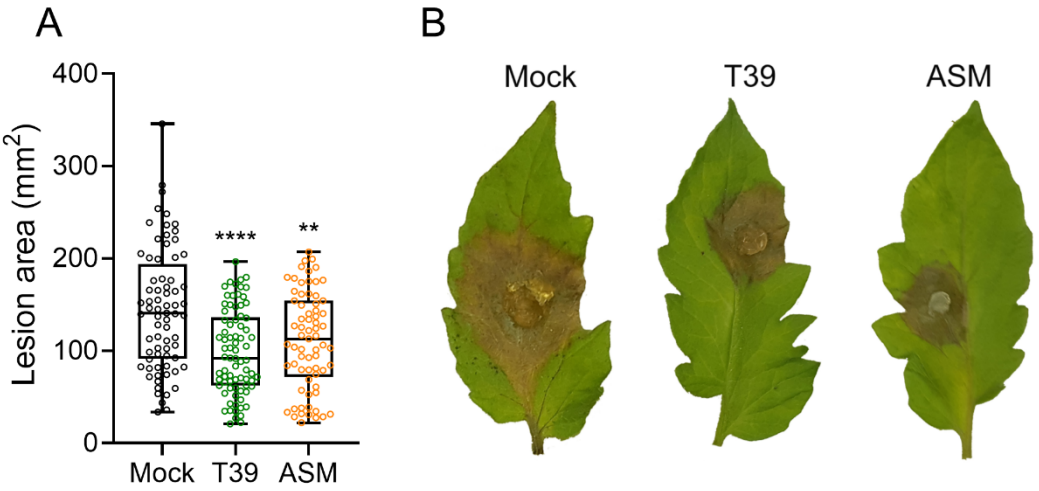


Figure 1: Treatment with T39 and ASM reduces *B. cinerea* disease.

S. lycopersicum cv M82 Tomato plants were soil-drenched twice with elicitors: 3 days and 4 h before *Botrytis cinerea* inoculation. Plant were inoculated with 3 day-old *B. cinerea* mycelia. Plants treated with DDW were used as mock. Lesion area was measured 5 days after inoculation. Experiment was repeated 5 independent times. **A** Lesion area. **B** representative images, 5 days after inoculation.

Box plots indicate inner quartile ranges (box), outer quartile ranges (whiskers), median (line), all points shown. Asterisks indicate significance from Mock treatment in an unpaired t test with Welch's correction, N = 70, *p<0.05; **p<0.01; ****p<0.0001.

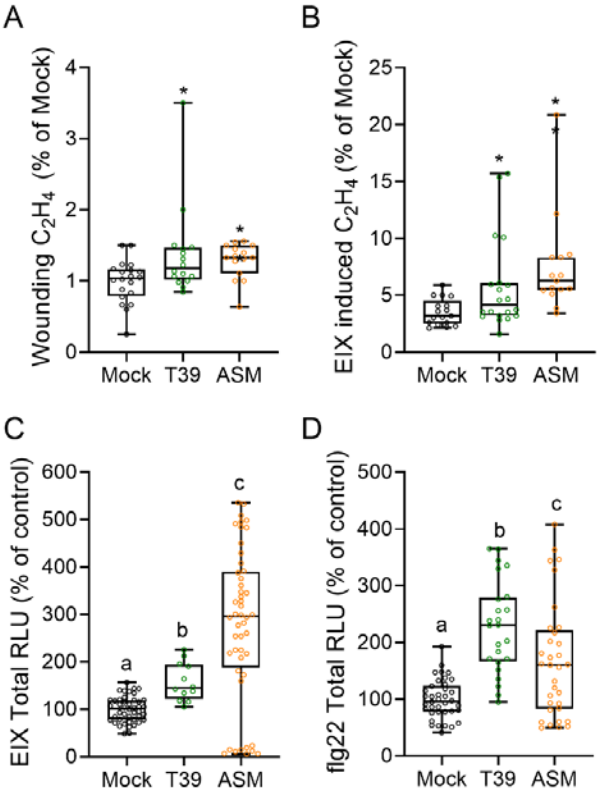


Figure 2: Treatment with T39 and ASM induces immune responses.

S. lycopersicum cv M82 Tomato plants were soil-drenched twice with elicitors: 3 days and 4 h before assays. Plants treated with DDW were used as mock. **A-B** Ethylene production was measured alone (A) or with the addition of 1 µg / mL Ethylene Inducing Xylanase (EIX, B), using gas-chromatography. **C-D** ROS production was measured every 5 minutes for 90 minutes (EIX) and every 3 minutes for 60 minutes (flg22) using the HRP luminol method, and expressed in terms of total RLU (Relative Luminescence Units).

Box plots indicate inner quartile ranges (box), outer quartile ranges (whiskers), median (line), all points shown. Asterisks indicate statistical significance from Mock treatment, and different letters indicate statistically significant differences among samples in Welch's ANOVA with a Dunnett post-hoc test , A-B: N=15, *$p<0.05$, C: N>12, $p<0.0007$, D: N>24 ,$p<0.045$.

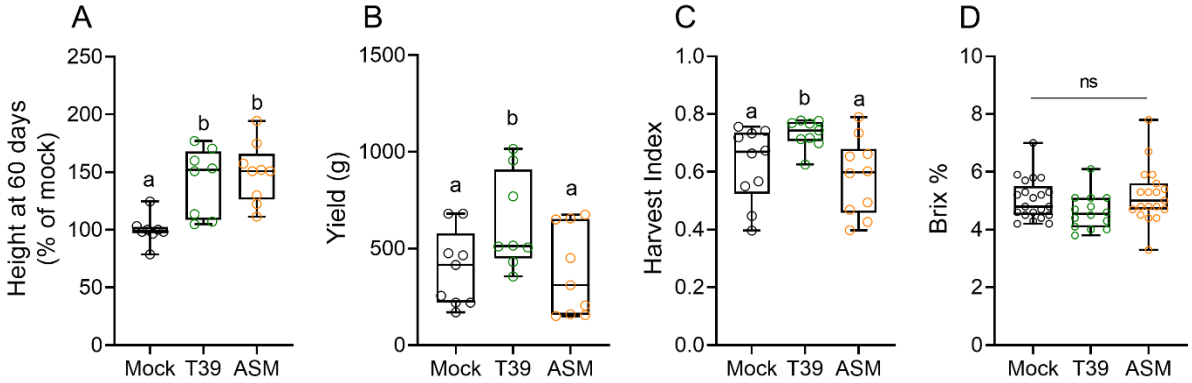


Figure 3: Treatment with T39 and ASM affects plant growth and agricultural parameters.

S. lycopersicum cv M82 Tomato plants were soil-drenched with elicitors once a week for four weeks. Plants treated with DDW were used as mock. **A** Height of 60 day old plants. **B** Average fruit weight per plant. **C** the amount of yield obtained per vegetative plant weight, expressed as Harvest Index. **D** Average tomato soluble sugar %, expressed as Brix.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Different letters indicate statistically significant differences among samples in an unpaired two-tailed t-test, A: N=8, $p<0.005$; B: N=9, $p<0.047$; C: N=10, $p<0.032$; D: N> 14, ns=not significant.

