The Molecular Interplay Between Climate Change and Plant Disease in Tomato

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Student name/number: Ryan Pendavingh (5514991)
Group: Translational Plant Biology, Utrecht University
Supervisor: dr. Dmitry Lapin
2nd Examiner: dr. Jason Gardiner
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Plain language summary

Due to the growing global population, food demand is expected to increase. At the same time, climate change is causing an increase in extreme weather like drought, heat waves, and heavy rain fall. Due to this, crop yield is projected to decrease globally. Climate change will also increase plant diseases through changes in the spread, growth rate and overwintering of plant pathogens, and thereby further increase the already existing food insecurity.

In the field, plant diseases are likely to co-occur with drought and heat. However, plant stresses are usually studied separately even though the combination of two stresses is not simply the sum of the individual stresses. Combined stress often leads to a worse outcome but in some cases the presence of one stress can also alleviate the other. This can vary depending on the severity and order of the stresses, the pathogen, and the plant species. The latter makes it hard to gain insights based on other plant species. Here, the combined effects of pathogen infection, and drought or heat on the tomato plant, one of the most important vegetable crops, are discussed.

For tomato, the ideal temperature is between 18-25°C during the day and 10-20°C at night. Above these temperatures, heat stress sets in. Various heat stress transcription factors (HSFs), heat shock proteins (HSPs), and other transcription factors and target genes are upregulated. Increased soil temperature (28°C) with normal air temperature strongly reduces the severity of grey mold in tomato plants. This is likely caused by the upregulation of three pathogen-associated-molecular-pattern (PAMP) recognition receptors (PRRs) and several defence-related genes. Exposure to longer periods of more severe heat (38°) after infection also strongly reduces grey mold. However, in this case, it is likely caused by the reduced fitness of the pathogen at 38°C.

A similar effect is seen with bacterial speck disease. Short exposure to extreme heat before infection causes upregulation of PRRs and defence-related genes and

reduces disease severity. Heat stress (31°C) after infection also reduces the disease, but this is likely due to the reduced fitness of the pathogen at that temperature.

Upon drought, tomato plants close their stomata to reduce water loss and prevent wilting. Drought leads to the upregulation of not only drought- but also defence-related genes and HSPs. Both drought and heat stress reduce powdery mildew in tomato as they upregulate defence-related genes and thereby prepare the tomato plant against infection.

The reverse is also possible. Several viruses reduce wilting and prevent plant death under prolonged drought stress. Tomato Yellow Leaf Curl Virus also protects tomato against heat stress by preventing the upregulation of HSFs and HSPs.

All in all, combined stress can lead to reduced disease severity because the defence mechanisms against pathogen infection, heat, and drought share transcription factors and target genes. Still, many questions on the molecular mechanisms behind combined plant disease and drought or heat stress remain. More research is needed to help protect tomato crops against climate change related increase in drought, heat, and plant diseases and to help feed the ever-increasing world population.

Abstract

In the next decades, climate change will lead to more frequent extreme weather while also increasing the burden of plant diseases. Together with the increase in the global population, climate change is expected to further increase the already existing food insecurity. In the field, extreme weather like drought and heat are likely to occur simultaneously with plant diseases. The combination of these two types of stresses is not simply the sum of their effects, both on the morpho-physiological and the molecular level, and can be perceived as a worse as well as a reduced stress. As the outcome of combined stresses can vary per plant species, insights into their molecular mechanisms cannot simply be transferred between species. Here, the separate and combined effects of pathogens, and drought or heat on the tomato plant (*Solanum lycopersicum*), one of the most important vegetable crops, are reviewed.

Introduction

Due to the growing global population, food demand is expected to increase up to 50% between 2010 and 2050 [1]. At the same time, climate change is causing an increase in extreme weather like drought, heat waves, and heavy rain fall [2]. This is paired with a decrease in the quality and availability of fresh water. While climate change can increase the crop yield in certain regions (mainly Northern countries), the crop yield is projected to decrease globally [2]. By 2050, crop loss is expected to increase with 30% in Africa adding to the already existing food insecurity [3]. In addition, the global rise in temperature and changes in precipitation will affect crop yield through changes in the spread, growth rate and overwintering of plant pathogens [4]. Currently, plant pathogens and pests already cause a yield loss of 20 to 30% globally [5], and any yield gains due to positive effects of climate change will likely be offset by the increase in plant diseases [3].

Tomato (*Solanum lycopersicum*) is one of most important crops worldwide accounting for just over 15% of the total vegetable production in 2022 [6]. Tomato plants can be affected by over 200 bacterial, fungal, oomycete, and viral pathogens causing a yield loss of roughly 18% each year [7]. In the field, plants are not only fighting pathogens, but also adverse environmental factors. However, most studies take into account only one of these two factors even though they are likely to occur simultaneously. A plants response to combined stress is hard to predict as it is not simply the sum of the individual stresses [8, 9]. Combined stress alters the expression of an additional set of genes compared to those altered under each individual stress leading to an alternative stress state. Often, the accumulation of two stress leads to a worse outcome [8]. However, the presence of one stress can also alleviate the other, for example heat stress reduces *Botrytis cinerea* infection in tomato [10]. The outcome of the combined stress can vary depending on the stress intensities, the order of the two stresses, and the plant species, e.g. *Sclerotinia sclerotiorum* infection is aggravated by drought in chickpeas but reduced in beans [11, 12]. The latter makes it hard to derive insights into combined stress responses from other species.

Here, an overview is given of the molecular response of tomato to the combinations of pathogen attack and two important factors in climate change, namely drought and heat. First, the response of tomato plants to the individual stresses are discussed followed by their combined effects.

Defence against pathogens

Tomato plants are susceptible to numerous diseases like powdery mildew (*Pseudoidium neolycopersici*), Fusarium wilt, grey mold (*B. cinerea*), yellow curl leaf disease (Tomato Yellow Leaf Curl Virus), and root rot (*Phytophthora* spp.). Common symptoms, in addition to visible growth of the pathogen, are chlorosis, wilting, necrosis, stunted growth, and reduced fruit yield. Traditionally, microbial plant pathogens have

been classified based on their mode of nutrition as biotrophs, hemibiotrophs and necrotrophs [13]. Biotrophs feed from living cells while necrotrophs feed from dead or necrotic cells. Hemibiotrophs switch from a biotrophic to a necrotrophic lifestyle during infection. It has become increasingly clear that many necrotrophic pathogens like *B. cinerea* also have a biotrophic phase in which they supress cell death [13]. As a rule of thumb, biotrophic and hemibiotrophic pathogens elucidate a salicylic acid (SA)-mediated defence response while necrotrophs induce a jasmonic acid (JA)-mediated response, but there are exceptions [14, 15].

