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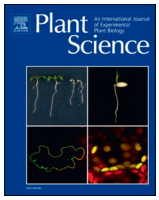

Review article

Advances and perspectives in the metabolomics of stomatal movement and the disease triangle

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ABSTRACT

Crops are continuously exposed to microbial pathogens that cause tremendous yield losses worldwide. Stomatal pores formed by pairs of specialized guard cells in the leaf epidermis represent a major route of pathogen entry. Guard cells have an essential role as a first line of defense against pathogens. Metabolomics is an indispensable systems biology tool that has facilitated discovery and functional studies of metabolites that regulate stomatal movement in response to pathogens and other environmental factors. Guard cells, pathogens and environmental factors constitute the "stomatal disease triangle". The aim of this review is to highlight recent advances toward understanding the stomatal disease triangle in the context of newly discovered signaling molecules, hormone crosstalk, and consequent molecular changes that integrate pathogens and environmental sensing into stomatal immune responses. Future perspectives on emerging single-cell studies, multiomics and molecular imaging in the context of stomatal defense are discussed. Advances in this important area of plant biology will inform rational crop engineering and breeding for enhanced stomatal defense without disruption of other pathways that impact crop yield.

1. Introduction

Metabolomics is the systematic study of all the low molecular weight chemical compounds (commonly known as metabolites or small molecules), including identification of their chemical structure, quantification, and flux in a biological system such as cells, tissues or organisms [1–4]. Metabolomics can be used to gauge cellular enzyme activities, the status of key regulatory proteins, and the activities of signaling and metabolic pathways [4–7]. Metabolomics has shown utility in bridging the gaps between genotypes and phenotypes [8–12] because it represents a comprehensive view of metabolites, which often cannot be predicted based on genomics and proteomics alone [13]. Metabolites and enzymes represent the physiological state of cells and have close links to phenotypes [14–16]. In addition, metabolomics enables discovery of new metabolites and novel metabolic pathways [17–20], and is a toolkit for systems biology and synthetic biology approaches [21,22]. Plants are exceptional biochemical factories, able to produce >200,000 metabolites, many with essential roles in plant growth, development, environmental interaction, and yield [11]. Therefore, plant metabolomics has immense potential and impact.

Crops suffer tremendous yield loss worldwide due to microbial pathogens. Many pathogens can be inhabitants of the phyllosphere, residing on the leaf surface and using stomata as their primary means of entry [23,24]. Stomatal pores formed by pairs of specialized guard cells in the leaf epidermis open and close to regulate CO_2 intake and transpirational water loss. Plant pathogens have long been known to exploit stomatal pores as major entry points to the intracellular leaf space, especially bacterial pathogens with no other means of entry [25]. Conversely, plants have evolved immune mechanisms to limit pathogen entry into the plant body. Efficient detection of pathogens and mounting of timely defense responses are essential for plant survival and crop viability. Upon detection of microbial pathogens, guard cells drive stomatal closure via decreasing cellular turgor pressure as an innate immune response [24–29] (Fig. 1). The plant innate immune system recognizes evolutionarily conserved microbial signatures, called microbe/pathogen-associated molecular patterns (MAMPs/PAMPs), and thus constitutes a first line of defense against pathogen invasion [30–34]. PAMP-triggered stomatal closure creates an effective barrier

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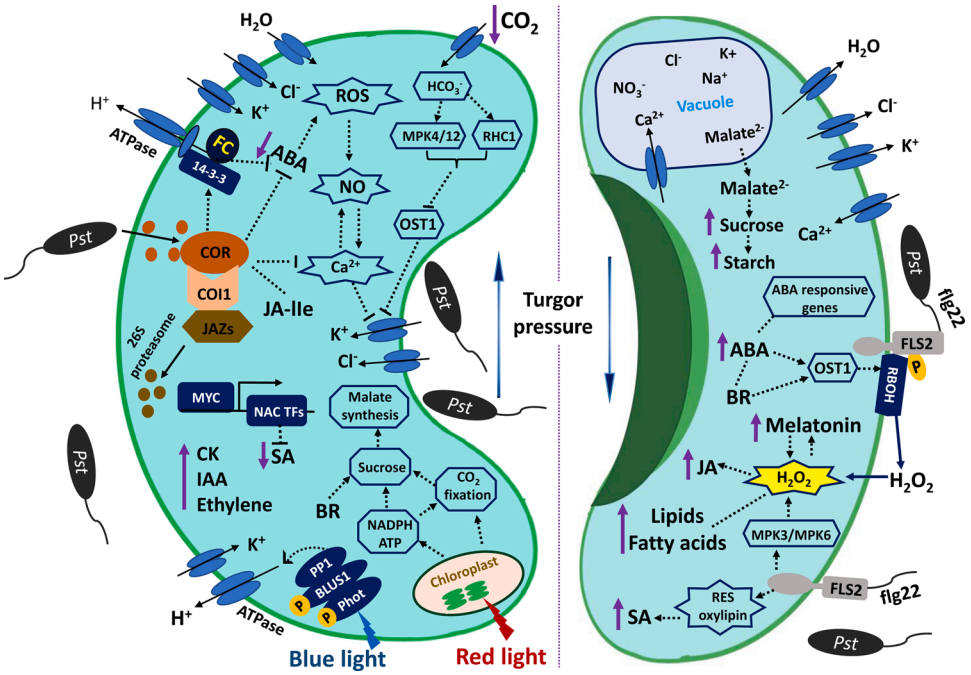

Fig. 1. A schematic diagram showing guard cell signaling and metabolic processes in response to environmental factors, especially to flg22 and *Pseudomonas syringae* pv. tomato (*Pst*) DC3000. Plasma membrane NADPH oxidase RBOH mediates flg22/bacterial-induced ROS production, followed by activation of downstream signaling components, MPK3/ MPK6 and crosstalk among multiple hormones (e.g., SA, JA, BR and melatonin), ultimately leading to efflux of K^+ and Cl^- and decrease of turgor pressure and stomatal closure. Both ABA-dependent and ABA-independent oxylipin pathways are shown (right panel). In countering stomatal closure, *Pst* DC3000 secretes coronatine (COR), which hijacks JA-Ile signaling by binding to the JA receptor COI1, leading to degradation of JAZ repressors. Next, downstream NAC transcription factors are activated, ABA and SA levels decrease, K^+ and Cl^- flow in, and turgor pressure increases, stimulating stomatal reopening. Key environmental factors mediate stomatal movement include, but are not limited to, light (red and blue), and CO_2 concentration. Red light coordinates guard cell and mesophyll cell photosynthetic responses. Blue light mediates guard cell volume increase through activating plasma membrane H^+-ATPases and driving K^+ and Cl^- uptake. Under low atmospheric CO_2 concentration, OST1 kinase activities are inhibited, promoting stomata opening (left panel). Abbreviations: *Pst*, *Pseudomonas syringae* pv. tomato; flg22, bacterial flagellin 22; 14-3-3, 14-3-3 protein; FC, Fusicoccin; FLS2, flagellin sensitive 2; RBOH, respiratory burst oxidase homolog; RBOHD, respiratory burst oxidase homolog D; JA-Ile, jasmonic acid-isoleucine; COR, coronatine; COI1, CORNATIVE INSENSITIVE 1; JAZs, JASMONATE-ZIM DOMAIN; NAC, NAM-ATAF-CUC2; PP1, protein phosphatase 1; BLUS1, blue light signal 1; Phot, phototropin; OST, OPEN STOMATA1; ABA, abscisic acid; SA, salicylic acid; CK, cytokinin; IAA, Indole-3-Acetic Acid; BR, Brassiosteroid; JA, jasmonic acid. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

against bacterial disease. The stomatal immune responses are regulated by the defense hormone salicylic acid (SA) and its homeostasis with jasmonic acid (JA) and abscisic acid (ABA), which together transduce signals involved in H_2O_2 and NO production [35]. PAMP-activated mitogen-activated protein kinase (MAPK) cascades and reactive oxygen species (ROS) that trigger stomatal closure are essential components in stomatal innate immune responses [29]. On the other hand, some pathogenic species have evolved virulence factors (e.g., coronatine, COR) to evade the innate immune system, reverse stomatal closure and thereby gain access to the leaf interior, which provides nutrients that favor pathogen proliferation [35–37]. For example, the plant bacterial pathogen *Pseudomonas syringae pv.* tomato (*Pst*) DC3000 and the human pathogen *Salmonella enterica* can counteract stomatal immunity, actively reopening stomata to facilitate entry [38–40].

Metabolites, including those elicited by microbial pathogens [41–44] are known to play important signaling and metabolic roles in stomatal movements. Although recent metabolomics efforts have increased the number of identified guard cell metabolites from a few [45] to more than 400 [46,47], the full size of the guard cell metabolome and the metabolites associated with pathogen entry remain unknown. Similar to the "plant disease triangle" [48], guard cell, pathogen and the environment form the "stomatal disease triangle". Past stomatal research has mostly focused on one environmental factor at a time, e.g., CO_2 [49,50] or drought [51,52], and thus knowledge of responses to multiple simultaneous environmental factors is lacking, particularly in the context of pathogen invasion. Deciphering the metabolic interactions between guard cells and pathogens under multiple environmental factors will provide a holistic view of the stomatal disease triangle [29,53]. This knowledge is essential toward enhancing crop defense in real-world situations. However, to date metabolomics of the stomatal disease triangle has been considerably under-studied. This review will highlight and analyze new findings, integrating pathogen and environmental sensing in stomatal immune responses with recently discovered signaling molecules, hormone crosstalk, and related metabolic changes. Advances in this important area of plant biology will aid the development of crops with improved disease resistance through biotechnology and molecular breeding.

2. Biotic factors in the stomatal disease triangle

In both managed lab conditions and natural environments, stomatal movements are affected by a number of external factors in conjunction with pathogens and other naturally occurring biota. Stomata close after sensing PAMPs/MAMPs (e.g., flagellin peptide). Flagellin peptide flg22 binds to its receptor FLAGELLIN SENSITIVE 2 (FLS2), which activates downstream signaling components, leading to the stomatal immune response (Fig. 1). The null mutant of *FLS2* is defective in the rapid flg22-dependent stomatal closure [32]. *Pst* DC3000 signaling represents the best studied example of pathogen interaction with Arabidopsis stomata [30,54]. COR, a phytotoxin produced by *Pst* DC3000, structurally mimics jasmonic acid-isoleucine (JA-Ile) and helps circumvent the innate immune response by causing stomata to reopen (Fig. 1). COR hijacks the native JA-Ile signal transduction pathway and is perceived by CORONATINE INSENSITIVE1 (COI1) (an F-box protein), causing poly-ubiquitination and degradation of JAZ proteins, releasing their repression of JA-responsive transcription factors [55]. Similar stomatal reopening following an immune response can be elicited by other bacteria, such as *Xanthomonas campestris* and *Salmonella enterica,* although the latter is not a plant pathogen and does not produce COR [56,57]. This indicates that other metabolites from the bacteria may have similar functions as COR in reopening the stomata, but their identities are unknown.

Many pathogenic fungi utilize stomata and affect stomatal movement. Fungi, especially the ones that cause rust diseases, have developed specific mechanisms to target leaf epidermal structures such as guard cells [58]. Arabidopsis has been shown to increase stomata density in new leaves following colonization by powdery mildew, an effect compounded by high CO_2 and possibly other environmental factors [50]. There are multiple fungal pathogens that impact stomatal movement, trigger immune responses, or utilize counteracting toxins. *Plasomopara viticola* enters and sporulates through stomata and non-mechanically locks them open, possibly through a toxin or through removing structural pressure from cells surrounding the guard cells [59]. Chitosan from *Blumeria graminis* has been shown to act as a PAMP/MAMP, allowing the plant to detect fungal hyphae [60]. Finally, fusicoccin (FC, a diterpene glycoside) is a well-studied fungal toxin produced by *Fusicoccum amygdali* during pathogenesis of peach and almond trees [61]. FC counteracts stomatal closure by activating plasma membrane H^+-ATPases, inducing guard cell K^+ and water uptake and thus stomatal opening [62]. FC is a useful laboratory tool, acts on the stomata of all higher plants to cause extreme stomatal opening and wilting and also irreversibly stabilizing H^+-ATPase complexes in both plants and animals [63]. Other fungi reopen stomata by producing an excess of a given metabolite common to both the plant and pathogen, such as *Sclerotinia sclerotiorum* which produces oxalic acid, likely interfering with ABA-induced stomatal closure [64].