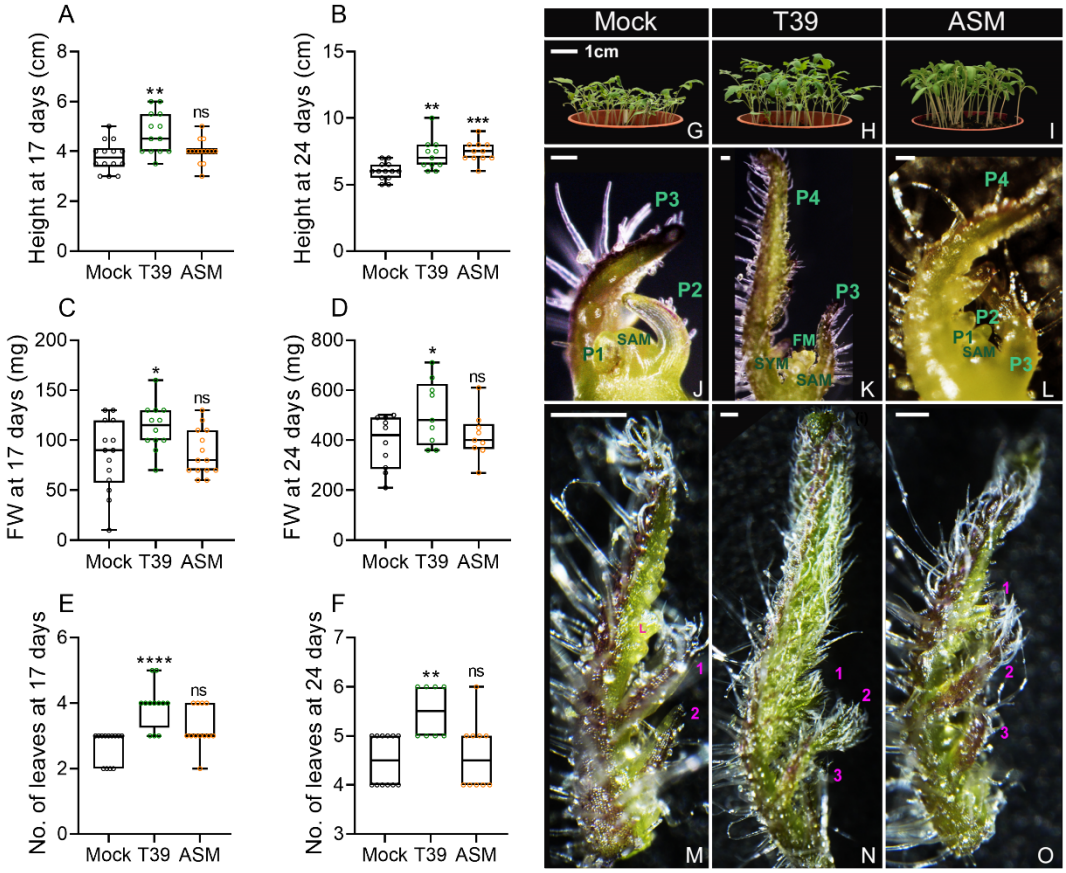


Figure 4: Treatment with T39 and ASM affects seedling growth and development.

S. lycopersicum cv M82 Tomato seedlings were soil-drenched with elicitors once (at 10 days of age, A,C,E) or twice (at 10 and 17 days of age, B,D,F). Seedlings treated with DDW were used as mock. **A,B** Height. **C, D** Fresh weight. **E, F** Number of unfurled leaves.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Asterisks indicate statistical significance from Mock treatment in one-way ANOVA with Tukey's post hoc test, A: N=14, $p<0.0012$; B: N=12, $p<0.0052$; C: N=12, $p<0.04$; D: N=9, $p<0.04$; E:N=12, $p<0.0001$, F: N=8, $p<0.003$.

G-O representative images at 24 days of seedlings (G-I), a single shoot with the two oldest leaves removed (J-L), and Leaf No. 5 (M-O), which was at the P5 stage in Mock and ASM (M, O), and at the P6 stage in T39 (N). J-O were acquired using a Nikon SMZ25 stereomicroscope equipped with a Nikon DS-RI2 camera and NIS elements software. Bars: G (For G-I): 1cm, J-L: 100 µM, M-O 250 µM.

SAM= shoot apical meristem; FM= floral meristem; SYM= Sympodial meristem (the vegetative meristem that continues to produce leaves after transition to flowering). P1/2/3/4 indicates developmental plastochron.

In M-O, numbers indicate the pairs of initiated leaflets on the developing leaf. "L" indicates lobes on the Mock leaf.

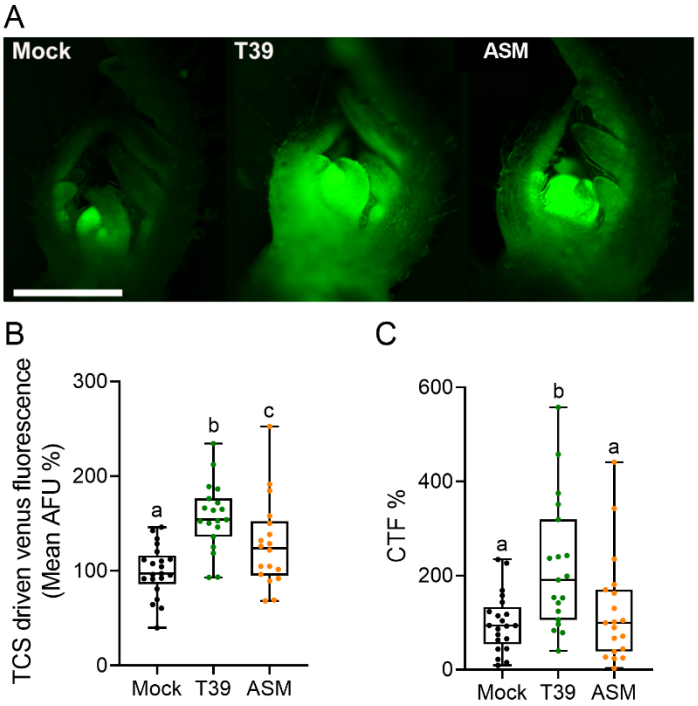


Figure 5: Treatment with T39 and ASM activates cytokinin response

S. lycopersicum cv M82 Tomato seedlings expressing the cytokinin response marker TCSv2::3XVENUS were soil-drenched with elicitors at 10 days of age, and imaged 24 h after treatment. Seedlings treated with DDW were used as mock. **A** Representative shoots. **B** Mean fluorescent signal, expressed as % of the Mock signal (AFU- arbitrary fluorescent units). **C** Corrected total fluorescence, CTF, measured in shoots (CTF= Integrated Density – (Area of selected cell X Mean fluorescence of background readings). Measurements were taken using FIJI-ImageJ.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Different letters indicate statistically significant differences among samples in one-way ANOVA with Tukey's post hoc test, B: N=18, $p<0.04$; C: N=18, $p<0.026$.