The defence system of plants can be divided into three parts: the constitutive defence, pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), and effector-triggered immunity (ETI) [16, 17]. The constitutive defence consists of secondary metabolites which are always present but are often also upregulated in response to pathogen attack. The main group of antimicrobial secondary metabolites are phenolic compounds which includes sesquiterpenes, coumarins, lignin, and the phytohormone SA. Lignin is a branched polymer of phenylpropanoid groups and is part of the plants cell wall where it provides a structural line of defence [16].

PAMPs are conserved molecular patterns which are usually a part of the pathogens cell wall, for instance flagellin, peptidoglycan and chitin, or secreted by the pathogen, e.g. elongation factor Tu or xylanase [18]. PAMPs can be recognised by their corresponding pattern recognition receptors (PRRs) (Figure 1). Two common types of PRRs are receptor like kinases (RLKs) and receptor-like proteins (RLPs) both of which often have a leucine-rich repeat (LRR) domain. Upon ligand binding, most of the PRRs associate with brassinosteroid insensitive 1-associated kinase 1 (BAK1). They phosphorylate each other which triggers downstream signalling [18]. There is an influx of Ca^{2+} and through calcium-dependent kinase (CDK) and mitogen-activated protein kinase (MAPK) signalling, various transcription factors are regulated which induce the expression of pathogenesis-related (pr) genes [17]. Not for all PR proteins their function is clear, but several PRs are chitinases, glucanases, and oxidases which degrade the fungal or bacterial cell wall. Activation of PRRs also leads to a rapid increase in reactive oxygen species (ROS). Among other things, ROS crosslink the plant cell wall to further strengthen it against penetration by pathogens. Sometimes, in response to the ROS burst, the expression and activity of anti-oxidative enzymes like polyphenol oxidase (PPO) and peroxidase (POX) are increased [19, 20].

In an evolutionary arms race, pathogens have developed effectors to interfere with PTI. In response, plants have developed ETI: resistance (R) proteins to recognise and block the function of these effectors [17]. After activation, SA signalling is upregulated which plays a crucial role in inducing systemic acquired resistance [21]. In addition, ETI also activates the hypersensitive response (HR) which triggers rapid cell death of neighbouring cells to prevent the spread of the pathogen [22].

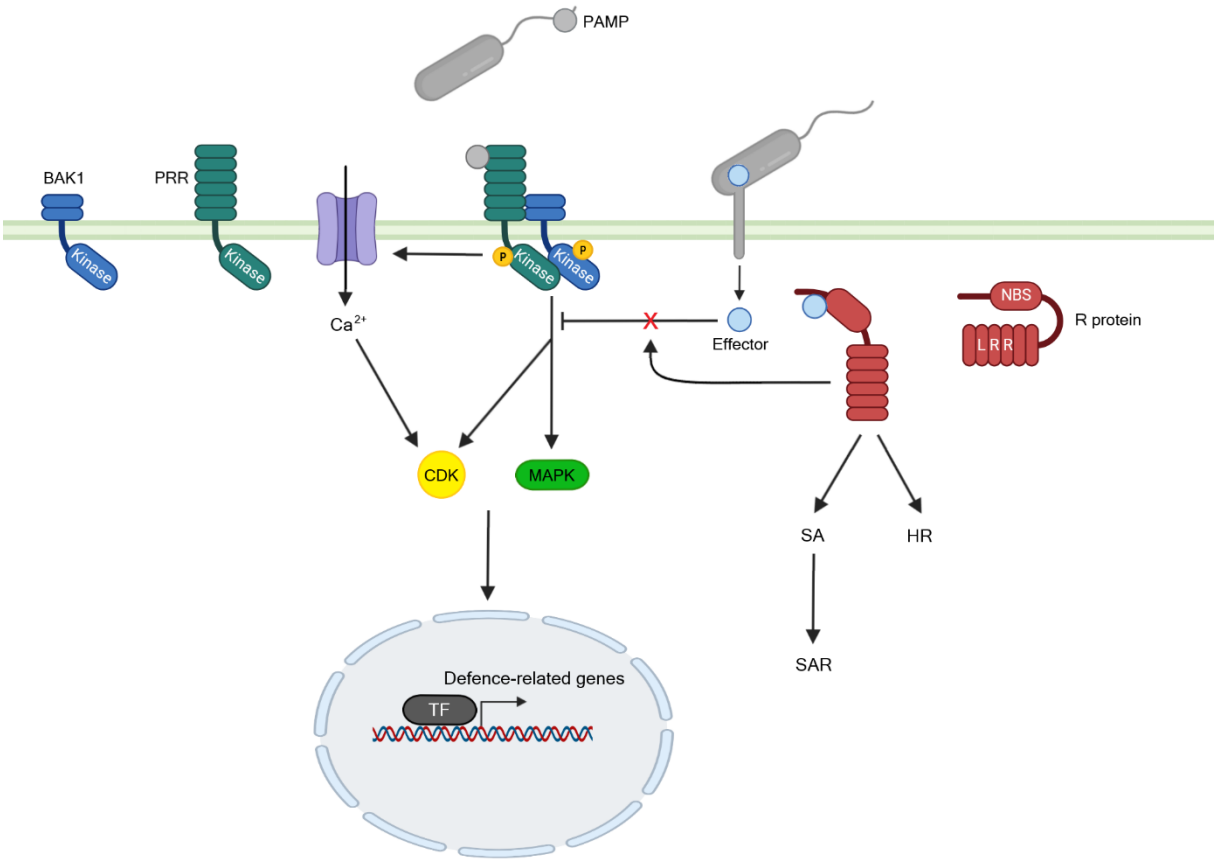

Figure 1: PAMP-triggered and effector triggered immunity. *PRRs are activated upon binding by their corresponding PAMP. Most of the PRRs require BAK1 for activation. After cross-phosphorylation, Ca2+ influx, and CDK and MAPK signalling is triggered which leads to the expression of defence-related genes. Pathogens have developed effector proteins to block PTI. In turn, plants have developed R proteins. Most R proteins contain a NBS and LLR domain and upon binding by their effectors, they activate the HR and upregulate SA signalling which leads to systemic acquired resistance. Made using Biorender icons.*

Response to heat stress

Heat stress sets in when a plant can no longer maintain an optimal tissue temperature. This can be caused by a strong but short increase in temperature, also called a heat shock, or a long-lasting milder increase in temperature. The second form of heat stress is often exacerbated by a combination of excessive sun exposure and reduced transpirational cooling [23]. The latter can be caused high air humidity and/or a low water potential, e.g. due to drought or high salinity. For tomato, the optimal temperature is 18-25°C during the day and 10-20°C at night [24]. When the temperature rises above these ranges, growth of tomato is reduced and flowering, pollination, and fruit development are negatively affected [25].