The phyllosphere microbiome is the microbial community existing on plants above the soil surface, both endophytically and epiphytically [24]. It provides balanced atmospheric carbon dioxide and oxygen to the most abundant habitats of microbial community. The phyllosphere has not been well-studied in terms of how plants interact with phyllosphere microbiota to prevent dysbiosis [65]. Many of the microbes reside in the apoplastic space, which is also a route for pathogen invasion. Bacterial community in phyllosphere health has shown a correlation with the host plant PAMP signaling [65]. Host genotype affects the leaf microbiome, which correlates with plant fitness/productivity [66,67]. Pathogenic and non-pathogenic bacteria interact with hosts in a humidity-dep endent manner [68], reinforcing the relevance of genotypes and environmental factors in the stomatal disease triangle.

Some beneficial microbiota likely improve stomatal resistance to pathogens, akin to rhizosphere microbes such as *Trichoderma* sp. and *Bacillus subtilis* [69,70], both of which modulate stomatal movements in an ABA and SA dependent manner. However, it is not known whether ABA and SA move from roots to shoots to mediate this process, or whether there are mobile long-distance signaling molecules that trigger ABA and SA biosynthesis in guard cells. In a recent study on Arabidopsis dehydration response, a small peptide CLAVATA3/EMBRYO-SURROUNDING REGION-RELATED 25 was found to transmit water-deficiency signals through vascular tissues, and modulate ABA accumulation and stomatal closure [71]. To search for the mobile signaling molecules, metabolite profiling should include identification of peptides, including those derived from microbes and pathogens. With an isotope-labeling technique [61], bacterial pathogens can be grown on isotopically heavy media and produce peptides and small molecules that can be distinguished from plant metabolites in mass despite identical chemical structures. Using this isotope labeling technique with untargeted/targeted metabolomics will help elucidate the roles of various metabolites produced by phyllosphere microbes vs. the plants themselves during plant immune responses.

3. Abiotic environmental factors in the stomatal disease triangle

Stomatal pores play an essential role in gaseous exchange between the leaf and the atmosphere, and represent a major route for transpiration and CO_2 uptake [72]. Guard cells constantly and rapidly adjust stomatal apertures in response to CO_2, light, humidity, and other environmental variables toward optimizing plant water use efficiency and productivity [73–75]. Abiotic environmental conditions are significant determinants of whether pathogens can successfully invade a plant. For example, light promotes bacterial chemotactic infiltration through stomatal pores [76], and high humidity aids pathogenesis [77]. When guard cells sense the bacterium *Pst* DC3000, stomata close as part of the innate immune response [32,35,78]. However, high humidity limits this immune response, thereby facilitating bacterial invasion [77]. In the dark, stomata tend to close. COR secreted by *Pst* DC3000 can open stomata in the dark [79,80]. Here we discuss the effect of the abiotic factors on stomatal immunity, a topic that has received much more attention than biotic factors.

3.1. CO_2

Guard cells enlarge stomatal apertures when intercellular CO_2 concentration (C_i) decreases, and narrow apertures when C_i rises [81]. High CO_2 activates K^+ efflux channels and anion channels like Slow Anion Channel 1 (SLAC1), driving ion and water loss and thus stomatal closure [82] (Fig. 1). *Arabidopsis thaliana* mutants lacking two β-carbonic anhydrases (CA1 and CA4) are hyposensitive to CO_2, implicating bicarbonate as an important metabolite in the CO_2 response [83–85]. Elevated bicarbonate arising from high CO_2 concentrations activates RESISTANT TO HIGH CO_2 1 (RHC1), which inhibits HIGH TEMPERATURE 1 (HT1) kinase, releasing the HT1 inhibition of stomatal closure and thus promoting high CO_2-induced stomatal closure. In addition, MPK4 and MPK12 also inhibit HT1 during promotion of stomatal closure by elevated CO_2 [86,87]. Because HT1 phosphorylates OPEN STOMATA 1 (OST1) and thereby inhibits the OST1 kinase activity, inhibition of HT1 activates OST1, a kinase that activates the SLAC1 channel and thus stomatal closure [88].

In response to changing atmospheric CO_2 conditions, CA1 and CA4 alter disease resistance levels [89]. Under low CO_2 when *Pst* was applied on the leaf surface, CA activity was relatively low and Arabidopsis wild-type plants showed resistance to *Pst* DC3000 [89], despite larger stomatal aperture under low CO_2 than under ambient CO_2 concentration [47]. Consistently, when *Pst* was surface-applied, the Arabidopsis *ca1ca4* double mutant exhibited enhanced resistance to *Pst* DC3000, as evidenced by lower bacterial growth and increased expression of defense-related genes [89]. In contrast, under ambient and elevated CO_2 conditions, *CA1* transcription increased compared to low CO_2, and the wild-type plants were vulnerable to surface-applied *Pst* bacteria [89].

Is ABA signaling involved in the stomatal CO_2 responses? Several studies have shown that under elevated CO_2, OST1 kinase activities were

strongly induced by ABA, and stomata closed rapidly [90–92]. And the *ost1* mutant showed impaired CO_2 induced stomatal closure [92]. These results appear to indicate that OST1 is essential for high CO_2-induced stomatal closure. However, recent results seem to contradict these findings. First, high CO_2-triggered stomatal closure is not prevented, but is delayed in ABA biosynthesis or receptor mutants [93]. Second, high CO_2 does not increase ABA levels in guard cells [94]. Third, recent studies indicate that high CO_2 does not activate OST1 kinase in guard cells [93,94]. These results point to an ABA-independent pathway in guard cell high CO_2 signal transduction. It is not clear what caused the conflicting results even from the same laboratory [92–94] and whether ABA-dependent pathway still plays a role in guard cell high CO_2 response.

High CO_2 levels usually maintain stomatal closure. Interestingly, in the presence of the foliar pathogen *Pst* DC3000 and elevated CO_2, Arabidopsis stomata reopened at 4 h [49]. This may partly explain the high level of *Pst* infection compared to low CO_2 [68]. In addition, high CO_2 increased leaf ABA levels, and ABA mutants *aba2-1* and *abi1-1* were resistant to *Pst* [49]. The result implies that the high leaf ABA levels under elevated CO_2 may weaken plant defense. This contradicts the general notion that ABA and high CO_2 would close stomata and enhance plant defense. Other hormones and metabolites were not profiled, so it is not known how high levels of ABA change other metabolites relevant to defense, such as SA and JA, or how cellular molecular networks are altered by CO_2 and *Pst* in different cell types, highlighting the importance of single-cell and single cell-type metabolomics [43,46,47,95]. In addition, sphingosine-1-phosphate (S1P) promotes stomatal closure and increases in guard cells following ABA treatment [96,97]. However, S1P may not be involved in stomatal CO_2 responses, as it was not detected to change in concentration in guard cells following a high CO_2 treatment [46,98]. Intriguingly, the G protein Gα mutant *gpa1* is insensitive to both S1P [96] and flg22 inhibition [32] of stomatal opening, while the G protein Gβ subunit functions downstream of the FERONIA receptor-like kinase [99,100], which functions in pathogen response [101]. How S1P plays a role in stomatal immunity is not clear.

3.2. Light

Light has important roles in both microbe pathogenesis and stomatal defense. Stomata open and close following the circadian day and night cycle. Pathogens and plants coordinate stomata interactions in response to this photoperiod. For example, a fungal pathogen *Cercospora zeae-maydis* infects maize leaves through stomatal pores, and light is required for the fungus to perceive stomata, infect and cause gray leaf spot disease [102]. This finding has led to identification of a fungal blue light photoreceptor, CRP1, that mediates stomatal tropism and infection [102]. Darkness closes stomata, thus effectively limiting *Pst* infection by stomata. In the dark, COR is usually required to reopen stomata and infect Arabidopsis leaves, with the COR-defective mutant *Pst DC3118* being incapable of inducing stomatal opening [103]. Moderate light intensity can induce bacterial chemotaxis towards and penetration through stomata, as bacteria traverse stomata along photosynthate gradients, with enhanced infiltration in the presence of white, blue and red light [76]. Moreover, light has a synergistic effect on stomatal opening with the fungal toxin FC [104], which is dependent on guard cell H^+-ATPase and ion channel activities [32,105,106]. A higher concentration of FC even causes stomatal opening in the dark [104].

Although both red light and blue light promote stomatal opening, they act through two distinct mechanisms. Blue light mediates guard cell volume change by activating a plasma membrane H^+-ATPase, with consequent membrane hyperpolarization driving K^+ and Cl^- uptake [107]. Red light acts through photosynthesis [108,109]. A stomatal red light response occurs when intercellular CO_2 is experimentally held constant and also occurs in isolated epidermal peels absent of mesophyll cells [95,110], suggesting that there is a direct guard cell response to red light. This direct response presumably functions in addition to guard cell response to decreasing intercellular CO_2 from the red light-driven mesophyll photosynthesis [111]. Red light-induced stomatal opening in epidermal peels is inhibited by 3-(3,4-dichlorophenyl)-1,1-dimethylurea, implicating photosynthesis in guard cell red-light perception [110,112]. The guard cell mechanisms downstream of red-light perception are largely unknown, although recent research shows that red light-activation of the H^+ ATPase is possible [62].

The contribution of photosynthetically-derived osmotica to red light-induced stomatal opening has been under debate. Guard cells are acknowledged to have an intact Calvin cycle [113], thus sugars derived from guard cell photosynthesis could be the osmotica driving stomatal opening under red light [109,113]. However, tobacco with antisense reduction in ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit or cytochrome b6f showed normal stomatal light responses [114,115], while antisense plants with reduced sedoheptulose-1, 7-bisphosphatase showed more rapid and larger stomatal responses to red light than control plants [116]. These results suggest that the guard cell Calvin cycle is not a necessary source of osmotica or signal. In the antisense plants, stomatal apertures under red light are proportional to products of the light reactions (ATP and NADPH) [116]. To address the sources of osmotica and/or signaling metabolites that regulate red light-triggered stomatal opening, Zhu et al. (2020) recently conducted a metabolomic study and identified Arabidopsis guard cell metabolic signatures in response to red light in the absence of the mesophyll cells [95]. Out of the 223 quantified metabolites in Arabidopsis guard cells, 104 were found to be red light responsive. It is interesting that a decrease in guard cell ABA and an increase in JA play important roles in the guard cell red light signaling process. Knowing the specific mechanisms of guard cell light response will help determine how pathogens manipulate and circumvent the normal guard cell light responses.

3.3. Water and humidity

Stomatal modulation of leaf water potential can impact water availability to foliar microbes and pathogens [117]. Guard cell perception and response to changes in vapor pressure difference (VPD) remain enigmatic, with both hydroactive and hydropassive stomatal movements having been proposed [118]. High relative humidity (RH) increases plant susceptibility to pathogens in the phyllosphere by facilitating pathogen proliferation and spread [77]. Bacteria can synthesize extracellular polymeric substances that become hydrated within the phyllosphere [119], in addition to producing biosurfactants that alter phyllosphere wettability [120]. RH not only affects microbial survival, but also influences phyllosphere nutrients, especially fructose and sucrose [121].

The drought hormone ABA is important to guard cell-pathogen interaction [122]. Under drought, ABA increases and promotes stomatal closure, but overall plant pathogen defense responses are suppressed [123]. In line with this result, ABA biosynthetic mutant *aba3-1* exhibited reduced susceptibility to *Pst* infection, whereas overexpression of ABA biosynthetic genes, such as *NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 5* (*NCED5*) caused enhanced disease susceptibility phenotype [124]. With increased *NCED5* expression, ABA strongly accumulated during the *Pst* infection [124]. Interestingly, ABA-modulated stomatal closure can be enhanced by a *Pst* effector, HopAM1, in response to phyllospheric water deficit [125]. HopAM1 may aid *Pst* infection and suppress Arabidopsis defense responses by helping close stomata under drought stress, protecting bacterial colonies inside the leaves from dehydration [125].

Studies of stomatal responses to water stress often utilize ABA biosynthesis mutants and are useful to investigate ABA's role in VPD responses. Under decreased atmospheric humidity, ABA biosynthesis mutants showed a decreased stomatal conductance leading to the hypothesis that ABA may not be involved in humidity signaling in guard cells [79,80]. Conversely, ABA synthesis mutant *aba2* and ABA (and possibly CO_2) signaling mutant *ost1* have shown reduced VPD responses

[80]. The ABA-regulated guard cell transcriptome and low RH-induced transcriptome do not completely overlap [126,127], indicating ABA-dependent and ABA-independent roles in stomatal movement [128]. To investigate the role of ABA production in different cells, cell-type dependent restoration of function was created for ABA biosynthetic mutants in guard cells and phloem companion cells [129]. These results indicate the redundancy of ABA sources in either companion cells or guard cells is adequate in regulating VPD-induced stomatal closure [129]. However, ABA and other metabolite changes in the guard cells of the ABA deficient mutants and rescued lines in response to different VPDs were not measured.