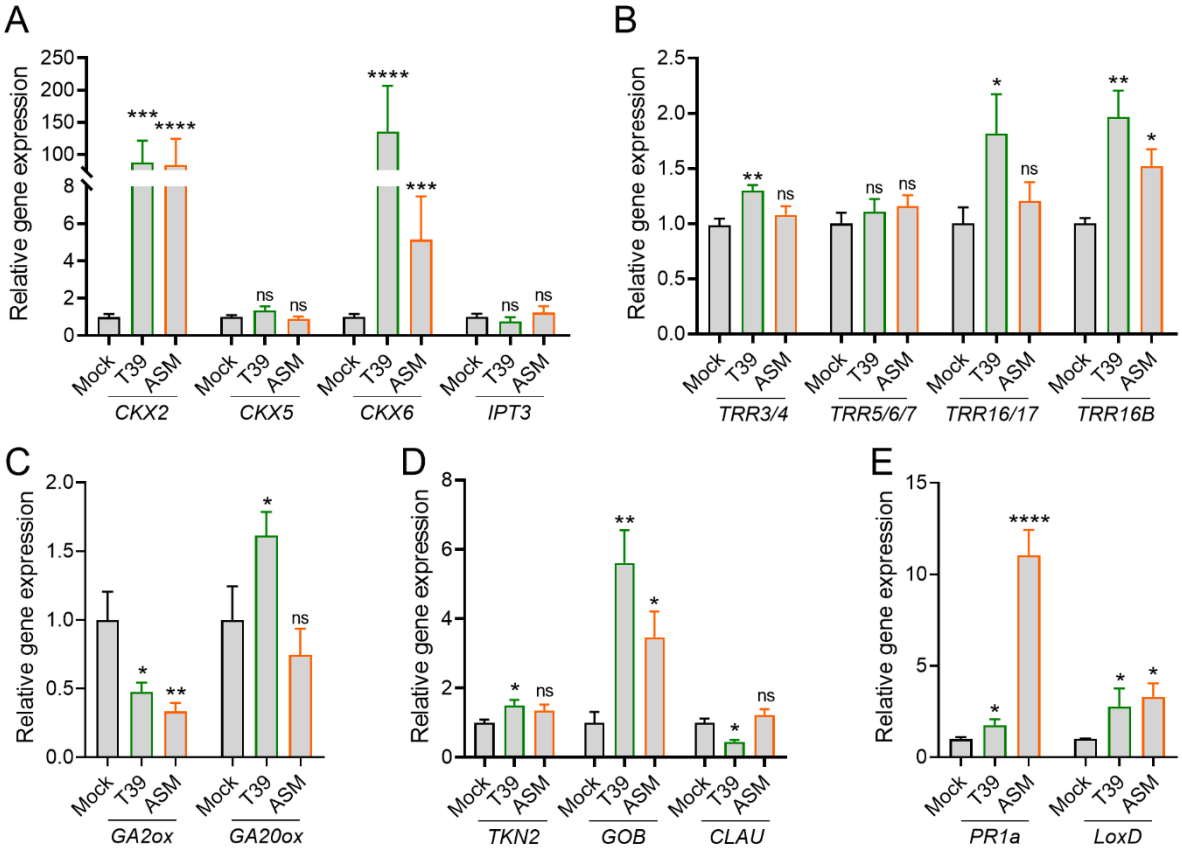


Figure 6: Treatment with T39 and ASM affects developmental gene expression

S. lycopersicum cv M82 Tomato seedlings were soil-drenched with elicitors twice, at 10 and 17 days of age. RNA was prepared 48 h after the second treatment. Seedlings treated with DDW were used as mock. qRT-PCR was conducted to examine gene expression, with relative expression normalized to the geometric mean of the expression of 3 normalizer genes: *EXP (*Solyc07g025390*)*, *CYP* (Solyc01g111170), and *RPL8* (Solyc10g006580). **A** Cytokinin biosynthesis (*IPT3*) and degradation (*CKXs*) genes. **B** Cytokinin signaling response regulators (*TRRs*). **C** Gibberellin biosynthesis regulation (*GA20ox*) and degradation (*CKX2ox*) genes. **D** Developmental transcription factors (*TKN2*- meristem maintenance; *GOB*- organ determination; *CLAU*- differentiation promotion). **E** Defense genes (*PR1a*- SAR; *LoxD*- ISR).

Bars represent mean ±SE, N=8. Asterisks represent statistical significance from Mock treatment in Welch's t-test, for each gene. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns- not significant.

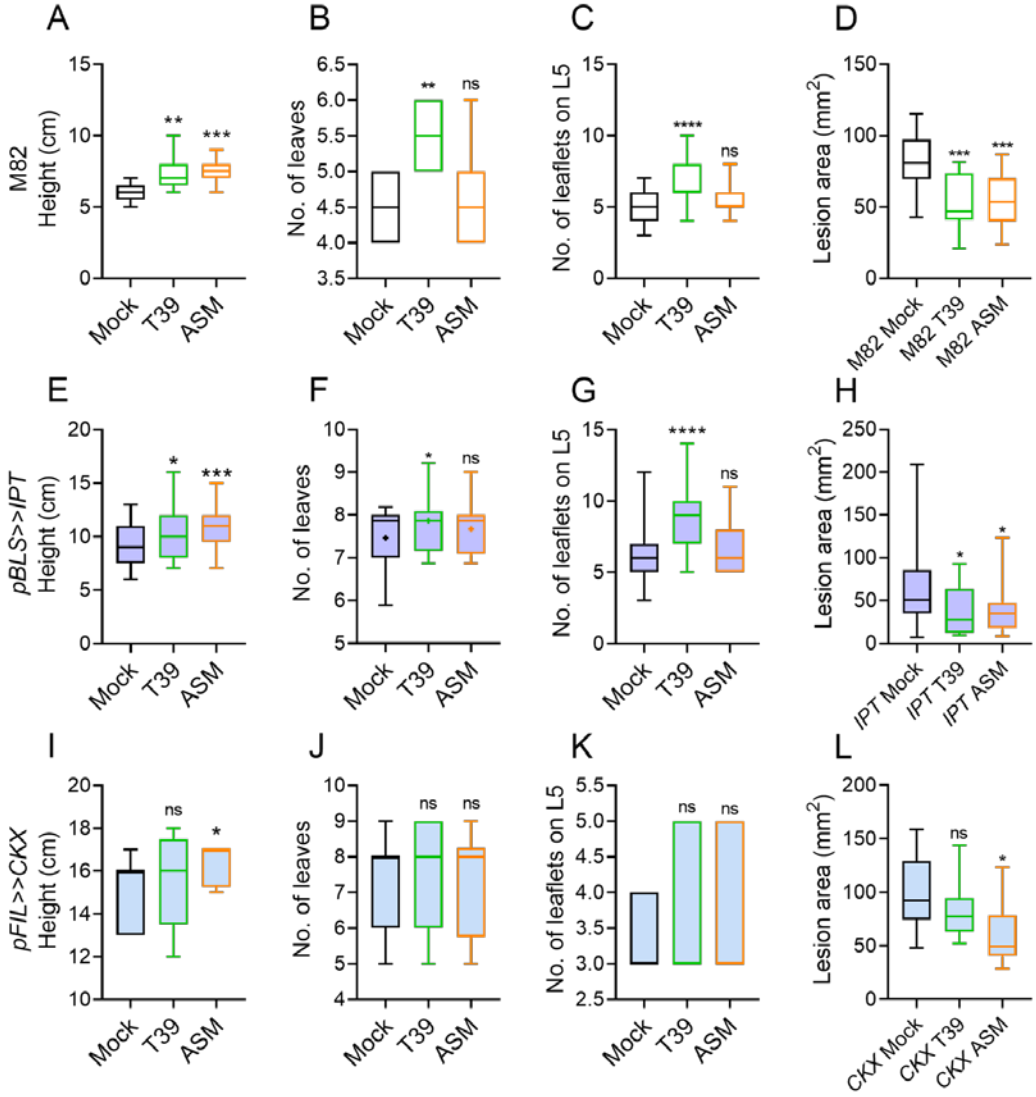
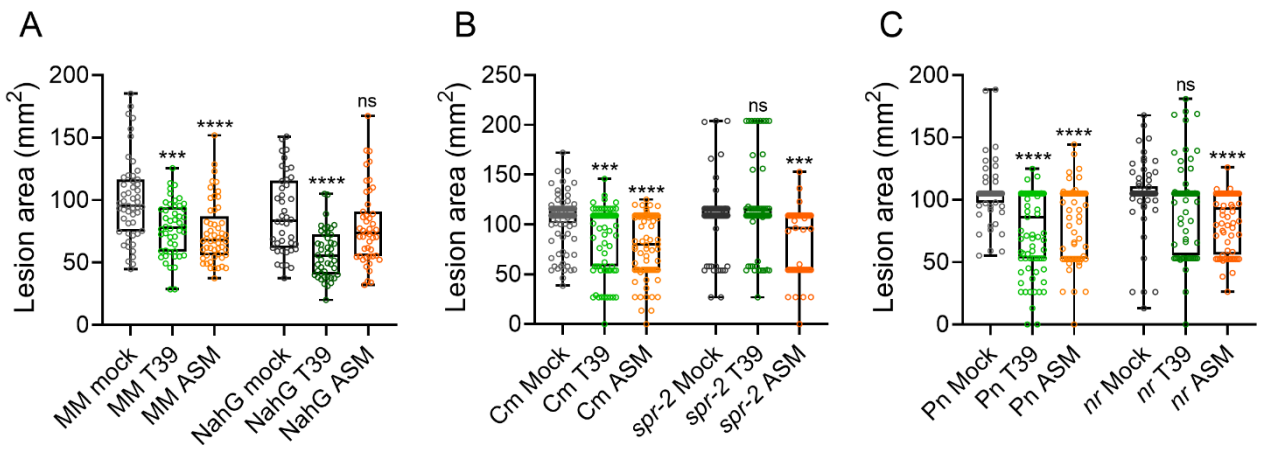