Plants can sense heat stress through an increase in membrane fluidity which through downstream signalling alters gene expression to induce heat tolerance (Figure 2). The increased membrane fluidity leads to ion leakage, the accumulation of ROS and

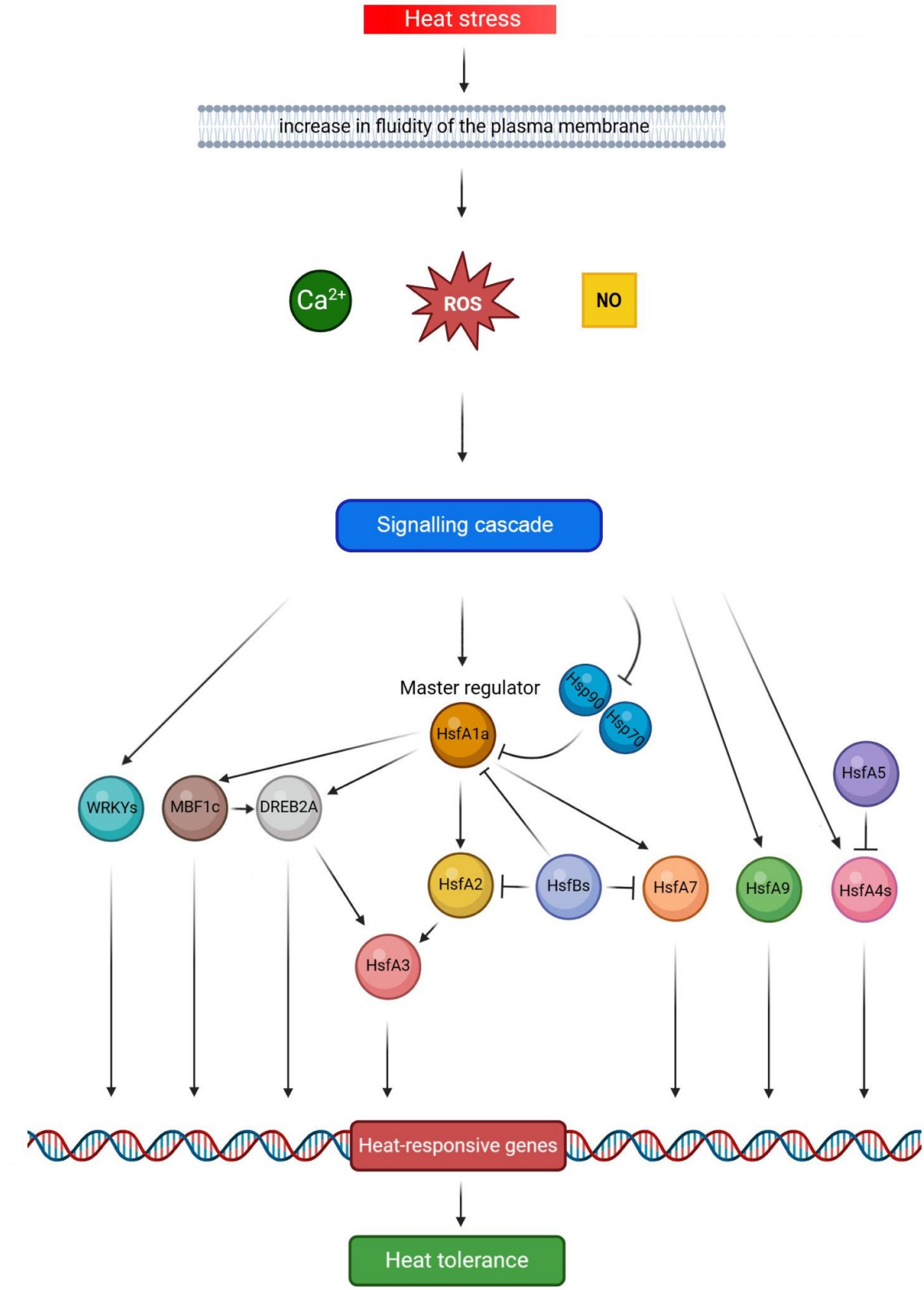

Figure 2: Heat stress is sensed through increased membrane fluidity and leads to the expression of heat stress related genes. *An increase in membrane fluidity triggers Ca2+ and ROS signalling. This leads to the activation of multiple HSFs and other heat stress responsive transcription factors. In turn, they increase the expression of other heat stress responsive genes which leads to heat tolerance. Adapted from* [26]

nitrogen oxide (NO), and an influx of Ca^{2+} through the activation of Ca^{2+} channels [23, 26]. Ca^{2+} signalling is mediated through calmodulin and CDPK28 and leads to an increase in ascorbate peroxidase (APX) and superoxide dismutase (SOD) activity, two anti-oxidative enzymes [27, 28]. The activity of other anti-oxidative enzymes like catalase (CAT) and POX is also increased, possibly through NO signalling [19, 29, 30]. Calmodulin and NO also positively influence the activity of heat shock transcription factors (HSFs) and expression of heat shock protein (HSP) 70 [30].

The increased membrane fluidity also affects other membrane proteins, including those for photosynthesis [23]. Overall, the photosynthesis rate decreases under heat stress [31]. Genes related to the synthesis of tetrapyrroles (which includes chlorophyll) are upregulated, and indeed the chlorophyll content is higher in plants under heat stress [31, 32]. Genes associated with the tricarboxylic acid (TCA) cycle, light reactions, and photorespiration, on the other hand, are downregulated [31].