Bacteria-triggered stomatal closure as an innate immune response could be compromised under high RH. When Arabidopsis was inoculated with *Pst* DC3000 or COR-defective mutant *Pst* DC3118 under low RH conditions, *Pst* DC3000 caused a typical increase in stomata aperture over *Pst* DC3118 due to the presence of COR in the former. In contrast, under high RH, stomata showed large apertures when treated with either bacteria, similar as with mock treatment, indicating that the stomata were insensitive to the presence of the pathogens [77]. The expression of JA-response genes was more rapidly induced in 95 % RH than in 60% RH. High RH also suppressed SA production and SA-responsive genes expression in guard cells, and promoted stomatal opening [77]. Given that plants impaired in SA responses are deficient in *Pst*-triggered stomatal closure [23], higher RH may aid *Pst* infection by altering plant JA and SA responses in the pathogen's favor.

4. Hormone signaling and crosstalk in stomatal immunity

Studying hormone signaling and crosstalk in the stomatal disease triangle is essential for understanding stomatal immunity [130]. The biosynthesis and signaling pathways of phytohormones in guard cells have not been fully elucidated, partly due to technical limitations in hormone analysis in single cells [35]. ABA, SA and JA were reported to mediate ROS production and induce stomatal closure [131–133]. Other hormones involved in stomatal closure include brassinosteroids [134] and strigolactone [135,136]. No one hormone can likely explain stomatal response to pathogen infection under specific temporal and spatial conditions [137]. Hormonal crosstalk requires components of multiple hormone signaling pathways. Here we discuss potential hormone crosstalk in stomatal immune responses.

Using reverse genetics with ABA synthesis and signaling mutants, ABA was found to be a key regulator in PAMP signal transduction through activation of G-protein-dependent K^+ channels, SLAC1-type anion channels and OST1 kinase, leading to stomatal closure [32,78,138]. Bacterial flagellin flg22 invokes PAMP triggered inhibition of light-induced stomatal opening [78]. In null mutants of the *FLS2* receptor, the flg22 regulation of stomatal movement and K^+ currents is abolished [78]. In addition, the flg22 inhibition of inward K^+ channels is eliminated in a mutant of G-protein α subunit (*gpa1*), leading to the conclusion that G proteins are important in transducing the PAMP signal [32]. Furthermore, null mutants of SLAC1 and SLAH3 channels showed no flg22-induced anion channel activities and lack of stomatal closure in response to flg22 or ABA [129], indicating the essential roles of the anion channels in flg22-induced rapid stomatal closure. Moreover, stomata of plants lacking OST1 failed to close in response to the elicitor flg22 as well as to intact *Pst* DC3000 [78]. These above results support a role for the ABA-dependent pathway in the stomatal immune response. A separate study identified an ABA-independent oxylipin pathway [40], wherein flg22-induced stomatal closure is mediated by MPK3 and MPK6, lipoxygenase 1, oxylipin and SA [23,38,40,78,139] (Fig. 1). Strong evidence for the ABA-independent pathway was that the mutants of *ost1* and *aba2-1* responded to the exogenously applied flg22 and closed stomata [40]. This result seems to contradict the earlier study [78]. However, it should be noted that the flg22 concentration used was much higher than that used for stomatal closure in the wild-type. The authors concluded that the ABA-independent pathway and ABA-dependent pathway converge at the SLAC1 component of the ABA signaling pathway [40]. It is not known whether OST1-independent activation of anion channels through other signaling mechanisms contributes to the stomatal immune response.

Recently, using in-gel kinase assay, MPK4 isolated from Arabidopsis leaves overexpressing a FLAG-tagged MPK4 was found to be activated by flg22. The overexpression plants showed hypersensitivity in flg22-induced stomatal closure and ROS production, indicating that MPK4 plays a positive role in stomatal immune response [140]. Overexpression of *MPK4* led to decrease of JA-Ile, but no change of SA, indicating the ratio of SA to JA, not the absolute amount, is important in pathogen defense [140,141]. The role of JA in stomatal response has been controversial. Most studies have shown that JA induces stomatal closure [46,133,142–148]. However, a recent study suggests that red light-induced stomatal opening is associated not only with declining ABA but also with increased JA, and JA-deficient mutants exhibited impaired stomatal opening [95]. In addition, the *P. syringae type* III effector protein AvrB that induces stomatal reopening requires the JA receptor COI1 and conserved NAM-ATAF-CUC2 (NAC) transcription factors [149]. One hypothesis for the inconsistency of stomatal JA response is that basal ABA levels vary between different studies and influence stomatal apertures [35]. The endogenous ABA threshold may influence JA-induced stomatal closure. Conversely, JA can stimulate the expression of *9-CIS-EPOXYCAROTENOID DIOXYGENASE* 3, encoding an important enzyme in ABA biosynthesis. When ABA level passes the threshold, stomatal closure can be observed [150]. Alternatively, the stomatal response to JA may be dose-dependent, with high concentration of MeJA promoting stomatal closure [133,143,146], and low concentration of endogenous JA-Ile inducing stomatal opening [35]. In addition, SA is critical for PAMP/MAMP-triggered immunity against bacterial pathogens, activating antimicrobial genes and suppressing antagonistic hormones like JA-lle [151]. Biosynthesis of SA was induced through transcriptional activation of a SA biosynthetic gene encoding isochorismate synthase within first hour after plant exposure to flg22 [152]. Whether SA is produced in the guard cells or transported from other cells remains unclear. Measurement of pathogen-induced changes of SA and other hormones in guard cells may resolve these contradictions. Similarly, despite evidence for involvement of metabolites such as cyclic GMP and cyclic ADP-ribose in ABA signaling [153], with respect to pathogen attack these metabolites have not been fully characterized in guard cells.

Melatonin, a newly discovered phytohormone [154,155] was shown to down-regulate ABA biosynthesis genes and up-regulate ABA catabolic genes, thus reducing ABA levels and promoting stomatal opening [156]. Exogenous application of melatonin to Arabidopsis seedlings increased invertase activity, enhanced sucrose metabolism, and reinforced cell wall structural barriers. These changes may partially account for the observed increase in resistance to *Pst* DC 3000 infection [157]. Melatonin also induces nitric oxide (NO) production and expression of SA-related genes (e.g., *ENHANCED DISEASE SUSCEPTIBILITY*, *PHYTOALEXIN DEFICIENT 4* and *PATHOGENESIS-RELATED PROTEIN (PR)*) in Arabidopsis leaves in response to *Pst* DC 3000 infection [158]. Whether these changes apply to stomatal immunity is not known because the metabolite and gene expression changes in guard cells were not measured.

In terms of the stomatal disease triangle, stomatal responses to multiple abiotic and biotic stimuli simultaneously involve crosstalk among different hormone pathways. For example, a plant growth-promoting soil bacterium *Bacillus amyloliquefaciens* FZB42 restricted pathogen-mediated stomatal reopening caused by *Phytophthora nicotianae* in tobacco leaves through increasing the contents of ABA and SA in the leaves [159]. Meanwhile, expression of SA and JA/ET responsive genes such as *PR-1*, *LIPOXYGENASE* and *ETHYLENE RESPONSE FACTOR 1* was induced in the leaves [159]. This result highlights the importance of hormone signaling networks in stomatal and/or apoplastic defenses. They also indicate a potential biocontrol application using soil

rhizobacteria/microbiota to control foliar diseases. High humidity is another environmental factor that weakens stomatal immunity through inhibiting JA responses by an ABA-dependent JA degradation pathway [77,160]. When studying multiple environmental factors, it can be challenging to understand the integration of hormone signaling networks in stomatal biotic versus abiotic responses. This area of research is currently lacking, and exciting outcomes will be highly relevant to real-life agricultural applications.

5. Specific metabolites in stomatal movement and immunity

Crosstalk between various hormone signaling and metabolic pathways affords numerous metabolites some degree of control over stomatal aperture and immunity, whether through potentially novel, independent pathways or well-described pathways such as ABA signaling [161–163]. Some hormones and metabolites have established roles in different plant tissues, but have only recently been associated with guard cell functions.

Low CO_2 generally causes stomata to open; conversely, higher CO_2 causes stomata to close [46,47]. This likely occurs through the crosstalk of different hormones. Under high CO_2, JA pathway metabolites were increased in guard cells, and JA was clearly associated with stomatal closure [46], in addition to potential involvement of ABA [88,92]. Interestingly, under low CO_2-induced stomata opening, JAs in the guard cells did not decreased as expected, and instead were found to remain at a constant level. However, traumatic acid rose significantly in the guard cells under low CO_2 [47]. Since both JA and traumatic acid are derived from linolenic acid, this result indicates the alternate synthesis of traumatic acid over JA, as has been demonstrated before in rice leaves [164]. Traumatic acid, along with JA, is a product of the oxylipin pathway and a known plant hormone [165].

Glucosinolates are a group of specialized metabolites in Brassicales. Together with their degradation enzymes, myrosinases, these metabolites are part of a well-regulated “mustard oil bomb”. This system has been well-described as a defense mechanism against insect pests and was recently shown to also play an important role in plant defense against bacterial pathogens [166,167]. Since pharmacological treatment of plant epidermal peels with a glucosinolate degradation product isothiocyanate caused stomatal closure, it is reasonable to hypothesize that the “mustard oil bomb” in guard cells may promote stomatal closure and inhibit reopening following *Pst* infection [167]. This hypothesis was supported by a recent study. The Arabidopsis glucosinolate degradation enzyme (myrosinase), β-thioglucoside glucohydrolase 1, (TGG1) overexpressed in broccoli was shown to be involved in the stomatal defense against *Pst* DC3000 [167]. When the epidermal peels were incubated with *Pst* DC3000, the TGG1-overexpression plants showed accelerated stomatal closure and reduced reopening. Compared with the wild-type, the overexpression plants had heightened stomatal closure in response to exogenous ABA or SA [167]. However, the guard cell glucosinolate degradation and hormone level changes were not measured. Thus, the biological relevance of the impact of these exogenous treatments is not clear.

It is known that degradation of starch and triacylglycerols [168,169], sucrose catabolism [170,171], and guard cell chloroplast lipids [172] are involved in stomatal responses to light and CO_2. For example, starch can be rapidly degraded into sugars and organic acids upon light exposure of guard cells, increasing turgor pressure and promoting stomatal opening [173,174]. Several fatty acids (FA) and lipids play an important role in plant defense signaling [175]. Monounsaturated FA (18:1) decrease in Arabidopsis leaves can stabilize a nitric oxide associate 1 protein, leading to increased NO synthesis and thereby enhancing NO-mediated signaling and resistance to bacterial pathogen invasion [176]. The role of this FA and NO signaling is worth exploring in guard cells in the context of stomatal immunity. In similar studies focused on tomato leaves, increased levels of polyunsaturated FAs (18:3) were associated with resistance to *P. syringae* [177] and increased FAs (16:1) were found to be important for resistance to powdery mildew [178]. Another lipid-derived metabolite, phosphatidic acid produced by phospholipase Dα1, stimulates plasma membrane respiratory burst oxidase homolog (RBOH) activity and lead to ROS production in ABA-mediated stomatal closure [179]. How these metabolites are involved in pathogen-triggered stomatal movement is unknown and would benefit from metabolomics/lipidomics studies.

Melatonin is newly discovered plant regulator that has been implicated in many different physiological functions [155] and is starting to be studied in guard cells. Melatonin has primarily been studied through exogenous pharmacological application; few studies have been reported that use biosynthetic mutants, and no mutant has been identified that does not produce melatonin. The first purported receptor for melatonin, CAND2 was recently described [180]. CAND2 was reported as a membrane protein that interacts with a heterotrimeric G-protein, stimulating H_2O_2 production and stomatal closure under certain melatonin concentrations [181]. A contrasting report indicated this may not be the case, as many known melatonin-mediated responses still occur within the *cand2* mutants [181]. In any case, the pathway(s) by which melatonin may influence guard cell functions, including immune response, requires further investigations. Specific environmental conditions or pathogen infections that cause a change in guard cell melatonin have not been reported. Knockouts of a melatonin biosynthetic gene, serotonin N-acetyl transferase, have been reported to result in increased susceptibility to an otherwise avirulent *Pst* strain. These infected plants also exhibited high SA levels [182]. Melatonin may be involved in stomatal immunity, but further studies will be required that test whether bacterial entry through stomata is altered in various melatonin signaling and biosynthetic mutants.