Figure 7: Treatment with T39, but not ASM, requires CK to promote growth and development.

Tomato seedlings of *S. lycopersicum* cv M82 (A-C), and the transgenic *pBLS>>IPT* (E-G) or *pFIL>>CKX* (I-K), both in the M82 background, were soil-drenched with elicitors twice, at 10 and 17 days of age. Seedlings treated with DDW were used as mock. Growth parameters were measured 1 week after the second treatment. **A, E, I** Height. **B, F, J** Number of leaves. **C, G, K** Leaf complexity, expressed as the number of leaflets on L5. **D, H, L** 4 week old tomato plants of the indicated genotypes were soil-drenched twice with elicitors: 3 days and 4 h before *Botrytis cinerea* inoculation. Plant were inoculated with 3 day-old *B. cinerea* mycelia. Plants treated with DDW were used as mock. Lesion area was measured 5 days after inoculation. Experiment was repeated 3 independent times.

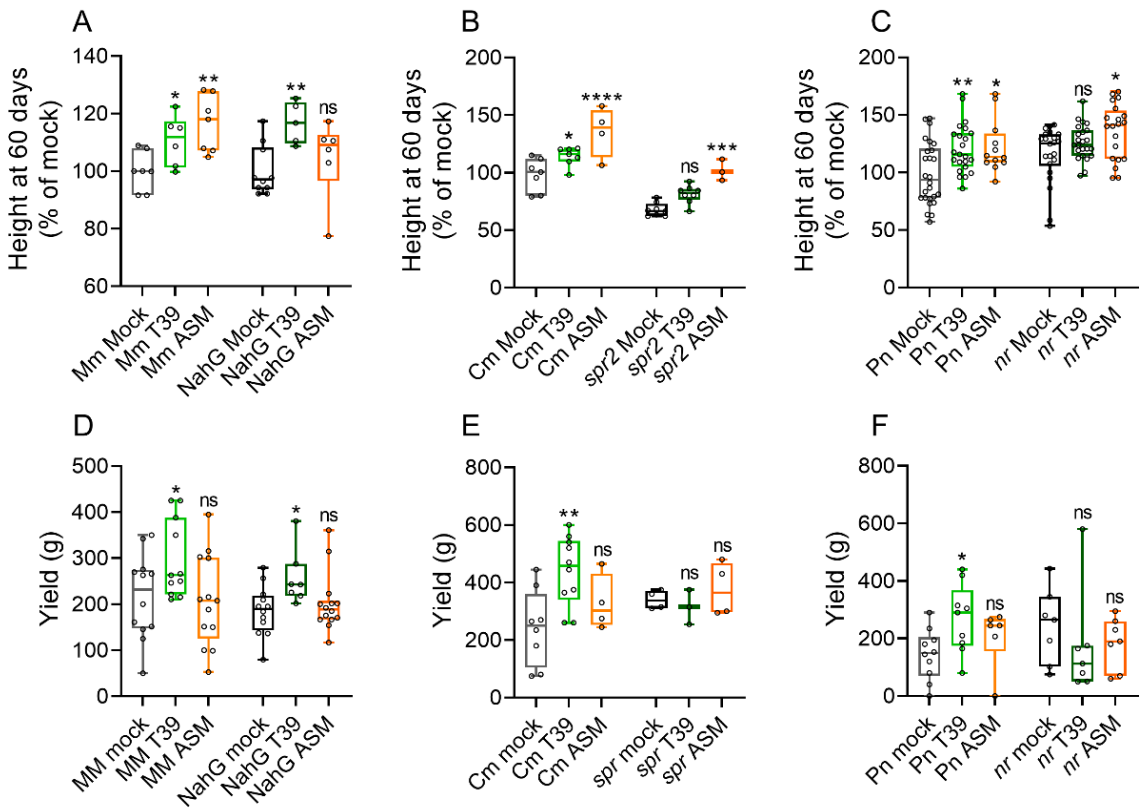
Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values. F: plus sign indicates mean. Asterisks indicate statistical significance from Mock treatment in one-way ANOVA with Bonferroni's post hoc test, A: N=12. B: N=12. C: N=26. D: N=16. E: N=50. F: N=35. G: N=35. H: N=30. I: N=12. J: N=25. K: N=12. L: N=14. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns-not significant.



<u>Figure 8: Treatment with T39 and ASM shows differential effects on *B. cinerea* disease reduction in defense pathway mutants.</u>

S. lycopersicum plants of the cultivars Moneymaker (MM) and the reduced SA transgene NahG (**A**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C**) were soil-drenched twice with elicitors: 3 days and 4 h before *Botrytis cinerea* inoculation. Plant were inoculated with 3 day-old *B. cinerea* mycelia. Plants treated with DDW were used as mock. Lesion area was measured 5 days after inoculation. Experiment was repeated 4 independent times.

Box plots indicate inner quartile ranges (box), outer quartile ranges (whiskers), median (line), all points shown. Asterisks indicate significance from Mock treatment within each genotype, in Welch's ANOVA with Dunnett's post hoc test, A: N> 40, B: N>55, C: N>60. ***p<0.001; ****p<0.0001; ns- non significant.



<u>Figure 9: Treatment with T39 and ASM shows differential effects on plant growth and agricultural parameters in defense pathway mutants.</u>

S. lycopersicum plants of the cultivars Moneymaker (MM) and the reduced SA transgene NahG (**A, D**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B, E**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C, F**) were soil-drenched with elicitors once a week for four weeks. Plants treated with DDW were used as mock. **A-C** Height of 60 day old plants. **D-F** Average fruit weight per plant.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Asterisks indicate significance from Mock treatment within each genotype, in Welch's ANOVA with Dunnett's post hoc test, A: N> 5, B: N>3, C: N>12, D: N>7, E: N>3 F: N>6. *p<0.05, **p<0.01, ***p<0.001; ns- non significant.