Heat stress leads to the activation and upregulation of numerous HSFs and HSPs [23, 26]. HSPs function as molecular chaperones and aid in the refolding denatured proteins. HSFA1a is the master regulator and initiates the transcriptional activation of HSFs and HSPs [26]. HSP70 and HSP90 also regulate the heat stress response through their interaction with HSFA1a. HSFA2 plays a role in the maintenance of thermotolerance while HSFB1 represses the activity of other HSFs [26]. Some HSPs are also induced by other abiotic stresses like wounding, drought or salinity which could explain the cross-tolerance between these stressors [23]. Additionally, some defence-related genes have cis-acting heat stress responsive regulatory elements and are upregulated after heat shock treatment through an HSF-dependent manner, for instance *pr1a*, *pr1b*, chitinases *chi3* and *chi9*, and β-glucosidases *gluA* and *gluB* [33].

In addition to HSFs, other types of transcription factors regulate the expression of heat-responsive genes. Under heat stress, multiprotein bridging factor 1c (*mbf1c*) is positively regulated by HSFA1a and in turn upregulates drought-responsive element binding protein 2a (*dreb2a*) [34] (Figure 2). The expression of several HSPs is positively regulated by the transcription factor WRKY33 [35]. Another mechanism to deal with denatured proteins is autophagy. Indeed, four autophagy-related genes, namely autophagy protein 5 (*atg5)*, *atg7*, neighbour of BRCA1 gene 1a (*nbr1a)* and *nbr1b*, are upregulated by WRKY33 [35]. *Wrky75*, also known for its role in pathogen defence, is upregulated after root zone warming (RZW), i.e. the air temperature remains normal but the soil is heated [36]. In addition, Pto-interacting protein 5 (*pti5)* and ethylene response factor 1 (*erf1)*, both pathogen- and ethylene (ET)-inducible transcription factors, are upregulated after RZW [36]. Multiple defence-related genes are also upregulated, namely *pr1a*, *pr1b*, and three PRR genes flagellin sensitive 2-receptor (*fls2)*, *fei1*, and extra sporogenous cells (*exs*) [36]. DREBA4 is induced after heat shock and upregulates genes involved in the synthesis of JA, SA, and ET [29].

To cope with heat stress, tomato plants adapt in several ways. They change the phospholipid content of the cell membrane to reduce the fluidity and restore membrane

protein function [26]. Leaf rolling and an increase in leaf hairs reduce sun exposure [23] while transpirational cooling is increased by stomatal opening, a process which is abscisic acid (ABA)-independent under heat stress [37]. However, the increase in evaporation can lead to a depletion of water from the soil and thus drought stress.

Response to drought stress

Tomato plants have maximal growth and yield when the soil water content is 80-90% of its maximum, a.k.a. the field capacity [38]. Below that, the water potential starts to drop due to a lack of water and drought stress sets in. The reduced water potential causes a loss of turgor which leads to hydropassive stomatal closure and ultimately decreased shoot height, leaf area, fruit yield, and to wilting [23]. The root weight, and root area are also decreased while the root length increases [20, 39]. This can be explained by the higher water content in deeper soil and thus a higher water potential, and turgor in the lower root apices [23], but is at least partially caused by an ABA-dependent mechanism [40].

Stomatal closure can also be triggered before the general loss of cell turgor through an hydroactive and ABA-dependent mechanism [23, 41] (Figure 3). During drought stress, the pH level rises, and protonated ABA is converted to its dissociated form (ABA^-). In this form, ABA can no longer passively cross the membranes of mesophyll cells, and thus more ABA reaches the guard cells. There, it is bound by ABA receptors which triggers an increase in intracellular Ca^{2+} in the guard cells through ROS, inositol trisphosphate (IP3), and cyclic ADP-ribose. This in turn causes an outflux of Cl^- and K^+ ions and the guard cells lose turgor due to osmosis [23, 41].

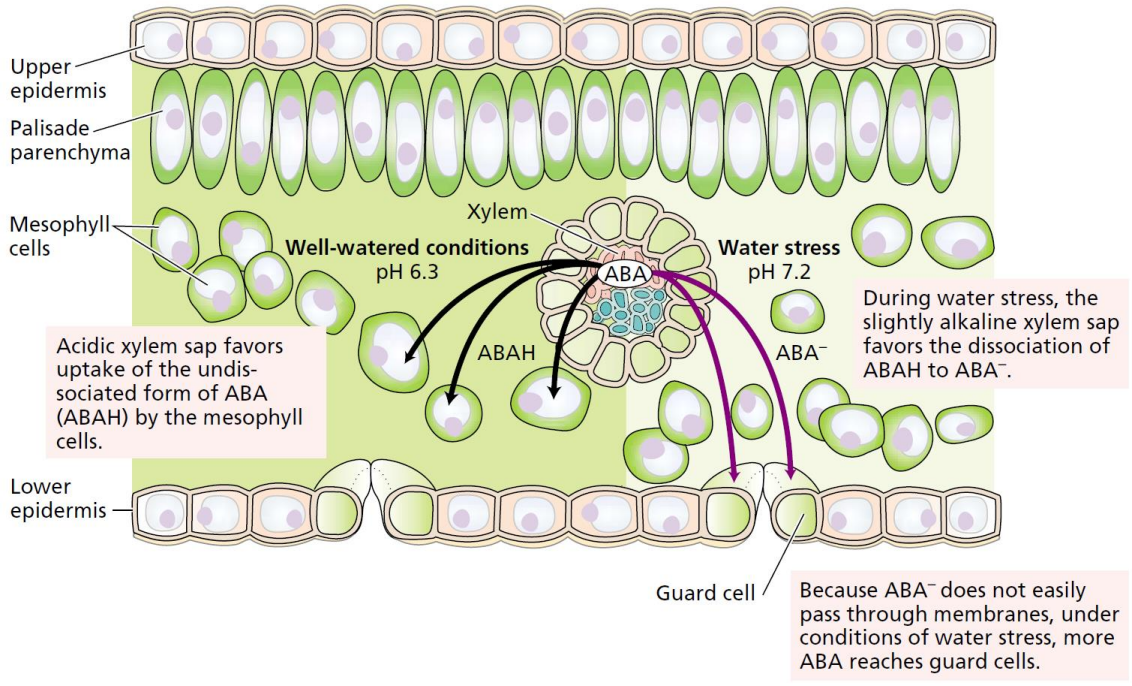

Figure 3: ABA-dependent closure of guard cells is caused by the redistribution of ABA. [23]