As mentioned above, guard cell ABA-triggered signaling is one of the best understood plant signaling cascades. ROS, reactive nitrogen species (RNS) and redox regulation play important roles in guard cell ABA signaling [161]. Under *Pst* invasion, PAMPs/MAMPs bind to different cell surface pattern-recognition receptors (e.g., FLS2), which may convergently activate a cytoplasmic botrytis-induced kinase (BIK1). BIK1 phosphorylates RBOH to produce ROS. Guard cell anion channels are then activated, leading to stomatal closure [138]. Production of ROS/RNS and perturbation of cellular redox homeostasis are conspicuous features of the immune response. Glutathione (GSH) and ascorbate are well-known redox buffers in plant cells. It was shown that increase of ABA led to decreased concentration of GSH in guard cells [183], and a GSH-deficient mutant had increased ROS in guard cells treated with ABA [75]. In addition, many specialized plant metabolites act as antioxidants, affecting the cellular redox state. For example, in Arabidopsis, flavonols accumulated in guard cells, acted as ROS scavengers and inhibited stomatal closure [184,185]. How guard cells regulate GSH, ascorbate and other antioxidant metabolites during pathogen infection is unknown but could be addressed by metabolomics [186].

6. Future perspectives on the stomatal disease triangle

In the past two decades, great progress has been made in studying how environmental factors affect plant defense outcomes. Obviously, research on the role of biotic factors in the stomatal disease triangle is still in its infancy. Studies on stomatal movement under multiple environmental factors that integrate underlying molecular processes in guard cells have also been scarce. It is critical to enhance this area of research in guard cell innate immunity and foliar pathogen defense. It should be emphasized that, within the stomatal disease triangle, pathogen counter-attack mechanisms in the presence of multiple environmental factors also should be considered.

Metabolomics of stomatal movement and the disease triangle is a new and fascinating area of exploration. The stomatal disease triangle encompasses many different metabolites, pathways, and environmental contexts. Given a rapidly changing climate and increasing agricultural demands, a better understanding of the disease triangle will be

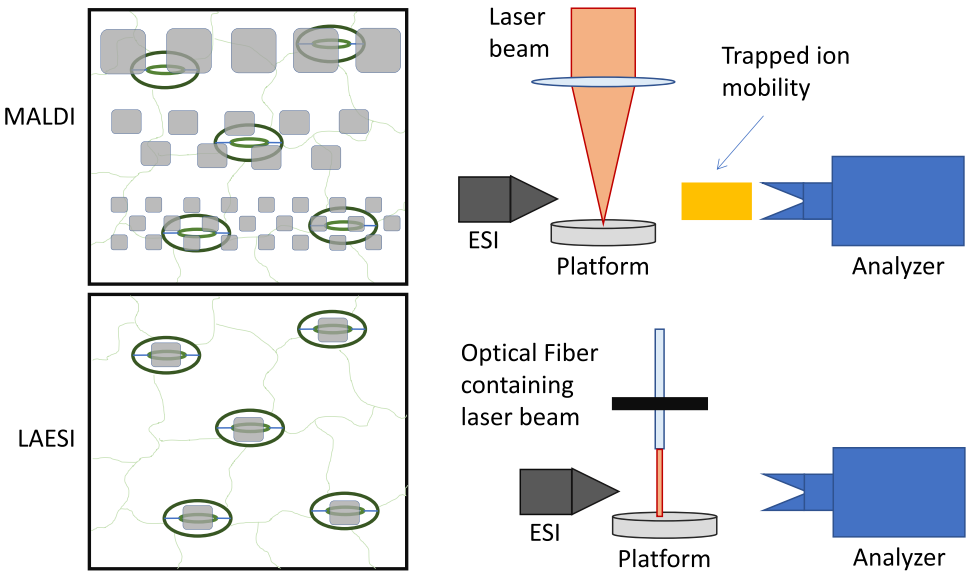

Fig. 2. Modern mass spectrometry technologies for single-cell metabolite imaging. MALDI imaging requires a matrix to be applied beforehand but can offer increasingly small ablation footprints able to target single cells through a simple grid. Ion mobility allows separation of isomeric metabolites. LAESI imaging requires no matrix and has a larger ablation footprint but can be automated to target specific cells through prior imaging. Abbreviations: MALDI, matrix-assisted laser desorption ionization; LAESI, laser ablation electrospray ionization.

important to limit diseases in many crops. Areas of study in the plant disease triangle, previously unexplored in guard cells, are likely to have increased prominence, as are new technologies that allow more detailed study of metabolomes in guard cells and many other types of cells. Metabolomics together with reverse genetics is an increasingly useful means of identifying new metabolites previously unassociated with guard cell function. Previous work was only able to assess about 100 guard cell metabolites [187], but newer studies have been able to identify 400+ metabolites and 1300+ proteins in this specialized cell type [46,47]. Although these newer studies analyzed guard cell responses to CO_2, similar studies are feasible to study pathogenesis, guard cell defense, environmental interactions, and other components of the disease triangle. Novel technologies are emerging in plant metabolomic analysis that will offer increased sensitivity and high resolution, as well as integration of metabolomics with other 'omics.

Single cell methods are particularly vital in the study of guard cell, epidermal cell, mesophyll cell, or other cell-type specific metabolic functions in the stomatal disease triangle. Mass spectrometry imaging (MSI) is a promising tool for imaging an array of metabolites *in situ* across a two- or three-dimensional space (Fig. 2). Ionization methods, including matrix-assisted laser desorption ionization (MALDI) and laser ablation electrospray ionization (LAESI) have achieved sufficient resolution for single-cell molecular imaging [67,188]. MSI techniques have been successfully utilized in mammalian metabolomics and lipidomics [189,190], but have not been widely employed in plants. Two limiting factors in MSI and metabolomics are limited metabolome coverage and differentiation between isobaric compounds. Recently developed mass spectrometers remedy this issue by integrating trapped ion mobility separation (TIMS) [190]. TIMS accumulates ion and produces an adjustable "ramp" to separate different ion species based on their size, shape and charge [191], providing higher sensitivity and an additional dimension to MS data. In addition, hyphenated metabolomics platforms including gas chromatography, liquid chromatography and capillary electrophoresis MS can enhance metabolome coverage [46,47]. Their integration with TIMS is very powerful. Alongside with MSI, they may be utilized in single-cell studies through laser-capture microdissection [192]. High resolution MS, particularly using MSn, provides confident compound identification and has become a critical component of untargeted metabolomics [193]. Improvements in metabolite spectral libraries (e.g., mzCloud) will continue to enhance MS spectra interpretation and MSI functionality. By applying these novel tools, cell-specific metabolomic responses to a given condition can be measured with deep coverage and in a high throughput manner. The contribution of each cell and the interactions of cells surrounding the guard cells in the stomatal disease triangle can be characterized. In a similar vein, multiomics, particularly using single cell or single cell-type information, can integrate data across the central dogma and reveal specific molecular mechanisms that were previously opaque. A better understanding of these mechanisms will be important to limit disease in crops and enhance food production for sustainable agriculture.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

[1] V. Shulaev, D. Cortes, G. Miller, R. Mittler, Metabolomics for plant stress response, Physiol. Plant. 132 (2008) 199–208, https://doi.org/10.1111/j.1399-3054.2007.01025.x.
[2] K. Saito, F. Matsuda, Metabolomics for functional genomics, systems biology, and biotechnology, Annu. Rev. Plant Biol. 61 (2010) 463–489, https://doi.org/10.1146/annurev.arplant.043008.092035.
[3] J.W. Allwood, D.I. Ellis, R. Goodacre, Metabolomic technologies and their application to the study of plants and plant-host interactions, Physiol. Plant. 132 (2008) 117–135, https://doi.org/10.1111/j.1399-3054.2007.01001.x.
[4] M.M. Rinschen, J. Ivanisevic, M. Giera, G. Siuzdak, Identification of bioactive metabolites using activity metabolomics, Nat. Rev. Mol. Cell Biol. 20 (2019) 353–367, https://doi.org/10.1038/s41580-019-0108-4.
[5] B. Peng, H. Li, X.X. Peng, Functional metabolomics: from biomarker discovery to metabolome reprogramming, Protein Cell 6 (2015) 628–637, https://doi.org/10.1007/s13238-015-0185-x.
[6] G.A. Prosser, G. Larrouy-Maumus, L.P.S. Carvalho, Metabolomic strategies for the identification of new enzyme functions and metabolic pathways, EMBO Rep. 15 (2014) 657–669, https://doi.org/10.15252/embr.201338283.
[7] D. Medina-Cleghorn, D.K. Nomura, Exploring metabolic pathways and regulation through functional chemoproteomic and metabolomic platforms, Chem. Biol. 21 (2014) 1171–1184, https://doi.org/10.1016/j.chembiol.2014.07.007.
[8] A.R. Fernie, J. Gutierrez-Marcos, From genome to phenome: genome-wide association studies and other approaches that bridge the genotype to phenotpe gap, Plant J. 97 (2019) 5–7, https://doi.org/10.1111/tpj.14219.
[9] J. Luo, Metabolite-based genome-wide association studies in plants, Curr. Opin. Plant Biol. 24 (2015) 31–38, https://doi.org/10.1016/j.pbi.2015.01.006.
[10] C. Fang, J. Luo, Metabolic GWAS-based dissection of genetic bases underlying the diversity of plant metabolism, Plant J. 97 (2019) 91–100, https://doi.org/10.1111/tpj.14097.

[11] O. Fiehn, Metabolomics - the link between genotypes and phenotypes, Plant Mol. Biol. 48 (2002) 155–171, https://doi.org/10.1023/A:1013713905833.

[12] P.P. Handakumbura, B. Stanfill, A. Rivas-Ubach, D. Fortin, J.P. Vogel, C. Jansson, Metabotyping as a stopover in genome-to-phenome mapping, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-38483-0.

[13] M. May, Big data, big picture: metabolomics meets systems biology, Science 356 (2017) 646–648, https://doi.org/10.1126/science.356.6338.646.

[14] C. Brunetti, R.M. George, M. Tattini, K. Field, M.P. Davey, Metabolomics in plant environmental physiology, J. Exp. Bot. 64 (2013) 4011–4020, https://doi.org/10.1093/jxb/ert244.

[15] Y. Liu, M. Li, J. Xu, X. Liu, S. Wang, L. Shi, Physiological and metabolomics analyses of young and old leaves from wild and cultivated soybean seedlings under low-nitrogen conditions, BMC Plant Biol. 19 (2019), https://doi.org/10.1186/s12870-019-2005-6.

[16] I. Alldritt, B. Whitham-Agut, M. Sipin, J. Studholme, A. Trentacoste, J.A. Tripp, M.G. Cappai, P. Ditchfield, T. Devièse, R.E.M. Hedges, J.S.O. McCullagh, Metabolomics reveals diet-derived plant polyphenols accumulate in physiological bone, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-44390-1.

[17] M.A. Farag, D.V. Huhman, R.A. Dixon, L.W. Sumner, Metabolomics reveals novel pathways and differential mechanistic and elicitor-specific responses in phenylpropanoid and isoflavonoid biosynthesis in *Medicago truncatula* cell cultures, Plant Physiol. 146 (2008) 387–402, https://doi.org/10.1104/pp.107.108431.

[18] V. Salim, B. Wiens, S. Masada-Atsumi, F. Yu, V. De Luca, 7-Deoxyloganetic acid synthase catalyzes a key 3 step oxidation to form 7-deoxyloganetic acid in *Catharanthus roseus* iridoid biosynthesis, Phytochemistry 101 (2014) 23–31, https://doi.org/10.1016/j.phytochem.2014.02.009.

[19] F. Geu-Flores, M.E. Moldrup, C. Böttcher, C.E. Olsen, D. Scheel, B.A. Halkier, Cytosolic γ-glutamyl peptidases process glutathione conjugates in the biosynthesis of glucosinolates and camalexin in arabidopsis, Plant Cell 23 (2011) 2456–2469, https://doi.org/10.1105/tpc.111.083998.

[20] S. Bocobza, L. Willmitzer, N.V. Raikhel, A. Aharoni, Discovery of new modules in metabolic biology using chemometabolomics, Plant Physiol. 160 (2012) 1160–1163, https://doi.org/10.1104/pp.112.203919.

[21] D.I. Ellis, R. Goodacre, Metabolomics-assisted synthetic biology, Curr. Opin. Biotechnol. 23 (2012) 22–28, https://doi.org/10.1016/j.copbio.2011.10.014.

[22] P.F. South, A.P. Cavanagh, H.W. Liu, D.R. Ort, Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field, Science 363 (2019), https://doi.org/10.1126/science.aat9077.