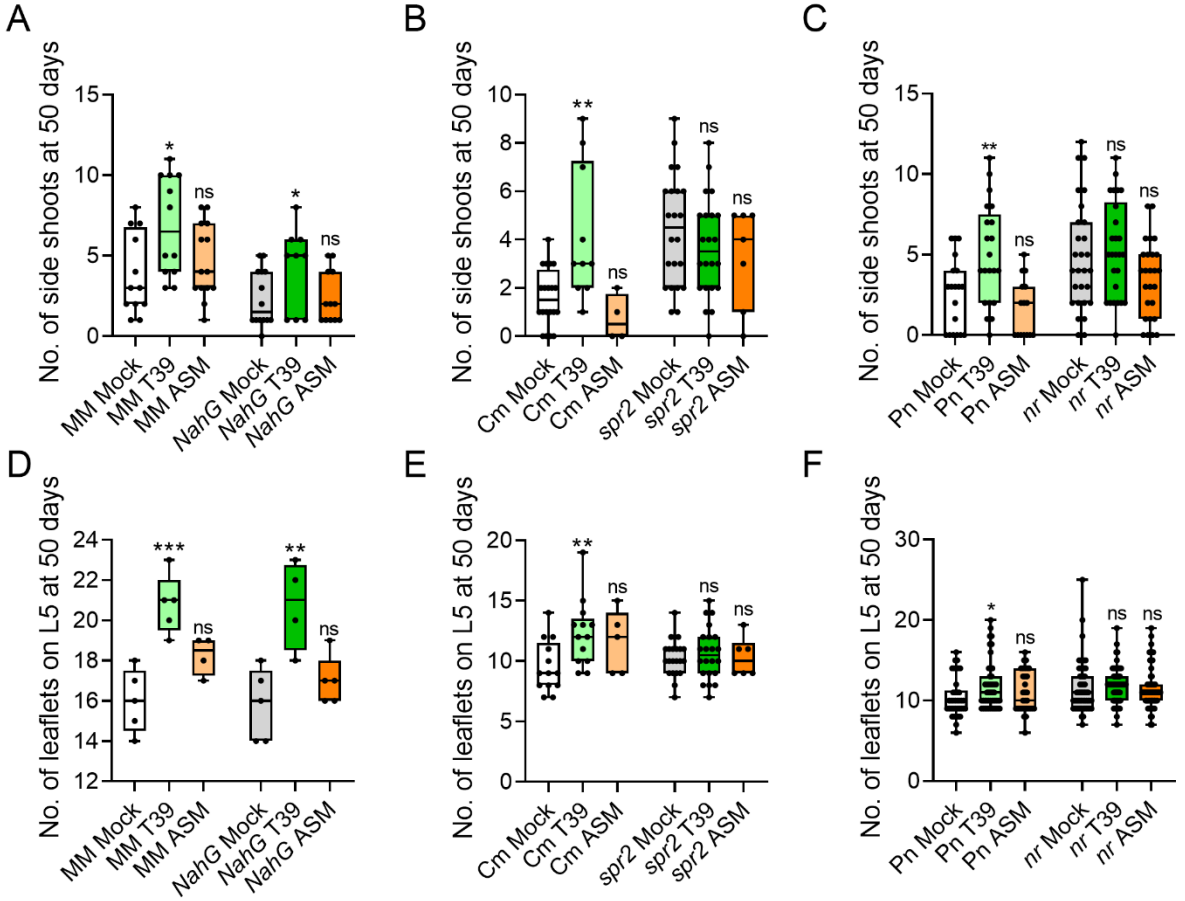


Figure 10: Treatment with T39 or ASM shows differential effects on plant development parameters in defense pathway mutants.

S. lycopersicum plants of the cultivars Moneymaker (MM) and the reduced SA transgene NahG (**A, D**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**B, E**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**C, F**) were soil-drenched with elicitors once a week for four weeks. Plants treated with DDW were used as mock. **A-C** Side shoot production in 50 day old plants. **D-F** Leaf complexity of leaf No. 5 on 50 day old plants.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown. Asterisks indicate significance from Mock treatment within each genotype, in Welch's ANOVA with Dunnett's post hoc test, A: N>9, B: N>5, C: N>12, D: N>5, E: N>5 F: N>18. *p<0.05, **p<0.01, ***p<0.001; ns- non significant.

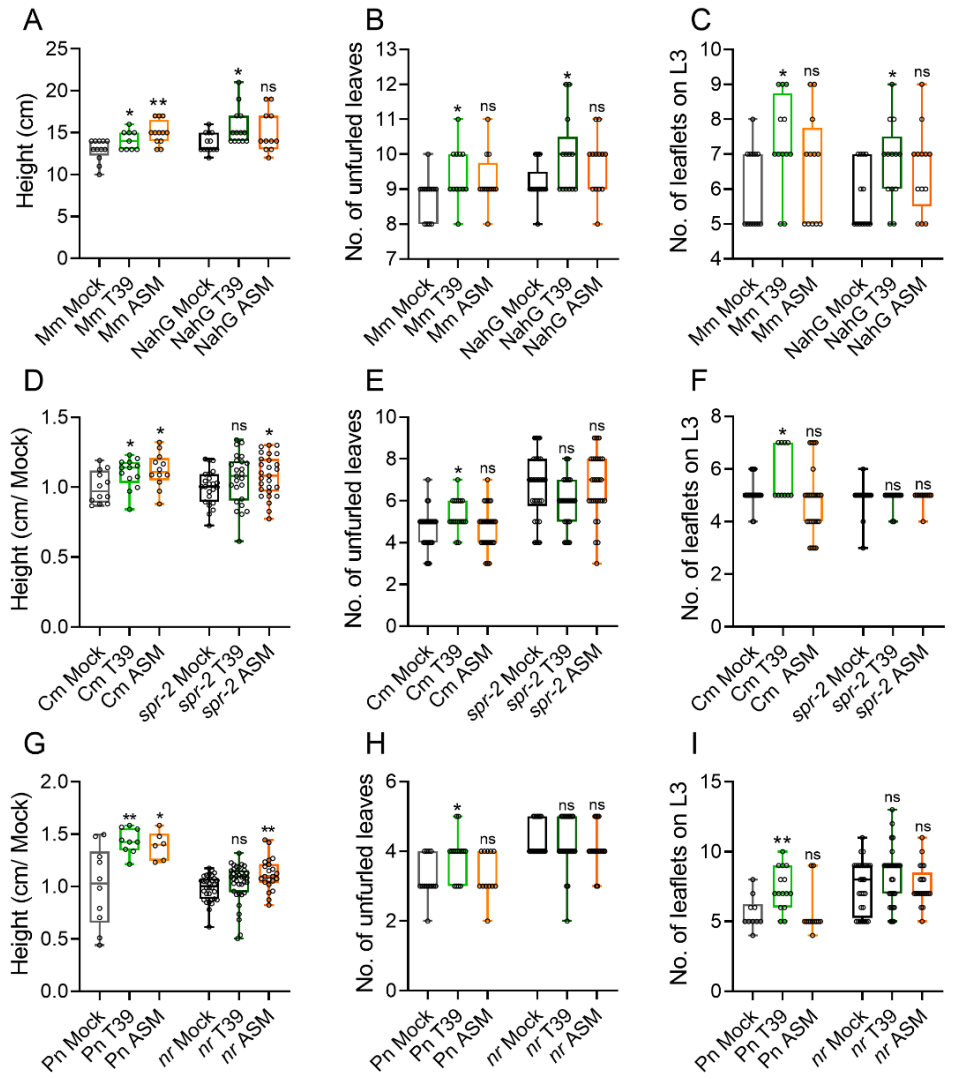


Figure 11: Treatment with T39 or ASM shows differential effects on plant development parameters in defense pathway mutant seedlings.