Photosynthesis is swiftly affected by the stomatal closure. The reduction in CO_2 availability, together with the reduced water potential disturbs the photosynthetic processes [42] (Figure 4). The oxygenation of RuBISCO, which normally catalyses the fixation of CO^2, leads to an increase in ROS. In turn, this damages membranes, proteins, etc. The chlorophyll content decreases and together with the photo inhibitory damage caused by ROS, this leads to a further decrease in photosynthesis [42–44]. To limit the damage of ROS, the activity of anti-oxidative enzymes like APX1/2, CAT, POX, and PPO is increased [20, 43]. Phenylalanine ammonia lyase (PAL) activity is also increased and plays a key role in the synthesis of phenolic compounds [16, 20].

Gene expression in response to drought is regulated by ABA-responsive and ABA-independent transcription factors. Almost all of the 10 ABA-responsive element binding proteins (AREBs) identified by Pan *et al.* [45] are induced by drought. *Dreb2a* is quickly upregulated when drought stress sets in and remains upregulated under prolonged drought stress [46, 47]. Wrky75 and 8 other WRKYs are strongly upregulated, while *wrky33*, *wrky39*, and some other WRKYs are downregulated [48]. However, in other experiments, *wrky33* and *wrky39* were upregulated as well as *wrky31* [47, 49]. *Wrky39* overexpression enhances root growth and drought resistance [47]. Overexpression of *wrky8* leads to an enhanced upregulation of *areb*, *dreb2a*, and responsive to desiccation 29 (*rd29)* under drought stress [50]. *Wrky75* and most other WRKYs have cis-acting ABA-, ET-, JA- and/or heat-responsive regulatory elements [48]. Acetyl-coenzyme A synthetase 2 (ACS2) and 9-cis-epoxycarotenoid dioxygenase 1 (NCED1), involved in the synthesis of ET and ABA respectively, are also upregulated upon drought stress. Additionally, several HSFs, including *hsfA1*, and multiple HSPs are upregulated [51–53]. This could be a secondary effect of drought-induced stomatal closures as the reduction in evaporation can lead to an increase in leaf temperature. Also similar to the response to heat stress, *atg10* and *atg18f* are upregulated by drought [51].

Plants have several mechanisms to mitigate drought stress, some of which overlap with the response to heat stress. They can limit the evaporation of water by vertical leaf placement, leaf rolling, an increase in leaf hairs, a thicker wax layer, and a decrease in leaf area [23]. The expansion of new leaves is passively reduced through a lack of turgor while an increase in ET causes leaf abscission [23]. The decrease in water potential can be compensated by increasing the osmolyte concentration, a slow process that takes several days [23]. Compatible osmolytes include betaine, proline and other amino acids, saccharides, and sorbitol [23, 54].

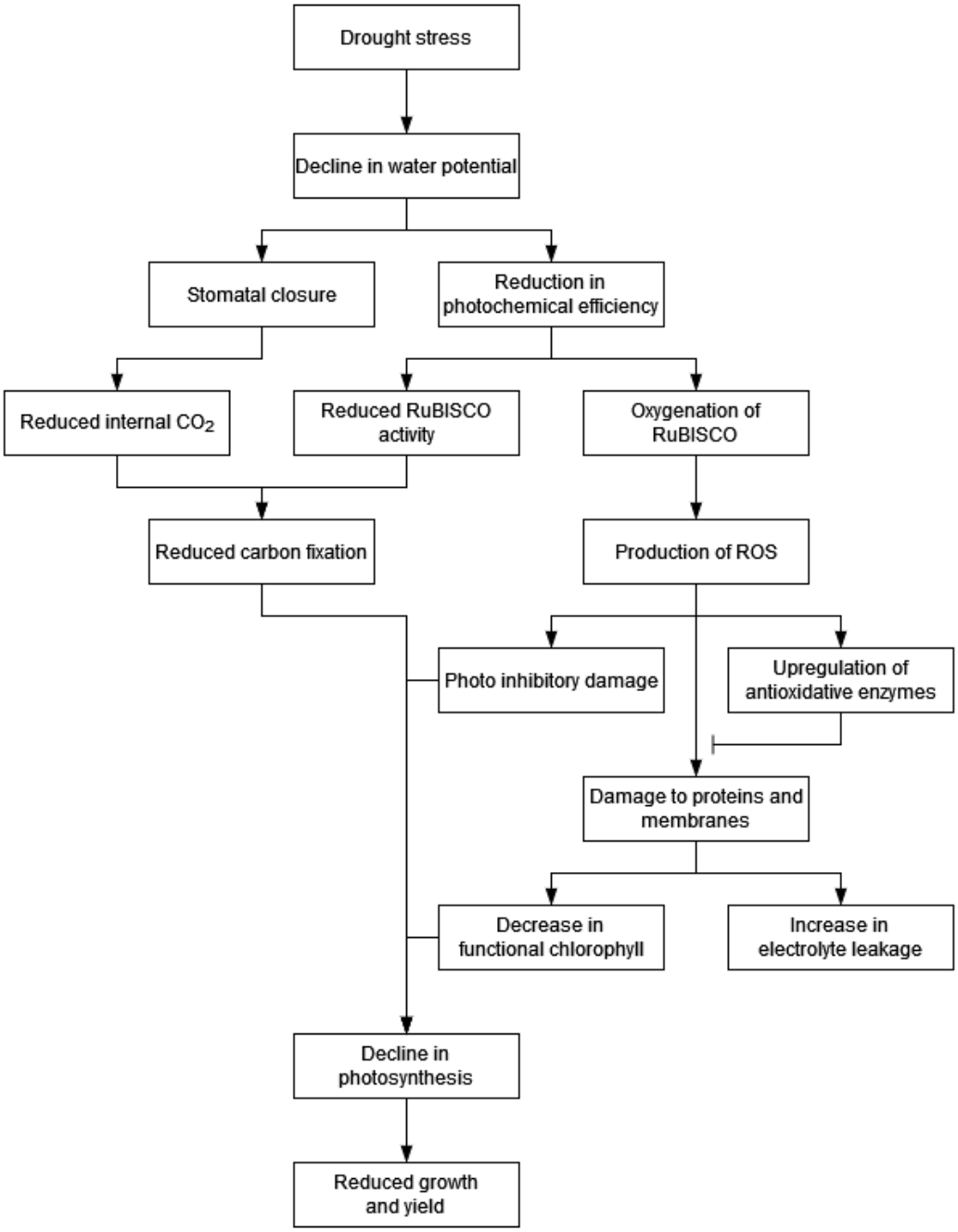

Figure 4: Drought negatively impacts photosynthesis.