[23] W. Zeng, M. Melotto, S.Y. He, Plant stomata: a checkpoint of host immunity and pathogen virulence, Curr. Opin. Biotechnol. 21 (2010) 599–603, https://doi.org/10.1016/j.copbio.2010.05.006.

[24] S.E. Lindow, M.T. Brandl, Microbiology of the phyllosphere, Appl. Environ. Microbiol. 69 (2003) 1875–1883, https://doi.org/10.1128/AEM.69.4.1875-1883.2003.

[25] T. Lawson, M.R. Blatt, Stomatal size, speed, and responsiveness impact on photosynthesis and water use efficiency, Plant Physiol. 164 (2014) 1556–1570, https://doi.org/10.1104/pp.114.237107.

[26] D.H. McLachlan, M. Kopischke, S. Robatzek, Gate control: guard cell regulation by microbial stress, New Phytol. 203 (2014) 1049–1063, https://doi.org/10.1111/nph.12916.

[27] W. Ye, Y. Murata, Microbe associated molecular pattern signaling in guard cells, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.00583.

[28] X. Zheng, S. Kang, Y. Jing, Z. Ren, L. Li, J.M. Zhou, G. Berkowitz, J. Shi, A. Fu, W. Lan, F. Zhao, S. Luan, Danger-associated peptides close stomata by OST1-independent activation of anion channels in guard cells, Plant Cell 30 (2018) 1132–1146, https://doi.org/10.1105/tpc.17.00701.

[29] J. Su, M. Zhang, L. Zhang, T. Sun, Y. Liu, W. Lukowitz, J. Xu, S. Zhang, Regulation of stomatal immunity by interdependent functions of a pathogen-responsive MPK3/MPK6 cascade and abscisic acid, Plant Cell 29 (2017) 526–542, https://doi.org/10.1105/tpc.16.00577.

[30] K. Buchmann, Evolution of innate immunity: clues from invertebrates via fish to mammals, Front. Immunol. 5 (2014), https://doi.org/10.3389/fimmu.2014.00459.

[31] S.H. Spoel, X. Dong, How do plants achieve immunity? Defence without specialized immune cells, Nat. Rev. Immunol. 12 (2012) 89–100, https://doi.org/10.1038/nri3141.

[32] W. Zhang, S.Y. He, S.M. Assmann, The plant innate immunity response in stomatal guard cells invokes G-protein-dependent ion channel regulation, Plant J. 56 (2008) 984–996, https://doi.org/10.1111/j.1365-313X.2008.03657.x.

[33] T. Boller, S.Y. He, Innate immunity in plants: an arms race between pattern recognition receptors in plants and effectors in microbial pathogens, Science 324 (2009) 742–743, https://doi.org/10.1126/science.1171647.

[34] B. Schwessinger, P.C. Ronald, Plant innate immunity: perception of conserved microbial signatures, Annu. Rev. Plant Biol. 63 (2012) 451–482, https://doi.org/10.1146/annurev-arplant-042811-105518.

[35] M. Melotto, L. Zhang, P.R. Oblessuc, S.Y. He, Stomatal defense a decade later, Plant Physiol. 174 (2017) 561–571, https://doi.org/10.1104/pp.16.01853.

[36] S.Q. An, N. Potnis, M. Dow, F.J. Vorhölter, Y.Q. He, A. Becker, D. Teper, Y. Li, N. Wang, L. Bleris, J.L. Tang, Mechanistic insights into host adaptation, virulence and epidemiology of the phytopathogen Xanthomonas, FEMS Microbiol. Rev. (2019), https://doi.org/10.1093/femsre/fuz024.

[37] S. Hok, A. Attard, H. Keller, Getting the most from the host: how pathogens force plants to cooperate in disease, Mol. Plant-microbe Interact. 23 (2010) 1253–1259, https://doi.org/10.1094/MPMI-04-10-0103.

[38] D. Arnaud, I. Hwang, A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens, Mol. Plant 8 (2015) 566–581, https://doi.org/10.1016/j.molp.2014.10.012.

[39] W. Zeng, S.Y. He, A prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to *Pseudomonas syringae* pv tomato DC3000 in Arabidopsis, Plant Physiol. 153 (2010) 1188–1198, https://doi.org/10.1104/pp.110.157016.

[40] J.L. Montillet, N. Leonhardt, S. Mondy, S. Tranchimand, D. Rumeau, M. Boudsocq, A.V. Garcia, T. Douki, J. Bigeard, C. Laurière, A. Chevalier, C. Castresana, H. Hirt, An abscisic acid-independent oxylipin pathway controls stomatal closure and immune defense in Arabidopsis, PLoS Biol. 11 (2013), https://doi.org/10.1371/journal.pbio.1001513.

[41] X. Qi, K.U. Torii, Hormonal and environmental signals guiding stomatal development, BMC Biol. 16 (2018), https://doi.org/10.1186/s12915-018-0488-5.

[42] M. Melotto, W. Underwood, S.Y. He, Role of stomata in plant innate immunity and foliar bacterial diseases, Annu. Rev. Phytopathol. 46 (2008) 101–122, https://doi.org/10.1146/annurev.phyto.121107.104959.

[43] B.B. Misra, B.R. Acharya, D. Granot, S.M. Assmann, S. Chen, The guard cell metabolome: functions in stomatal movement and global food security, Front. Plant Sci. 6 (2015) 1–13, https://doi.org/10.3389/fpls.2015.00334.

[44] R. Peyraud, U. Dubiella, A. Barbacci, S. Genin, S. Raffaele, D. Roby, Advances on plant–pathogen interactions from molecular toward systems biology perspectives, Plant J. 90 (2017) 720–737, https://doi.org/10.1111/tpj.13429.

[45] S. Li, S.M. Assmann, R. Albert, Predicting essential components of signal transduction networks: a dynamic model of guard cell abscisic acid signaling, PLoS Biol. 4 (2006) 1732–1748, https://doi.org/10.1371/journal.pbio.0040312.

[46] S. Geng, B.B. Misra, E. de Armas, D.V. Huhman, H.T. Alborn, L.W. Sumner, S. Chen, Jasmonate-mediated stomatal closure under elevated CO_2 revealed by time-resolved metabolomics, Plant J. 88 (2016) 947–962, https://doi.org/10.1111/tpj.13296.

[47] S. Geng, B. Yu, N. Zhu, C. Dufresne, S. Chen, Metabolomics and proteomics of *Brassica napus* guard cells in response to low CO_2, Front. Mol. Biosci. 4 (2017), https://doi.org/10.3389/fmolb.2017.00051.

[48] G. Agrios, Plant Pathology, fifth edition, 2004, https://doi.org/10.1016/C2009-0-02037-6.

[49] Y. Zhou, I. Vroegop-Vos, R.C. Schuurink, C.M.J. Pieterse, S.C.M. Van Wees, Atmospheric CO_2 alters resistance of Arabidopsis to *Pseudomonas syringae* by affecting abscisic acid accumulation and stomatal responsiveness to coronatine, Front. Plant Sci. 8 (2017), https://doi.org/10.3389/fpls.2017.00700.

[50] J.A. Lake, R.N. Wade, Plant-pathogen interactions and elevated CO_2: morphological changes in favour of pathogens, J. Exp. Bot. 60 (2009) 3123–3131, https://doi.org/10.1093/jxb/erp147.

[51] R. Sinha, V. Irulappan, B. Mohan-Raju, A. Suganthi, M. Senthil-Kumar, Impact of drought stress on simultaneously occurring pathogen infection in field-grown chickpea, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-41463-z.

[52] C.W. Lim, W. Baek, J. Jung, J.H. Kim, S.C. Lee, Function of ABA in stomatal defense against biotic and drought stresses, Int. J. Mol. Sci. 16 (2015) 15251–15270, https://doi.org/10.3390/ijms160715251.

[53] X. Meng, S. Zhang, MAPK cascades in plant disease resistance signaling, Annu. Rev. Phytopathol. 51 (2013) 245–266, https://doi.org/10.1146/annurev-phyto-082712-102314.

[54] R. Athman, D. Philpott, Innate immunity via Toll-like receptors and Nod proteins, Curr. Opin. Microbiol. 7 (2004) 25–32, https://doi.org/10.1016/j.mib.2003.12.013.

[55] S. Gimenez-Ibanez, M. Boter, A. Ortigosa, G. García-Casado, A. Chini, M. G. Lewsey, J.R. Ecker, V. Ntoukakis, R. Solano, JAZ2 controls stomata dynamics during bacterial invasion, New Phytol. 213 (2017) 1378–1392, https://doi.org/10.1111/nph.14354.

[56] D. Roy, S. Panchal, B.A. Rosa, M. Melotto, *Escherichia coli* O157:H7 induces stronger plant immunity than *Salmonella enterica* typhimurium SL1344, Phytopathology 103 (2013) 326–332, https://doi.org/10.1094/PHYTO-09-12-0230-FI.

[57] G.E. Gudesblat, P.S. Torres, A.A. Vojnov, *Xanthomonas campestris* overcomes Arabidopsis stomatal innate immunity through a DSF cell-to-cell signal-regulated virulence factor, Plant Physiol. 149 (2009) 1017–1027, https://doi.org/10.1104/pp.108.126870.

[58] R.C. Staples, The development of infection structures by the rusts and other fungi, Microbiol. Sci. 2 (1985) 193–198.

[59] M. Allègre, X. Daire, M.C. Héloir, S. Trouvelot, L. Mercier, M. Adrian, A. Pugin, Stomatal deregulation in *Plasmopara viticola*-infected grapevine leaves, New Phytol. 173 (2007) 832–840, https://doi.org/10.1111/j.1469-8137.2006.01959.x.

[60] S. Koers, A. Guzel-Deger, I. Marten, M.R.G. Roelfsema, Barley mildew and its elicitor chitosan promote closed stomata by stimulating guard-cell S-type anion channels, Plant J. 68 (2011) 670–680, https://doi.org/10.1111/j.1365-313X.2011.04719.x.

[61] E. Marre, Fusicoccin: a tool in plant physiology, Annu. Rev. Plant Physiol. 30 (1979) 273–288, https://doi.org/10.1146/annurev.pp.30.060179.001421.

[62] T. Kinoshita, K.I. Shimazaki, Analysis of the phosphorylation level in guard-cell plasma membrane H^+-ATPase in response to fusicoccin, Plant Cell Physiol. 42 (2001) 424–432, https://doi.org/10.1093/pcp/pce055.

[63] L. Camoni, S. Visconti, P. Aducci, M. Marra, From plant physiology to pharmacology: fusicoccin leaves the leaves, Planta 249 (2019) 49–57, https://doi.org/10.1007/s00425-018-3051-2.

[64] R.L. Guimarães, H.U. Stotz, Oxalate production by *Sclerotinia sclerotiorum* deregulates guard cells during infection, Plant Physiol. 136 (2004) 3703–3711, https://doi.org/10.1104/pp.104.049650.

[65] T. Chen, K. Nomura, X. Wang, R. Sohrabi, J. Xu, L. Yao, B.C. Paasch, L. Ma, J. Kremer, Y. Cheng, L. Zhang, N. Wang, E. Wang, X.F. Xin, S.Y. He, A plant genetic network for preventing dysbiosis in the phyllosphere, Nature 580 (2020) 653–657, https://doi.org/10.1038/s41586-020-2185-0.

[66] S. D'Alessandro, Y. Mizokami, B. Légeret, M. Havaux, The apocarotenoid β-cyclocitric acid elicits drought tolerance in plants, IScience 19 (2019) 461–473, https://doi.org/10.1016/j.isci.2019.08.003.

[67] B. Shrestha, J.M. Patt, A. Vertes, In situ cell-by-cell imaging and analysis of small cell populations by mass spectrometry, Anal. Chem. 83 (2011) 2947–2955, https://doi.org/10.1021/ac102958x.

[68] X.F. Xin, K. Nomura, K. Aung, A.C. Velásquez, J. Yao, F. Boutrot, J.H. Chang, C. Zipfel, S.Y. He, Bacteria establish an aqueous living space in plants crucial for virulence, Nature 539 (2016) 524–529, https://doi.org/10.1038/nature20166.

[69] H.A. Contreras-Cornejo, L. Macías-Rodríguez, A.G. Vergara, J. López-Bucio, Trichoderma modulates stomatal aperture and leaf transpiration through an abscisic acid-dependent mechanism in Arabidopsis, J. Plant Growth Regul. 34 (2015) 425–432, https://doi.org/10.1007/s00344-014-9471-8.