S. lycopersicum seedlings of the cultivars Moneymaker (MM) and the reduced SA transgene NahG (**A-C**), Castelmart (Cm) and the reduced JA mutant *spr-2* (**D-F**), and Pearson (Pn) and the reduced ET sensitivity mutant *neveripe* (*nr*) (**G-I**), were soil-drenched with elicitors twice, at 10 and 17 days of age. Seedlings treated with DDW were used as mock. Parameters were measured 1 week after the second treatment. **A, D, G** Height. **B, E, H** Number of leaves. **G, H, I** Leaf complexity, expressed as the number of leaflets on L3.

Boxplots are shown with inter-quartile-ranges (box), medians (line in box) and outer quartile whiskers, minimum to maximum values, all points shown, N>12. Asterisks indicate statistical significance from Mock treatment of the same genotype in one-way ANOVA with Bonferroni's post hoc test, *p<0.05, **p<0.01, ns- not significant.

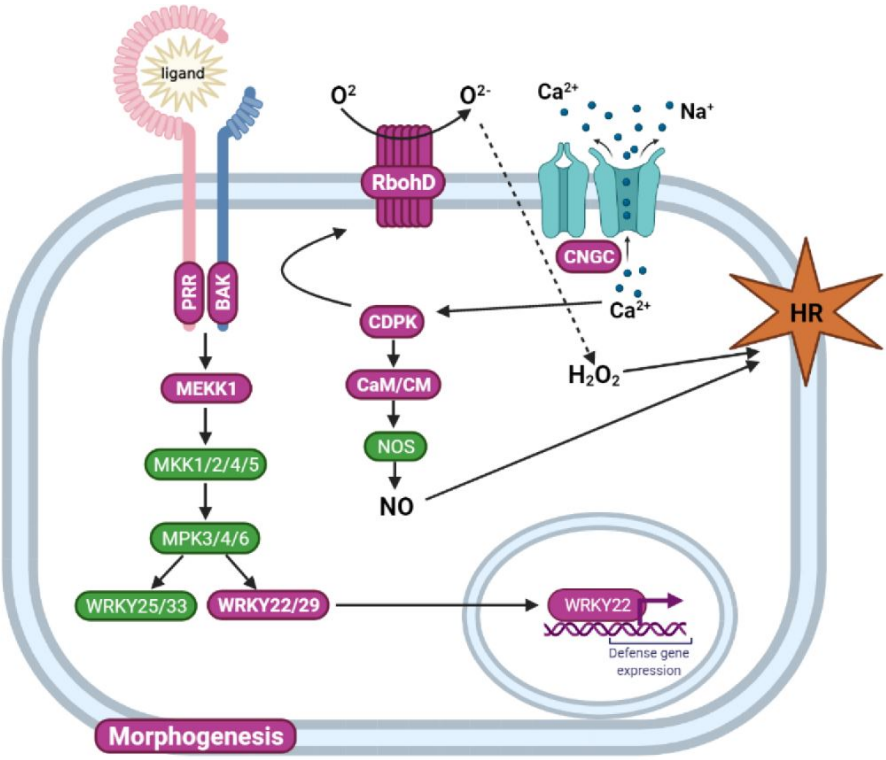


Figure 12: Cartoon model of the overlap between developmental morphogenesis and defense responses.

The model details different genetic groups involved in defense responses within the cell:

PRR- Pattern Recognition Receptor: recognition of MAMPs, PAMPs

BAK- Bri-Associated Kinase: immune co-receptor for several PRRs

MEKK/ MKK/ MPK- Mitogen activated protein kinases: transduction of immune signals

WRKY- Transcriptional regulation of biotic stress responses

RbohD- Membrane localized NADPH oxidase: generation of ROS

CDPK- Calcium Dependent protein Kinase: stress response

CNGC- cyclic nucleotide-gated channel: PAMP-induced calcium signaling

CaM/ CM- Calmodulin: Calcium sensors, involved in calcium-dependent regulation of gene expression during plant immune responses.

NOS- Nitric Oxide Synthase: required for HR, involved in both SA and ET/JA signaling

Generated defense responses: Defense gene expression, reactive oxygen (H_2O_2), Nitric Oxide (NO), Hypersensitive response (HR).

Gene groups highlighted in fuchsia are also found in the group of genes promoting morphogenesis (Israeli et al. 2021). Illustration created with BioRender.com.

Supplemental Materials

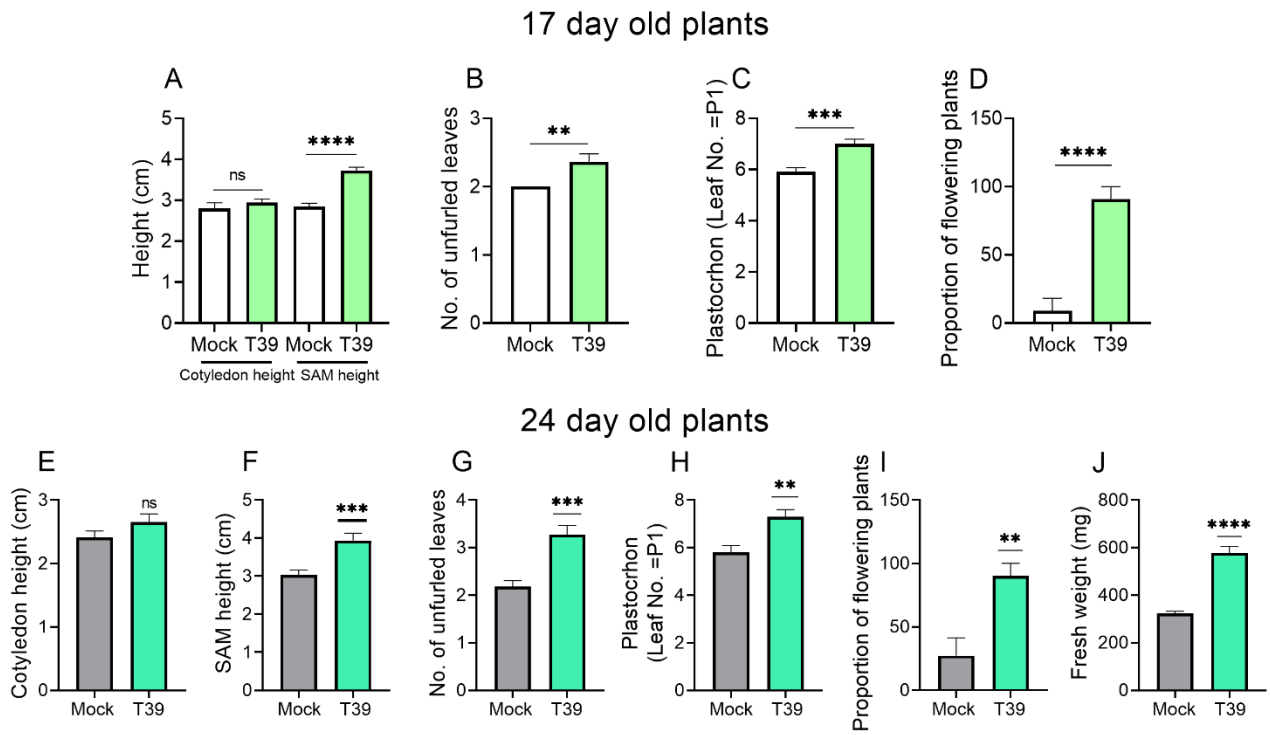


Figure S1: Treatment with T39 affects seedling growth and development.

S. lycopersicum cv M82 Tomato seedlings were soil-drenched with T39 once (at 10 days of age, **A-D**) or twice (at 10 and 17 days of age, **E-J**). Seedlings treated with DDW were used as mock. **A, E ,F** Height parameters. **B, G** Number of unfurled leaves. **C, H** Leaf developmental plastochron, i.e., the leaf number that has just initiated from the SAM and is at the P1 stage. **D, I** the proportion of plants in which the SAM has differentiated into a floral meristem. **J** Fresh weight.