Interactions between tomato plant diseases and drought or heat

As outlined in the previous sections, the defence mechanism against pathogen infection, heat stress, and drought stress have overlapping symptoms, signalling pathways, transcription factors, and target genes. This can lead to an additive or synergistic increase in stress symptoms. However, this also allows for the priming of tomato plants for one stress by the other, similar to the priming of mild heat or drought

stress to their more severe stresses and the systemic acquired resistance from one pathogen to other pathogens. An overview of plant disease and drought or heat interactions are outlined in Table 1. For most of these interactions, only the morphological and/or physiological effects have been studied. The studies that do go into the molecular mechanism behind the interaction are all on beneficial interactions. Some of those are discussed in further detail below.

B. cinerea and heat stress

Botrytis cinerea is the causal agent of grey mold. Infection by *B. cinerea* leads to a reduction in stomatal conductance, photosynthesis, and plant height [55]. The transgenic line expressing bacterial *NahG* is SA-deficient has a reduced resistance to *B. cinerea*, while the ABA-deficient *sitiens*-mutant has an increased resistance [55, 56]. Upon infection, two ET-responsive transcription factors, namely *erf1* and *pti5*, are upregulated [36]. *Wrky75* is also upregulated and positively regulates JA-mediated defence [57]. Notably, *wrky31* is JA-responsive and its expression is also increased by *B. cinerea* infection [58]. Genes known to be involved in pathogen defence are upregulated: *pr1a*, *pr1b*, proteinase inhibitor 2 (*pi2*), ET-responsive chitinase *chi9*, and 1-aminocyclopropane-1-carboxylate oxidase 1 (*aco1)* which is involved in the ET biosynthesis pathway [36].

Tomato plants inoculated with *B. cinerea* simultaneous with an increase in temperature to 38°C/25°C day/night, showed a reduced disease incidence and severity [29]. When compared to inoculated plants at normal temperatures (28°C/15°C), genes involved in starch biosynthesis, tetrapyrrole biosynthesis, and light reactions were upregulated under combined stress and TCA, and sucrose biosynthesis were downregulated. The photosynthetic rate was increased under combined stress even though it is decreased under heat stress and *B. cinerea* infection alone. The reduced disease incidence and severity at high temperatures, is likely to be caused by a reduced fitness of *B. cinerea*, not enhanced resistance of the tomato plant, as the growth of *B. cinerea* and the expression of virulence genes *in vitro* is reduced at 38°C/25°C versus 28°C/15°C [29].

Mild heat stress through RZW reduces the severity of grey mold disease regardless of the timing of the heat stress. Two cycles of 10 h RZW to 28°C followed by 14 h recovery at 21°C with or without an additional 3- or 5-day recovery period prior to inoculation was effective in reducing the disease severity [36]. The same treatment when applied up to 4 dpi were able to reduce grey mold symptoms. RZW on day 3 and 4 post inoculation showed the strongest reduction with almost no necrotic lesions 10 dpi. Both one and seven cycles of RZW upregulated three PRRs, namely *fls2*, *fei1*, and *exs*. When primed with seven cycles of RZW, the expression of *wrky75*, *erf1*, *aco1*, and *chi9* at 24 hpi were higher than after RZW alone and similar to non-primed inoculated plants. The expression levels of *pti5*, *pr1a*, and *pi2* were in between those of the single stresses or equal to RZW alone.

Table 1: Overview of interactions between pathogen infection and drought or heat stress in tomato plants. *D= drought stress, H = heat stress, I= inoculation or infection, hpi = hours post-inoculation, dpi= days post-inoculation, WHC= water holding capacity, FC= field capacity, VWC= volumetric water content, RWC= relative water content*

PATHOGEN	*TYPE*	*ORDER OF STRESS*	*ABIOTIC STRESS*	*EFFECT*	*REF & NOTES*
Ralstonia solanacearum	Bacterium	D & I	25, 30, 40, 45, 50% WHC	Reduction in wilting (disease symptom) and bacterial growth in soil with increasing drought. Plant growth not affected	[59]
Botrytis cinerea	Fungus	D - R - I	3x water withholding until wilting followed by recovery	Reduced disease symptoms	[55]
Fusarium oxysporum	Fungus	I - D	3, 5, 7 or 9 d irrigation intervals	Increasing drought severity correlated with shorter time till onset of disease symptoms and mortality. Fungal infection aggravated drought symptoms	[60]
Pseudoidium neolycopersici	Fungus	D - D & I	200 or 120mL every 2 d control: 400 mL	Infection mitigates drought-induced reduction in height but not the reductions in fresh weight and stomatal conductance. Drought strongly reduces fungal growth	[14]
Phytophthora capsici	Oomycete	D - R - I	3x water withholding until wilting followed by recovery	Reduced disease symptoms	[55]
		D - R - I	15% or 10% VWC for 2, 4, or 6 w; 7 d recovery control: 20%	Shorter drought starting at a later stage led to a stronger growth reduction, and more wilting but less root discolouration	[39]
		D & I	15% or 10% VWC for 4 w control: 20%	No effect of mild drought conditions. Stronger growth reduction, more wilting, and more root discolouration under more severe drought	[39]
Phytophthora parasitica	Oomycete	D - I	water withholding for 5 d	Increased disease severity for both stages	[61]
		I -D	water withholding for 6 d	Increased disease severity in seedlings, but not during early flowering stage	[61]
Cucumber mosaic virus	Virus	I - D	80%, 60%, 40% FC	Delayed onset of drought symptoms. Virus infection mitigated loss in RWC at 80% FC	[44]
		I - D - R	water withholding from 8 dpi till wilting; 1-2 w recovery	Delay of drought symptoms	[62]
Tobacco mosaic virus	Virus	D & I	10-15% FC control: 40-45%	Reduced viral load, disease incidence, and disease severity. Reduced growth and fruit yield in drought conditions, not measured for infection alone and combined stress	[43] Field experiment
		D & I	40-50% WHC control: 80-90%	Delayed and reduced disease symptoms, effects on growth under combined stress same as under drought only	[20]