[70] A.S. Kumar, V. Lakshmanan, J.L. Caplan, D. Powell, K.J. Czymmek, D.F. Levia, H. P. Bais, Rhizobacteria *Bacillus subtilis* restricts foliar pathogen entry through stomata, Plant J. 72 (2012) 694–706, https://doi.org/10.1111/j.1365-313X.2012.05116.x.

[71] F. Takahashi, T. Suzuki, Y. Osakabe, S. Betsuyaku, Y. Kondo, N. Dohmae, H. Fukuda, K. Yamaguchi-Shinozaki, K. Shinozaki, A small peptide modulates stomatal control via abscisic acid in long-distance signaling, Nature 556 (2018) 235–238, https://doi.org/10.1038/s41586-018-0009-2.

[72] E.L. Harrison, L. Arce Cubas, J.E. Gray, C. Hepworth, The influence of stomatal morphology and distribution on photosynthetic gas exchange, Plant J. (2020), https://doi.org/10.1111/tpj.14560.

[73] J. Schroeder, G. Allen, V. Hugouvieux, J. Kwak, D. Waner, Guard cell signal transduction, Annu. Rev. Plant Biol. 52 (2001) 627–658.

[74] M. Israelsson, R.S. Siegel, J. Young, M. Hashimoto, K. Iba, J.I. Schroeder, Guard cell ABA and CO_2 signaling network updates and Ca^{2+} sensor priming hypothesis, Curr. Opin. Plant Biol. 9 (2006) 654–663, https://doi.org/10.1016/j.pbi.2006.09.006.

[75] S. Munemasa, D. Muroyama, H. Nagahashi, Y. Nakamura, I.C. Mori, Y. Murata, Regulation of reactive oxygen species-mediated abscisic acid signaling in guard cells and drought tolerance by glutathione, Front. Plant Sci. 4 (2013), https://doi.org/10.3389/fpls.2013.00472.

[76] M. Ranjbaran, M. Solhtalab, A.K. Datta, Mechanistic modeling of light-induced chemotactic infiltration of bacteria into leaf stomata, PLoS Comput. Biol. 16 (2020), https://doi.org/10.1371/journal.pcbi.1007841.

[77] S. Panchal, R. Chitrakar, B.K. Thompson, N. Obulareddy, D. Roy, W.S. Hambright, M. Melotto, Regulation of stomatal defense by air relative humidity, Plant Physiol. 172 (2016) 2021–2032, https://doi.org/10.1104/pp.16.00696.

[78] M. Melotto, W. Underwood, J. Koczan, K. Nomura, S.Y. He, Plant stomata function in innate immunity against bacterial invasion, Cell 126 (2006) 969–980, https://doi.org/10.1016/j.cell.2006.06.054.

[79] S.M. Assmann, J.A. Snyder, Y.R.J. Lee, ABA-deficient (*aba1*) and ABA-insensitive (*abi1-1*, *abi2-1*) mutants of Arabidopsis have a wild-type stomatal response to humidity, Plant Cell Environ. 23 (2000) 387–395, https://doi.org/10.1046/j.1365-3040.2000.00551.x.

[80] X. Xie, Y. Wang, L. Williamson, G.H. Holroyd, C. Tagliavia, E. Murchie, J. Theobald, M.R. Knight, W.J. Davies, H.M.O. Leyser, A.M. Hetherington, The identification of genes involved in the stomatal response to reduced atmospheric relative humidity, Curr. Biol. 16 (2006) 882–887, https://doi.org/10.1016/j.cub.2006.03.028.

[81] C.B. Engineer, M. Hashimoto-Sugimoto, J. Negi, M. Israelsson-Nordström, T. Azoulay-Shemer, W.J. Rappel, K. Iba, J.I. Schroeder, CO_2 sensing and CO_2 regulation of stomatal conductance: advances and open questions, Trends Plant Sci. 21 (2016) 16–30, https://doi.org/10.1016/j.tplants.2015.08.014.

[82] J. Brearley, M.A. Venis, M.R. Blatt, The effect of elevated CO_2 concentrations on K^+ and anion channels of *Vicia faba* L. guard cells, Planta 203 (1997) 145–154, https://doi.org/10.1007/s004250050176.

[83] W.B. Frommer, CO_2mmon sense, Science 327 (2010) 275–276, https://doi.org/10.1126/science.1186022.

[84] H. Hu, W.J. Rappel, R. Occhipinti, A. Ries, M. Böhmer, L. You, C. Xiao, C. B. Engineer, W.F. Boron, J.I. Schroeder, Distinct cellular locations of carbonic anhydrases mediate carbon dioxide control of stomatal movements, Plant Physiol. 169 (2015) 1168–1178, https://doi.org/10.1104/pp.15.00646.

[85] H. Hu, A. Boisson-Dernier, M. Israelsson-Nordström, M. Böhmer, S. Xue, A. Ries, J. Godoski, J.M. Kuhn, J.I. Schroeder, Carbonic anhydrases are upstream regulators of CO_2-controlled stomatal movements in guard cells, Nat. Cell Biol. 12 (2010) 87–93, https://doi.org/10.1038/ncb2009.

[86] L. Jakobson, L. Vaahtera, K. Tõldsepp, M. Nuhkat, C. Wang, Y.S. Wang, H. Hõrak, E. Valk, P. Pechter, Y. Sindarovska, J. Tang, C. Xiao, Y. Xu, U. Gerst Talas, A. T. García-Sosa, S. Kangasjärvi, U. Maran, M. Remm, M.R.G. Roelfsema, H. Hu, J. Kangasjärvi, M. Loog, J.I. Schroeder, H. Kollist, M. Brosché, Natural variation in Arabidopsis Cvi-0 accession reveals an important role of MPK12 in guard cell CO_2 signaling, PLoS Biol. 14 (2016), https://doi.org/10.1371/journal.pbio.2000322.

[87] H. Hõrak, M. Sierla, K. Tõldsepp, C. Wang, Y.S. Wang, M. Nuhkat, E. Valk, P. Pechter, E. Merilo, J. Salojärvi, K. Overmyer, M. Loog, M. Brosché, J. I. Schroeder, J. Kangasjärvi, H. Kollist, A dominant mutation in the ht1 kinase uncovers roles of MAP kinases and GHR1 in CO_2-induced stomatal closure, Plant Cell 28 (2016) 2493–2509, https://doi.org/10.1105/tpc.16.00131.

[88] W. Tian, C. Hou, Z. Ren, Y. Pan, J. Jia, H. Zhang, F. Bai, P. Zhang, H. Zhu, Y. He, S. Luo, L. Li, S. Luan, A molecular pathway for CO_2 response in Arabidopsis guard cells, Nat. Commun. 6 (2015), https://doi.org/10.1038/ncomms7057.

[89] Y. Zhou, I.A. Vroegop-Vos, A.J.H. Van Dijken, D. Van der Does, C. Zipfel, C.M. J. Pieterse, S.C.M. Van Wees, Carbonic anhydrases CA1 and CA4 function in atmospheric CO_2-modulated disease resistance, Planta 251 (2020), https://doi.org/10.1007/s00425-020-03370-w.

[90] R. Yoshida, T. Hobo, K. Ichimura, T. Mizoguchi, F. Takahashi, J. Aronso, J. R. Ecker, K. Shinozaki, ABA-activated SnRK2 protein kinase is required for dehydration stress signaling in Arabidopsis, Plant Cell Physiol. 43 (2002) 1473–1483, https://doi.org/10.1093/pcp/pcf188.

[91] A.C. Mustilli, S. Merlot, A. Vavasseur, F. Fenzi, J. Giraudat, Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production, Plant Cell 14 (2002) 3089–3099, https://doi.org/10.1105/tpc.007906.

[92] S. Xue, H. Hu, A. Ries, E. Merilo, H. Kollist, J.I. Schroeder, Central functions of bicarbonate in S-type anion channel activation and OST1 protein kinase in CO_2 signal transduction in guard cell, EMBO J. 30 (2011) 1645–1658, https://doi.org/10.1038/emboj.2011.68.

[93] P.K. Hsu, Y. Takahashi, S. Munemasa, E. Merilo, K. Laanemets, R. Waadt, D. Pater, H. Kollist, J.I. Schroeder, Abscisic acid-independent stomatal CO_2 signal transduction pathway and convergence of CO_2 and ABA signaling downstream of OST1 kinase, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) E9971–E9980, https://doi.org/10.1073/pnas.1809204115.

[94] L. Zhang, Y. Takahashi, P.K. Hsu, H. Kollist, E. Merilo, P.J. Krysan, J.I. Schroeder, FRET kinase sensor development reveals SnRK2/OST1 activation by ABA but not by MeJA and high CO_2 during stomatal closure, ELife 9 (2020) 1–74, https://doi.org/10.7554/eLife.56351.

[95] M. Zhu, S. Geng, D. Chakravorty, Q. Guan, S. Chen, S.M. Assmann, Metabolomics of red-light-induced stomatal opening in *Arabidopsis thaliana*: coupling with abscisic acid and jasmonic acid metabolism, Plant J. 101 (2020) 1331–1348, https://doi.org/10.1111/tpj.14594.

[96] S. Coursol, L.M. Fan, H. Le Stunff, S. Splegel, S. Gilroy, S.M. Assman, Sphingolipid signalling in Arabidopsis guard cells involves heterotrimeric G proteins, Nature 423 (2003) 651–654, https://doi.org/10.1038/nature01643.

[97] S. Coursol, H. Le Le Stunff, D.V. Lynch, S. Gilroy, S.M. Assmann, S. Spiegel, Arabidopsis sphingosine kinase and the effects of phytosphingosine-1- phosphate on stomatal aperture, Plant Physiol. 137 (2005) 724–737, https://doi.org/10.1104/pp.104.055806.

[98] X. Geng, W.J. Horst, J.F. Golz, J.E. Lee, Z. Ding, Z.B. Yang, LEUNIG_HOMOLOG transcriptional co-repressor mediates aluminium sensitivity through PECTIN METHYLESTERASE46-modulated root cell wall pectin methylesterification in Arabidopsis, Plant J. (2017), https://doi.org/10.1111/tpj.13506.

[99] D. Chakravorty, Y. Yu, S.M. Assmann, A kinase-dead version of FERONIA receptor-like kinase has dose-dependent impacts on rosette morphology and RALF1-mediated stomatal movements, FEBS Lett. 592 (2018) 3429–3437, https://doi.org/10.1002/1873-3468.13249.

[100] Y. Yu, D. Chakravorty, S.M. Assmann, The G protein β-subunit, AGB1, interacts with FERONIA in RALF1-regulated stomatal movement, Plant Physiol. 176 (2018) 2426–2440, https://doi.org/10.1104/pp.17.01277.

[101] M. Stegmann, J. Monaghan, E. Smakowska-Luzan, H. Rovenich, A. Lehner, N. Holton, Y. Belkhadir, C. Zipfel, The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling, Science 355 (2017) 287–289, https://doi.org/10.1126/science.aal2541.

[102] H. Kim, J.B. Ridenour, L.D. Dunkle, B.H. Bluhm, Regulation of stomatal tropism and infection by light in cercospora zeae-maydis: evidence for coordinated host/pathogen responses to photoperiod? PLoS Pathog. 7 (2011) https://doi.org/10.1371/journal.ppat.1002113.

[103] S. Panchal, D. Roy, R. Chitrakar, L. Price, Z.S. Breitbach, D.W. Armstrong, M. Melotto, Coronatine facilitates *Pseudomonas syringae* infection of Arabidopsis leaves at night, Front. Plant Sci. 7 (2016) 1–11, https://doi.org/10.3389/fpls.2016.00880.

[104] S.M. Assmann, A. Schwartz, Synergistic effect of light and fusicoccin on stomatal opening: epidermal peel and patch clamp experiments, Plant Physiol. 98 (1992) 1349–1355, https://doi.org/10.1104/pp.98.4.1349.

[105] S. Merlot, N. Leonhardt, F. Fenzi, C. Valon, M. Costa, L. Piette, A. Vavasseur, B. Genty, K. Boivin, A. Müller, J. Giraudat, J. Leung, Constitutive activation of a plasma membrane H^+-ATPase prevents abscisic acid-mediated stomatal closure, EMBO J. 26 (2007) 3216–3226, https://doi.org/10.1038/sj.emboj.7601750.

[106] R. Ali, W. Ma, F. Lemtiri-Chlieh, D. Tsaltas, Q. Leng, S. Von Bodman, G. A. Berkowitz, Death don't have no mercy and neither does calcium: arabidopsis CYCLIC NUCLEOTIDE GATED CHANNEL2 and innate immunity, Plant Cell 19 (2007) 1081–1095, https://doi.org/10.1105/tpc.106.045096.