Bars represent mean ±SE, N=10. Asterisks indicate statistical significance from Mock treatment in an unpaired two-tailed t-test. **p<0.01, ***p<0.001, ****p<0.0001.

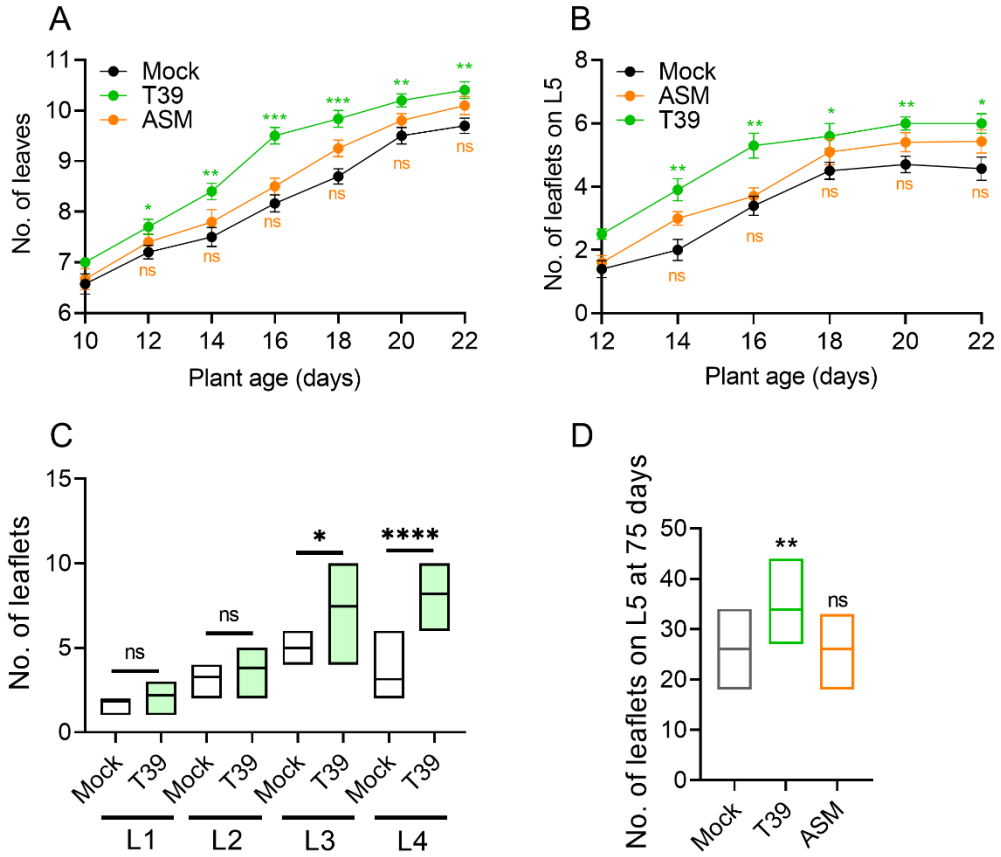


Figure S2: Treatment with T39 accelerates leaf development.

S. lycopersicum cv M82 Tomato seedlings were soil-drenched with elicitors twice (at 10 and 17 days of age, **A-C**), or 4 times, once a week, starting at the age of 10 days (**D**). Seedlings treated with DDW were used as mock. **A** Number of initiated leaves over time, starting from the day of the first elicitor treatment. **B** Number of leaflets on L5 over time, starting from the day of the first elicitor treatment. **C** Number of leaflets on leaves 1-4, one week after the second treatment. **D** Number of leaflets on L5 of mature 75 day old plants.

A-B Points on kinetic graph represent mean ±SE, N=10. Asterisks indicate statistical significance from Mock treatment in multiple t-tests with Holm-Sidak correction. *p<0.05, **p<0.01, ***p<0.001, ns- non significant.

C-D Floating bars represent minimum to maximum values, line in box indicates mean, C: N=10, D: N=7. Asterisks indicate statistical significance from Mock treatment in unpaired two-tailed t-test. *p<0.05, **p<0.01, ****p<0.0001, ns- non significant.

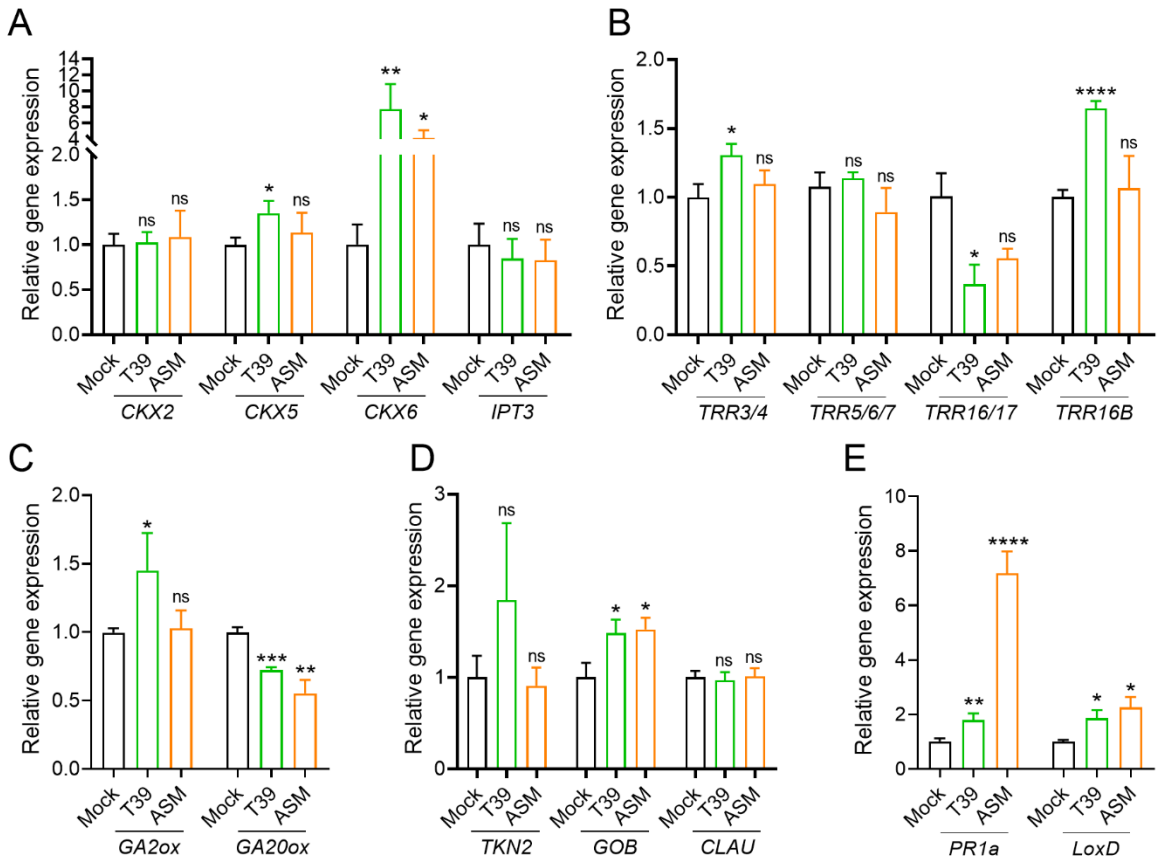


Figure S3: Treatment with T39 and ASM affects developmental gene expression after one treatment.