PATHOGEN	*TYPE*	*ORDER OF STRESS*	*ABIOTIC STRESS*	*EFFECT*	*REF & NOTES*
Tomato chlorosis virus	Virus	I - D	3x 50 mL in 25 d	Combined stress reduced the drought- and virus-induced growth impairments, reduced drought symptoms, and prevented plant death	[63]
Tomato spotted wilt virus	Virus	I - D	starting at 48 hpi 50mL/1L-pot every 2 d	Combined stress almost fully restored RWC. Drought strongly reduced virus levels and disease symptoms	[64]
		D - I - R	water withholding for 7 d; recovery at 48 hpi	Single stresses increased elongation rate while combined stress reduced elongation rate. Drought priming prevented virus-induced decrease in RWC. Drought priming delayed root colonisation. Removal of inoculated leaf before recovery prevented infection	[64]
		D - I - R - D	water withholding for 7 d; recovery at 48 hpi followed by 50mL/1L-pot every 2 d	Combined stress led to a reduction in RWC larger than drought, but less than virus infection. Drought delayed root colonisation and systemic infection, and strongly reduced disease symptoms. Removal of inoculated leaf before recovery prevented infection	[64]
Tomato yellow leaf curl virus	Virus	D & I	multiple experiments	Virus infection increases water use efficiency, reallocated metabolites from shoots to roots, prevented *hsfa1/2* upregulation, and suppressed drought-induced autophagy. Drought reduced viral replication	Reviewed in [10]
Clavibacter michiganensis	Bacterium	H & I	winter 15-18°C, spring 21-24°C, summer 28-31°C, autumn 18-23°C	T50 = 58-64d when planted in April, May or September, and 109-142d when planted in December January, June, or July	[65] Field experiment
		I - H I - H - R	35°C for 8 h at 0-96 hpi control: 28°C	Temporary reduction in bacterial population	[65]
		H - H & I - R	35°C for 2 d pre- and 14 d post- inoculation control: 28°C	Reduced spreading and disease progression. Reduced pathogen fitness	[65]
Pseudomonas syringae	Bacterium	H - H & I	31/23°C control: 26/18°C	Reduced disease severity. Reduced pathogen fitness *in vitro*	[32]
		H - R - I	dipped in 45°C water for 2 m; 12, 24, 48, or 72 h recovery	Reduced disease severity. Strongest effect after 12 h recovery	[33]
Xanthomonas campestris	Bacterium	H - R - I	2 cycles of 10 h RZW to 28°C and 14 h recovery; 3 or 5 d recovery	Reduced bacterial growth	[36]

PATHOGEN	*TYPE*	*ORDER OF STRESS*	*ABIOTIC STRESS*	*EFFECT*	*REF & NOTES*
Botrytis cinerea	Fungus	H & I	38/25°C control: 28/15°C	Reduced disease incidence and severity. Reduced pathogen fitness *in vitro*	[31]
		H - I H - R - I	2 cycles of 10 h RZW to 28°C and 14 h recovery; 0, 3, or 5 d recovery	Reduced disease severity	[36]
		I - H	2 cycles of 10 h RZW to 28°C and 14 h recovery within 7 dpi	Reduced disease severity when applied within 5 dpi, strongest effect when applied on 4 and 5 dpi	[36]
Fusarium solani	Fungus	H - I	40-55°C control: 20-40°C	Increased disease severity	[66] Greenhouse experiment
Pseudoidium neolycopersici	Fungus	H - I	40°C for 2 h control: 20°C	Reduced fungal growth	[67] Leaf discs
		H - I	40.5°C for 2 h	Heat temporarily decreased fungal growth, increased infection-induced SA and JA response, and caused appearance of chlorosis and necrosis	[19]
		H & I	22, 24, 26, 28°C	Reduced (22/26°C) to no (28°C) disease symptoms	[68] Natural infection
		H & I	25, 30, 35°C for 12 h	Fungal growth reduced with increasing heat stress	[69]
		H & I	Different greenhouse set-ups leading to differences in temp and humidity	Disease severity was lowest in the greenhouse set-up with the highest temperatures	[69] Greenhouse experiment
		I - H	2 cycles of 10h RZW to 28°C and 14h recovery	Reduced disease severity	[36] Natural infection
Tobacco mosaic virus	Virus	H - H & I	33°C for 8 d pre-/post-inoculation control: 25°C	Reduced viral replication, but increased disease symptoms	[70]
		H - H & I H - H & I - R	33°C for 7 d pre- and 5 d post-inoculation; recovery at 23°C	Temporary reduction in viral load upon recovery, and reduced disease symptoms compared to no recovery	[70]
Tomato yellow leaf curl virus	Virus	H & I	multiple experiments	Virus infection prevented *hsfa2* upregulation, and HSP90-induced cell death	Reviewed in [10]

The upregulation of both PRRs and defence-related target genes by RZW is likely to contribute to the protective effect of heat stress to *B. cinerea* infection. RZW also increased the ethylene production in response to wounding and ethylene-inducing xylanase (EIX), a fungal effector protein. Priming with RZW is not effective in the ET-insensitive *Nr*-mutant, indicating that the priming relies on ET signalling [36]. Since RZW is already effective at 28°C, a temperature not considered to negatively affect tomato plant growth or yield, together with the fact that it is also effective several days post infection when the first necrotic lesions start to appear, RZW can be a potentially effective treatment against *B. cinerea* infection in greenhouses.

P. syringae and heat

Bacterial speck disease is caused by *Pseudomonas syringae*. After infection, the levels of ABA, JA, JA-ile, and the JA precursor OPDA increase and *pr1a*, *pr1b*, and *pr7* are upregulated [32, 33, 50]. The biosynthesis of putrescine and spermine are enhanced through upregulation of arginine decarboxylase (*adc)* and spermidine synthase (*spds)*, respectively [32]. The precise function of putrescine and spermine are still unclear but they play a role in defence as well as in abiotic stress responses [71, 72]. Several WRKY transcription factors including *wrky8*, *wrky39*, and *wrky75* are upregulated upon infection by *P. syringae* [49, 73]. Overexpression of these genes leads to an enhanced upregulation of *pr1a*, *pr1b*, *pr7*, *cat*, *sod*, and *pod* after infection and reduces bacterial speck disease [47, 50, 73].