[107] S.M. Assmann, L. Simoncini, J.I. Schroeder, Blue light activates electrogenic ion pumping in guard cell protoplasts of *Vicia faba*, Nature 318 (1985) 285–287, https://doi.org/10.1038/318285a0.

[108] S.I. Inoue, T. Kinoshita, Blue light regulation of stomatal opening and the plasma membrane H^+-ATPase, Plant Physiol. 174 (2017) 531–538, https://doi.org/10.1104/pp.17.00166.

[109] J.S.A. Matthews, S. Vialet-Chabrand, T. Lawson, Role of blue and red light in stomatal dynamic behaviour, J. Exp. Bot. 71 (2020) 2253–2269, https://doi.org/10.1093/jxb/erz563.

[110] A. Schwartz, E. Zeiger, Metabolic energy for stomatal opening. Roles of photophosphorylation and oxidative phosphorylation, Planta 161 (1984) 129–136, https://doi.org/10.1007/BF00395472.
[111] M.R.G. Roelfsema, K.R. Konrad, H. Marten, G.K. Psaras, W. Hartung, R. Hedrich, Guard cells in albino leaf patches do not respond to photosynthetically active radiation, but are sensitive to blue light, CO_2 and abscisic acid, Plant Cell Environ. 29 (2006) 1595–1605, https://doi.org/10.1111/j.1365-3040.2006.01536.x.
[112] Z. Sun, X. Jin, R. Albert, S.M. Assmann, Multi-level modeling of light-induced stomatal opening offers new insights into its regulation by drought, PLoS Comput. Biol. 10 (2014), https://doi.org/10.1371/journal.pcbi.1003930.
[113] T. Lawson, Guard cell photosynthesis and stomatal function, New Phytol. 181 (2009) 13–34, https://doi.org/10.1111/j.1469-8137.2008.02685.x.
[114] G.S. Hudson, J.R. Evans, S. Von Caemmerer, Y.B.C. Arvidsson, T.J. Andrews, Reduction of ribulose-1,5-bisphosphate carboxylase/oxygenase content by antisense RNA reduces photosynthesis in transgenic tobacco plants, Plant Physiol. 98 (1992) 294–302, https://doi.org/10.1104/pp.98.1.294.
[115] I. Baroli, G.D. Price, M.R. Badger, S. Von Caemmerer, The contribution of photosynthesis to the red light response of stomatal conductance, Plant Physiol. 146 (2008) 737–747, https://doi.org/10.1104/pp.107.110924.
[116] T. Lawson, S. Lefebvre, N.R. Baker, J.I.L. Morison, C.A. Raines, Reductions in mesophyll and guard cell photosynthesis impact on the control of stomatal responses to light and CO_2, J. Exp. Bot. 59 (2008) 3609–3619, https://doi.org/10.1093/jxb/ern211.
[117] G.A. Beattie, Water relations in the interaction of foliar bacterial pathogens with plants, Annu. Rev. Phytopathol. 49 (2011) 533–555, https://doi.org/10.1146/annurev-phyto-073009-114436.
[118] F. Pantin, M.R. Blatt, Stomatal response to humidity: blurring the boundary between active and passive movement, Plant Physiol. 176 (2018) 485–488, https://doi.org/10.1104/pp.17.01699.
[119] J.M. Monier, S.E. Lindow, Differential survival of solitary and aggregated bacterial cells promotes aggregate formation on leaf surfaces, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 15977–15982, https://doi.org/10.1073/pnas.2436560100.
[120] L. Schreiber, U. Krimm, D. Knoll, M. Sayed, G. Auling, R.M. Kroppenstedt, Plant-microbe interactions: identification of epiphytic bacteria and their ability to alter leaf surface permeability, New Phytol. 166 (2005) 589–594, https://doi.org/10.1111/j.1469-8137.2005.01343.x.
[121] J.H.J. Leveau, S.E. Lindow, Appetite of an epiphyte: quantitative monitoring of bacterial sugar consumption in the phyllosphere, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 3446–3453, https://doi.org/10.1073/pnas.061629598.
[122] B. Asselbergh, D. De Vleesschauwer, M. Höfte, Global switches and fine-tuning-ABA modulates plant pathogen defense, Mol. Plant-Microbe Interact. 21 (2008) 709–719, https://doi.org/10.1094/MPMI-21-6-0709.
[123] M. Fujita, Y. Fujita, Y. Noutoshi, F. Takahashi, Y. Narusaka, K. Yamaguchi-Shinozaki, K. Shinozaki, Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks, Curr. Opin. Plant Biol. 9 (2006) 436–442, https://doi.org/10.1016/j.pbi.2006.05.014.
[124] J. Fan, L. Hill, C. Crooks, P. Doerner, C. Lamb, Abscisic acid has a key role in modulating diverse plant-pathogen interactions, Plant Physiol. 150 (2009) 1750–1761, https://doi.org/10.1104/pp.109.137943.
[125] A.K. Goel, D. Lundberg, M.A. Torres, R. Matthews, C. Akimoto-Tomiyama, L. Farmer, J.L. Dangl, S.R. Grant, The *Pseudomonas syringae* type III effector HopAM1 enhances virulence on water-stressed plants, Mol. Plant-Microbe Interact. 21 (2008) 361–370, https://doi.org/10.1094/MPMI-21-3-0361.
[126] H. Bauer, P. Ache, S. Lautner, J. Fromm, W. Hartung, K.A.S. Al-Rasheid, S. Sonnewald, U. Sonnewald, S. Kneitz, N. Lachmann, R.R. Mendel, F. Bittner, A. M. Hetherington, R. Hedrich, The stomatal response to reduced relative humidity requires guard cell-autonomous ABA synthesis, Curr. Biol. 23 (2013) 53–57, https://doi.org/10.1016/j.cub.2012.11.022.
[127] H. Bauer, P. Ache, F. Wohlfart, K.A.S. Al-Rasheid, S. Sonnewald, U. Sonnewald, S. Kneitz, A.M. Hetherington, R. Hedrich, How do stomata sense reductions in atmospheric relative humidity? Mol. Plant 6 (2013) 1703–1706, https://doi.org/10.1093/mp/sst055.
[128] M. Okamoto, Y. Tanaka, S.R. Abrams, Y. Kamiya, M. Seki, E. Nambara, High humidity induces abscisic acid 8′-hydroxylase in stomata and vasculature to regulate local and systemic abscisic acid responses in Arabidopsis, Plant Physiol. 149 (2009) 825–834, https://doi.org/10.1104/pp.108.130823.
[129] E. Merilo, D. Yarmolinsky, P. Jalakas, H. Parik, I. Tulva, B. Rasulov, K. Kilk, H. Kollist, Stomatal VPD response: there is more to the story than ABA, Plant Physiol. 176 (2018) 851–864, https://doi.org/10.1104/pp.17.00912.
[130] P.J. Franks, J.A. Berry, D.L. Lombardozzi, G.B. Bonan, Stomatal function across temporal and spatial scales: deep-time trends, land-atmosphere coupling and global models, Plant Physiol. 174 (2017) 583–602, https://doi.org/10.1104/pp.17.00287.
[131] L. Bacete, H. Mélida, E. Miedes, A. Molina, Plant cell wall-mediated immunity: cell wall changes trigger disease resistance responses, Plant J. 93 (2018) 614–636, https://doi.org/10.1111/tpj.13807.
[132] I.C. Mori, R. Pinontoan, T. Kawano, S. Muto, Involvement of superoxide generation in salicylic acid-induced stomatal closure in *Vicia faba*, Plant Cell Physiol. 42 (2001) 1383–1388, https://doi.org/10.1093/pcp/pce176.
[133] D. Suhita, A.S. Raghavendra, J.M. Kwak, A. Vavasseur, Cytoplasmic alkalization precedes reactive oxygen species production during methyl jasmonate- and abscisic acid-induced stomatal closure, Plant Physiol. 134 (2004) 1536–1545, https://doi.org/10.1104/pp.103.032250.
[134] J.G. Li, M. Fan, W. Hua, Y. Tian, L.G. Chen, Y. Sun, M.Y. Bai, Brassinosteroid and hydrogen peroxide interdependently induce stomatal opening by promoting guard cell starch degradation, Plant Cell 32 (2020) 984–999, https://doi.org/10.1105/tpc.19.00587.
[135] M. Kalliola, L. Jakobson, P. Davidsson, V. Pennanen, C. Waszczak, D. Yarmolinsky, O. Zamora, E.T. Palva, T. Kariola, H. Kollist, M. Brosché, The role of strigolactones in regulation of stomatal conductance and plant-pathogen interactions in *Arabidopsis thaliana*, BioRxiv (2019), https://doi.org/10.1101/573873, 573873.
[136] S. Lv, Y. Zhang, C. Li, Z. Liu, N. Yang, L. Pan, J. Wu, J. Wang, J. Yang, Y. Lv, Y. Zhang, W. Jiang, X. She, G. Wang, Strigolactone-triggered stomatal closure requires hydrogen peroxide synthesis and nitric oxide production in an abscisic acid-independent manner, New Phytol. 217 (2018) 290–304, https://doi.org/10.1111/nph.14813.
[137] S.H. Spoel, X. Dong, Making sense of hormone crosstalk during plant immune responses, Cell Host Microbe 3 (2008) 348–351, https://doi.org/10.1016/j.chom.2008.05.009.
[138] A. Guzel Deger, S. Scherzer, M. Nuhkat, J. Kedzierska, H. Kollist, M. Brosché, S. Unyayar, M. Boudsocq, R. Hedrich, M.R.G. Roelfsema, Guard cell SLAC1-type anion channels mediate flagellin-induced stomatal closure, New Phytol. 208 (2015) 162–173, https://doi.org/10.1111/nph.13435.
[139] M. Issak, E. Okuma, S. Munemasa, Y. Nakamura, I.C. Mori, Y. Murata, Neither endogenous abscisic acid nor endogenous jasmonate is involved in salicylic acid-, yeast elicitor-, or chitosan-induced stomatal closure in *Arabidopsis thaliana*, Biosci. Biotechnol. Biochem. 77 (2013) 1111–1113, https://doi.org/10.1271/bbb.120980.
[140] T. Zhang, J.D. Schneider, C. Lin, S. Geng, T. Ma, S.R. Lawrence, C.P. Dufresne, A. C. Harmon, S. Chen, MPK4 phosphorylation dynamics and interacting proteins in plant immunity, J. Proteome Res. 18 (2019) 826–840, https://doi.org/10.1021/acs.jproteome.8b00345.
[141] Q. Zhai, C. Li, The plant mediator complex and its role in jasmonate signaling, J. Exp. Bot. 70 (2019) 3415–3424, https://doi.org/10.1093/jxb/erz233.
[142] S. Munemasa, K. Oda, M. Watanabe-Sugimoto, Y. Nakamura, Y. Shimoishi, Y. Murata, The coronatine-insensitive 1 mutation reveals the hormonal signaling interaction between abscisic acid and methyl jasmonate in Arabidopsis guard cells. Specific impairment of ion channel activation and second messenger production, Plant Physiol. 143 (2007) 1398–1407, https://doi.org/10.1104/pp.106.091298.
[143] M. Zhu, S. Dai, N. Zhu, A. Booy, B. Simons, S. Yi, S. Chen, Methyl jasmonate responsive proteins in *Brassica napus* guard cells revealed by iTRAQ-based quantitative proteomics, J. Proteome Res. 11 (2012) 3728–3742, https://doi.org/10.1021/pr300213k.
[144] M. Desclos-Theveniau, D. Arnaud, T.Y. Huang, G.J.C. Lin, W.Y. Chen, Y.C. Lin, L. Zimmerli, The Arabidopsis lectin receptor kinase LecRK-V.5 represses stomatal immunity induced by *Pseudomonas syringae* pv. tomato DC3000, PLoS Pathog. 8 (2012), https://doi.org/10.1371/journal.ppat.1002513.
[145] D. Hua, C. Wang, J. He, H. Liao, Y. Duan, Z. Zhu, Y. Guo, Z. Chen, Z. Gong, A plasma membrane receptor kinase, GHR1, mediates abscisic acid- and hydrogen peroxide-regulated stomatal movement in Arabidopsis, Plant Cell 24 (2012) 2546–2561, https://doi.org/10.1105/tpc.112.100107.
[146] C. De Ollas, V. Arbona, A. Gómez-Cadenas, I.C. Dodd, Attenuated accumulation of jasmonates modifies stomatal responses to water deficit, J. Exp. Bot. 69 (2018) 2103–2116, https://doi.org/10.1093/jxb/ery045.
[147] C. de Ollas, I.C. Dodd, Physiological impacts of ABA–JA interactions under water-limitation, Plant Mol. Biol. 91 (2016) 641–650, https://doi.org/10.1007/s11103-016-0503-6.
[148] T. Savchenko, V.A. Kolla, C.Q. Wang, Z. Nasafi, D.R. Hicks, B. Phadungchob, W. E. Chehab, F. Brandizzi, J. Froehlich, K. Dehesh, Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought, Plant Physiol. 164 (2014) 1151–1160, https://doi.org/10.1104/pp.113.234310.
[149] Z. Zhou, Y. Wu, Y. Yang, M. Du, X. Zhang, Y. Guo, C. Li, J.M. Zhou, An arabidopsis plasma membrane proton ATPase modulates JA signaling and is exploited by the *Pseudomonas syringae* effector protein AvrB for stomatal invasion, Plant Cell 27 (2015) 2032–2041, https://doi.org/10.1105/tpc.15.00466.
[150] M.A. Hossain, S. Munemasa, M. Uraji, Y. Nakamura, I.C. Mori, Y. Murata, Involvement of endogenous abscisic acid in methyl jasmonate-induced stomatal closure in Arabidopsis, Plant Physiol. 156 (2011) 430–438, https://doi.org/10.1104/pp.111.172254.
[151] K. Tsuda, M. Sato, J. Glazebrook, J.D. Cohen, F. Katagiri, Interplay between MAMP-triggered and SA-mediated defense responses, Plant J. 53 (2008) 763–775, https://doi.org/10.1111/j.1365-313X.2007.03369.x.
[152] X.Y. Zheng, M. Zhou, H. Yoo, J.L. Pruneda-Paz, N.W. Spivey, S.A. Kay, X. Dong, Spatial and temporal regulation of biosynthesis of the plant immune signal salicylic acid, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 9166–9173, https://doi.org/10.1073/pnas.1511182112.
[153] T. Joudoi, Y. Shichiri, N. Kamizono, T. Akaike, T. Sawa, J. Yoshitake, N. Yamada, S. Iwai, Nitrated cyclic GMP modulates guard cell signaling in Arabidopsis, Plant Cell 25 (2013) 558–571, https://doi.org/10.1105/tpc.112.105049.
[154] D. Zhao, Y. Yu, Y. Shen, Q. Liu, Z. Zhao, R. Sharma, R.J. Reiter, Melatonin synthesis and function: evolutionary history in animals and plants, Front. Endocrinol. 10 (2019), https://doi.org/10.3389/fendo.2019.00249.
[155] M.B. Arnao, J. Hernández-Ruiz, Is phytomelatonin a new plant hormone? Agronomy 10 (2020) https://doi.org/10.3390/agronomy10010095.
[156] C. Li, D.X. Tan, D. Liang, C. Chang, D. Jia, F. Ma, Melatonin mediates the regulation of ABA metabolism, free-radical scavenging, and stomatal behaviour in