S. lycopersicum cv M82 Tomato seedlings were soil-drenched with elicitors once, at 10 days of age. RNA was prepared 48 h after treatment. Seedlings treated with DDW were used as mock. qRT-PCR was conducted to examine gene expression, with relative expression normalized to the geometric mean of the expression of 3 normalizer genes: *EXP (*Solyc07g025390*), CYP* (Solyc01g111170), and *RPL8* (Solyc10g006580). **A** Cytokinin biosynthesis (*IPT3*) and degradation (*CKXs*) genes. **B** Cytokinin signaling response regulators (*TRRs*). **C** Gibberellin biosynthesis regulation (*GA20ox*) and degradation (*CKX2ox*) genes. **D** Developmental transcription factors (*TKN2*- meristem maintenance; *GOB*- organ determination; *CLAU*- differentiation promotion). **E** Defense genes (*PR1a*- SAR; *LoxD*- ISR).

Bars represent mean ±SE, N=8. Asterisks represent statistical significance from Mock treatment in Welch's t-test, for each gene. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns- non significant.

Supplemental Table 1: Genes recognized as "plant-pathogen interaction" by KEGG that are defined as morphogenetic

Listed are genes belonging to the morphogenetic dataset (Israeli et al., 2021) that were recognized as belonging to the KEGG group "plant-pathogen interaction" (Fishers exact test, $p<0.05$). The table lists the relative expression levels of each gene in successive developmental stages.

Gene_ID	Description	m+P3	P4-d	P5-d	P6-d
Solyc10g074570	Calcium dependent protein kinase 6 (AHRD V1 **** B9MZ11_POPTR); contains Interpro domain(s) IPR002290 Serine/threonine protein kinase	79.0	50.7	40.0	29.3
Solyc04g015980	FLS2 homolog Receptor kinase-like protein (AHRD V1 **** Q7DMC2_ORYLO); contains Interpro domain(s) IPR002290 Serine/threonine protein kinase	11.3	7.0	5.3	4.3
Solyc12g010010	Cyclic nucleotide gated channel (AHRD V1 ***- A9CRE4_MALDO); contains Interpro domain(s) IPR000595 Cyclic nucleotide-binding	125.7	117.7	102.0	89.3
Solyc03g083520	Calmodulin (AHRD V1 ***- B6T4U8_MAIZE); contains Interpro domain(s) IPR011992 EF-Hand type	68.0	63.7	58.7	52.3
Solyc07g065840	Heat shock protein 90 -2	8570.3	7991.7	5098.0	4105.7
Solyc12g099990	SlCaM5 is a member of the calmodulin gene family which modulates the response to calcium signalling. Calmodulin 2 (AHRD V1 ***- Q710C9_BRAOL); contains Interpro domain(s) IPR011992 EF-Hand type	417.0	315.7	260.3	222.3
Solyc11g071740	Calmodulin-like protein	19.7	7.7	5.3	1.3
Solyc04g018110	Calmodulin-like protein 1	43.0	38.3	21.7	19.0
Solyc01g008950	CaM1 is a member of the Calmodulin gene family which modulate responses to calcium signalling. Calmodulin 5/6/7/8-like protein	328.7	277.7	219.7	213.3
Solyc01g104970	BAK/SERK3B Receptor-like kinase (AHRD V1 **** A7VM44_MARPO); contains Interpro domain(s) IPR002290 Serine/threonine	49.3	48.3	46.7	46.3

	protein kinase Receptor like kinase, RLK				
Solyc03g098050	SlCaM6 is a member of the calmodulin gene family which modulates the response to calcium signalling. Calmodulin 3 protein (AHRD V1 **** Q712P2_CAPAN); contains Interpro domain(s) IPR011992 EF-Hand type	297.0	234.0	169.3	169.0
Solyc04g009800	Calcium-dependent protein kinase 2 (AHRD V1 **** Q93YF4_TOBAC); contains Interpro domain(s) IPR002290 Serine/threonine protein kinase	72.0	71.3	62.0	60.0
Solyc08g081690	RBOH1 NADPH oxidase	13.7	5.0	4.7	1.7
Solyc07g053170	SlMAPKKK56 is a part of the MAPKKK gene family known to be involved in the response to various biotic and abiotic stresses in plants. Protein serine/threonine kinase	109.3	105.0	85.7	82.7
Solyc12g098820	Pto homolog Receptor-like kinase (AHRD V1 ***- A7VM24_MARPO); contains Interpro domain(s) IPR002290 Serine/threonine protein kinase	127.7	126.0	121.7	115.0
Solyc01g104530	SlMAPKKK10 is a part of the MAPKKK gene family known to be involved in the response to various biotic and abiotic stresses in plants. Protein serine/threonine kinase	103.0	100.3	87.3	82.7
Solyc01g095100	SlWRKY22 is a member of WRKY transcription factor gene family which has been implicated in multiple biological processes in plants. It has the C2H2 Zinc-finger domain. Computational annotation: WRKY transcription factor 23 (AHRD V1 ***- C9DI12_9ROSI); contains Interpro domain(s) IPR003657 DNA-binding WRKY	14.0	10.0	8.0	7.0

Supplemental Table 2: qPCR primers used in this work

Locus	Name	Forward	Reverse
Solyc07g025390	*EXP*	TGGGTGTGCCTTTCTGAATG	GCTAAGAACGCTGGACCTAATG
Solyc10g006580	*RPL8*	TGGAGGGCGTACTGAGAAAC	TCATAGCAACACCACGAACC
Solyc01g111170	*CYP*	TGAGTGGCTCAACGGAAAGC	CCAACAGCCTCTGCCTTCTTA
Solyc01g106620	*PR1a*	CTGGTGCTGTGAAGATGTGG	TGACCCTAGCACAACCAAGA
Solyc03g122340	*LoxD*	CCATCCTCACCACCCTCATC	TACTCGGGATCGTTCTCGTC
Solyc01g088160	*CKX2*	CCCCGAAAATGGTGAAATG	CAAAGTGGCTTGCTTGAACA
Solyc04g016430	*CKX5*	TGTCACTGGTAAAGGAGAGGTG	GAGCAATCCTAGCCCTTGTG
Solyc12g008900	*CKX6*	CAGGTGCTAAGCCATACTCTAGG	GGACATTCCATTAGGGGACA
Solyc01g080150	*IPT3*	TTCCATGCTTGATGTGCTTC	GCTTGCTGTCAACGTCAAAA
Solyc05g006420	*TRR3/4*	CGTCCCCTAAAGCATTCTCA	CGTCTTGTTGGTGATGTTGG
Solyc03g113720	*TRR5/6/7*	GGGATTGATGGTTTGAAGGT	ATCTTGCTCAACACCGATGA
Solyc06g048930	*TRR16/17*	GGTCTAAGGGCGTTGGAGTA	TCCTGGCATGCAATAATCTG
Solyc06g048600	*TRR 16b*	CATCAATGCATGGAAGAAGG	GCATTGCATTATTTGGCATC
Solyc07g061720	*GA2*	CCAACAACACTTCCGGTCTT	CATTCGTCATCACCTGTAATGAG
Solyc03g006880	*GA20*	AGATTGTGTTGGTGGACTTCAA	TAGCGCCATAAATGTGTCG
Solyc02g081120	*TKN2*	CCATATCCATCGGAATCTCAG	TGGTTTCCAATGCCTCTTTC
Solyc07g062840	*GOB*	CAGGAGTTCGAAGGACGAGTGG	TTGGCTGTAGTGTATGCAAGGTG
Solyc04g008480	*CLAU*	CCTCTCACAACAAGCAATGAACTT	AGGACGATGCAATGAGAGAGAC