Heat shock by dipping the plant into 45°C water for 2 min, reduces *P. syringae* infection when inoculated 12 to 72 h later, but not when inoculated within 6 h [33]. This corresponds to the time frame in which the heat shock treatment locally increased the expression of *pr1a* and *pr1b*, namely between 12 and 72 h. Infection also induces systemic upregulation of *pr1a*. As mentioned earlier, expression of the two *pr1* genes and the defence-related genes *chi3*, *chi9*, *gluA*, and *gluA* can be induced by HSFs. Foliar application of KRIBB11 blocks binding of HSFs to heat shock responsive elements and reduces the expression of these six genes, confirming the mechanism of heat shock induced resistance [33].

Longer exposure to milder heat stress, 2 weeks at 31°C pre- and post-inoculation, also reduces bacterial speck disease [32]. The combined stress leads to higher ABA and JA-ile levels than either stress alone, while *pr1* expression and OPDA levels are much lower than heat stress alone and only slightly higher than after infection. The growth of *P. syringae*, its mobility, and the expression of its virulence genes at 31°C are compromised in vitro. Therefore, it is likely that the reduced disease severity is due to impaired virulence of the pathogen and not due to heat stress induced resistance of tomato plants [32].

P. neolycopersici and heat

Pseudoidium neolycopersici, previously classified as *Odium neolycopersici*, is one of the causal agents of powdery mildew and can infect over 60 plant species [69].

The first symptoms are pale yellow spots which quickly become covered with the white spores of the fungus, hence the name of the disease. Infected tomato plants grow slightly higher and have a reduced fresh weight. The latter can be attributed to the increase in stomatal conductance [14]. The main defence response of tomato is the HR, as *P. neolycopersici* is a biotrophic fungus [69]. Infection of tomato has been reported to increase SA levels, and *pal* expression and decrease the expression of isochorismate synthase (*ics*) [19, 74]. Both PAL and ICS are involved in SA biosynthesis. ET biosynthesis enzymes ACO1 and ACS2 are upregulated, and ABA hydroxylase is downregulated, possibly leading to an increase in ET and ABA levels [14, 74]. Respiratory burst oxidase homolog protein D (*rbohd*), but not *rbohf*, is increased and *apx* is decreased leading to an increase in ROS [74]. The defence related genes *pr1a*, beta-fructofuranosidase *lin6*, and *chi9* are also upregulated [14, 74].

The severity of powdery mildew is reduced by both heat shock and prolonged heat stress [19, 36, 67–69]. Chlorosis, which is a sign of the HR, appears much earlier and there is a stronger accumulation of SA and JA when tomato plants are inoculated after heat shock (40-41°C for 2 h) [19]. 2 cycles of RZW, as described earlier, reduces disease severity in wild type tomato plants, but not in the *NahG*-line which indicates that heat stress induced resistance is SA-dependent [69].

P. neolycopersici and drought

In addition to heat, drought stress also affects powdery mildew disease. The priming of tomato with three consecutive cycles of water withholding until wilting and subsequent recovery reduces powdery mildew infection [55]. The *sitiens*-mutant has lower levels of ABA and is more resistant to *P. neolycopersici*. Application of ABA to *sitiens* plans restores the endogenous ABA levels to wild type levels, decreases its resistance to wild type level, and restores its stunted growth. The application of higher concentrations had no further negative effects [55].

In another set of experiments, powdery mildew infection was reduced when plants grown under moderate or severe drought pre- and post-inoculation [14]. The expression of *pr1*, *lin6*, and *nced1* was similar to moderate or severe drought, or infection alone, while the expression of *tas14*, *ac2* and *apx* were higher than either stress alone and highest under the combination of severe drought and infection [14]. Thus, the drought treatment and infection seem to have an additive or synergistic as well as an intensity-dependent effect on several drought and defence responsive genes.

The resistance of sitiens mutants seems to conflict with the strong increase of ABA under drought conditions, and the strongly increased expression of the ABA-responsive gene *tas14* under combined stress. One should note that the endogenous ABA levels at the highest concentration of exogenously applied ABA did not reach the ABA levels as seen under drought conditions [55]. This leaves the possibility that ABA can induce resistance at levels below base line as well as far above, but not at basal or moderately increased levels.

Conclusion

In tomato plants, the combination of pathogen attack, and drought or heat can be detrimental in some cases and beneficial in others. Research into the positive interactions on the molecular level remains limited, while that on negative interactions seems completely absent. The reduction of disease severity due to heat stress was not always due to increased resistance of the tomato plant, but sometimes at least partially due to reduced pathogen fitness at a higher temperature. More specifically, this was the case for *B. cinerea* and *P. syringae* under prolonged heat stress.

A correlation between pathogens lifestyle (biotrophic vs necrotrophic) and the outcome of the interaction with drought or heat stress has been suggested [21]. However, based on the studies reviewed here, the only link that can be made is that viral pathogens almost always reduce the effects of drought and/or heat stress and protected tomato plants against dying. This is not surprising since viruses need a living host to reproduce and survive.

Hardly any papers assessed the effect of the combined stresses on fruit yield, let alone fruit quality, despite that those are the two most important traits for a crop. Due to climate change, drought and heat will become more frequent and thus are more likely to co-occur. Nevertheless, the research on plant disease, drought, and heat is very sparse. Studies on combined stress have led to (possible) ways to improve or protect crop yield either through breeding or treatments. For instance, a TYLCV-mutant which is unable to infect its vector has been suggested as a way to alleviate drought stress as well as the foliar application of trehalose [10, 75]. The lack of a significant correlation between heat-induced fruit set inhibition and Fusarium wilt susceptibility, makes it possible to optimise both through breeding [66]. Still, many questions on the molecular mechanisms behind combined plant disease and drought or heat stress remain. More research is needed to help protect tomato crop yield against the climate change related increase in drought and heat as well as changes in the occurrence of plant diseases, and to help feed the ever-increasing world population.

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