two Malus species under drought stress, J. Exp. Bot. 66 (2015) 669–680, https://doi.org/10.1093/jxb/eru476.
[157] H. Zhao, L. Xu, T. Su, Y. Jiang, L. Hu, F. Ma, Melatonin regulates carbohydrate metabolism and defenses against *Pseudomonas syringae* pv. tomato DC3000 infection in *Arabidopsis thaliana*, J. Pineal Res. 59 (2015) 109–119, https://doi.org/10.1111/jpi.12245.
[158] H. Shi, Y. Chen, D.X. Tan, R.J. Reiter, Z. Chan, C. He, Melatonin induces nitric oxide and the potential mechanisms relate to innate immunity against bacterial pathogen infection in Arabidopsis, J. Pineal Res. 59 (2015) 102–108, https://doi.org/10.1111/jpi.12244.
[159] L. Wu, Z. Huang, X. Li, L. Ma, Q. Gu, H. Wu, J. Liu, R. Borriss, Z. Wu, X. Gao, Stomatal closure and SA-, JA/ET-signaling pathways are essential for Bacillus amyloliquefaciens FZB42 to restrict leaf disease caused by *Phytophthora nicotianae* in *Nicotiana benthamiana*, Front. Microbiol. 9 (2018), https://doi.org/10.3389/fmicb.2018.00847.
[160] S. Panchal, M. Melotto, Stomate-based defense and environmental cues, Plant Signal. Behav. (2017), https://doi.org/10.1080/15592324.2017.1362517.
[161] Z.M. Pel, Y. Murata, G. Benning, S. Thomine, B. Klüsener, G.J. Allen, E. Grill, J. I. Schroeder, Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells, Nature 406 (2000) 731–734, https://doi.org/10.1038/35021067.
[162] M. Sierla, C. Waszczak, T. Vahisalu, J. Kangasjärvi, Reactive oxygen species in the regulation of stomatal movements, Plant Physiol. 171 (2016) 1569–1580, https://doi.org/10.1104/pp.16.00328.
[163] N. Singh, S.C. Bhatla, Signaling through reactive oxygen and nitrogen species is differentially modulated in sunflower seedling root and cotyledon in response to various nitric oxide donors and scavengers, Plant Signal. Behav. 12 (2017), https://doi.org/10.1080/15592324.2017.1365214.
[164] X. Liu, F. Li, J. Tang, W. Wang, F. Zhang, G. Wang, J. Chu, C. Yan, T. Wang, C. Chu, C. Li, Activation of the jasmonic acid pathway by depletion of the hydroperoxide lyase OsHPL3 reveals crosstalk between the HPL and AOS branches of the oxylipin pathway in rice, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0050089.
[165] R.A. Creelman, R. Mulpuri, The oxylipin pathway in Arabidopsis, Arabidopsis Book 1 (2002) e0012, https://doi.org/10.1199/tab.0012.
[166] P. Bednarek, Chemical warfare or modulators of defence responses - the function of secondary metabolites in plant immunity, Curr. Opin. Plant Biol. 15 (2012) 407–414, https://doi.org/10.1016/j.pbi.2012.03.002.
[167] K. Zhang, H. Su, J. Zhou, W. Liang, D. Liu, J. Li, Overexpressing the myrosinase gene TGG1 enhances stomatal defense against *Pseudomonas syringae* and delays flowering in Arabidopsis, Front. Plant Sci. 10 (2019), https://doi.org/10.3389/fpls.2019.01230.
[168] D. Horrer, S. Flütsch, D. Pazmino, J.S.A. Matthews, M. Thalmann, A. Nigro, N. Leonhardt, T. Lawson, D. Santelia, Blue light induces a distinct starch degradation pathway in guard cells for stomatal opening, Curr. Biol. 26 (2016) 362–370, https://doi.org/10.1016/j.cub.2015.12.036.
[169] D.H. McLachlan, J. Lan, C.M. Geilfus, A.N. Dodd, T. Larson, A. Baker, H. Hõrak, H. Kollist, Z. He, I. Graham, M.V. Mickelbart, A.M. Hetherington, The breakdown of stored triacylglycerols is required during light-induced stomatal opening, Curr. Biol. 26 (2016) 707–712, https://doi.org/10.1016/j.cub.2016.01.019.
[170] D.M. Daloso, W.C. Antunes, D.P. Pinheiro, J.P. Waquim, W.L. Araújo, M. E. Loureiro, A.R. Fernie, T.C.R. Williams, Tobacco guard cells fix CO_2 by both Rubisco and PEPcase while sucrose acts as a substrate during light-induced stomatal opening, Plant Cell Environ. 38 (2015) 2353–2371, https://doi.org/10.1111/pce.12555.
[171] D.M. Daloso, T.C.R. Williams, W.C. Antunes, D.P. Pinheiro, C. Müller, M. E. Loureiro, A.R. Fernie, Guard cell-specific upregulation of sucrose synthase 3 reveals that the role of sucrose in stomatal function is primarily energetic, New Phytol. 209 (2016) 1470–1483, https://doi.org/10.1111/nph.13704.
[172] J. Negi, S. Munemasa, B. Song, R. Tadakuma, M. Fujita, T. Azoulay-Shemer, C. B. Engineer, K. Kusumi, I. Nishida, J.I. Schroeder, K. Iba, Eukaryotic lipid metabolic pathway is essential for functional chloroplasts and CO_2 and light responses in Arabidopsis guard cells, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 9038–9043, https://doi.org/10.1073/pnas.1810458115.
[173] D.M. Daloso, D.B. Medeiros, L. dos Anjos, T. Yoshida, W.L. Araújo, A.R. Fernie, Metabolism within the specialized guard cells of plants, New Phytologist. 216 (2017) 1018–1033, https://doi.org/10.1111/nph.14823.
[174] D. Santelia, T. Lawson, Rethinking guard cell metabolism, Plant Physiol. 172 (2016) 1371–1392, https://doi.org/10.1104/pp.16.00767.
[175] G.-H. Lim, R. Singhal, A. Kachroo, P. Kachroo, Fatty acid- and lipid-mediated signaling in plant defense, Annu. Rev. Phytopathol. 55 (2017) 505–536, https://doi.org/10.1146/annurev-phyto-080516-035406.
[176] M.K. Mandal, A.C. Chandra-Shekara, R.D. Jeong, K. Yu, S. Zhu, B. Chanda, D. Navarre, A. Kachroo, P. Kachroo, Oleic acid-dependent modulation of NITRIC OXIDE ASSOCIATED1 protein levels regulates nitric oxide-mediated defense signaling in Arabidopsis, Plant Cell 24 (2012) 1654–1674, https://doi.org/10.1105/tpc.112.096768.
[177] T. Yaeno, O. Matsuda, K. Iba, Role of chloroplast trienoic fatty acids in plaint disease defense responses, Plant J. 40 (2004) 931–941, https://doi.org/10.1111/j.1365-313X.2004.02260.x.
[178] C. Wang, C.K. Chin, A. Chen, Expression of the yeast δ-9 desaturase gene in tomato enhances its resistance to powdery mildew, Physiol. Mol. Plant Pathol. 52 (1998) 371–383, https://doi.org/10.1006/pmpp.1998.0158.
[179] Y. Zhang, H. Zhu, Q. Zhang, M. Li, M. Yan, R. Wang, L. Wang, R. Welti, W. Zhang, X. Wang, Phospholipase Dα1 and phosphatidic acid regulate NADPH oxidase activity and production of reactive oxygen species in ABA-mediated stomatal closure in Arabidopsis, Plant Cell 21 (2009) 2357–2377, https://doi.org/10.1105/tpc.108.062992.
[180] J. Wei, D.X. Li, J.R. Zhang, C. Shan, Z. Rengel, Z.B. Song, Q. Chen, Phytomelatonin receptor PMTR1-mediated signaling regulates stomatal closure in *Arabidopsis thaliana*, J. Pineal Res. 65 (2018), https://doi.org/10.1111/jpi.12500.
[181] H.Y. Lee, K. Back, The phytomelatonin receptor (PMRT1) Arabidopsis Cand2 is not a bona fide G protein–coupled melatonin receptor, Melatonin Res. 3 (2020) 177–186, https://doi.org/10.32794/mr11250055.
[182] H.Y. Lee, Y. Byeon, D.X. Tan, R.J. Reiter, K. Back, Arabidopsis serotonin N-acetyltransferase knockout mutant plants exhibit decreased melatonin and salicylic acid levels resulting in susceptibility to an avirulent pathogen, J. Pineal Res. 58 (2015) 291–299, https://doi.org/10.1111/jpi.12214.
[183] E. Okuma, M.S. Jahan, S. Munemasa, M.A. Hossain, D. Muroyama, M.M. Islam, K. Ogawa, M. Watanabe-Sugimoto, Y. Nakamura, Y. Shimoishi, I.C. Mori, Y. Murata, Negative regulation of abscisic acid-induced stomatal closure by glutathione in Arabidopsis, J. Plant Physiol. 168 (2011) 2048–2055, https://doi.org/10.1016/j.jplph.2011.06.002.
[184] J.M. Watkins, P.J. Hechler, G.K. Muday, Ethylene-induced flavonol accumulation in guard cells suppresses reactive oxygen species and moderates stomatal aperture, Plant Physiol. 164 (2014) 1707–1717, https://doi.org/10.1104/pp.113.233528.
[185] Y. An, X. Feng, L. Liu, L. Xiong, L. Wang, ALA-induced flavonols accumulation in guard cells is involved in scavenging H_2O_2 and inhibiting stomatal closure in Arabidopsis cotyledons, Front. Plant Sci. 7 (2016), https://doi.org/10.3389/fpls.2016.01713.
[186] L. David, J. Kang, S. Chen, Targeted metabolomics of plant hormones and redox metabolites in stomatal immunity. Methods in Molecular Biology, 2020, pp. 79–92, https://doi.org/10.1007/978-1-0716-0142-6_6.
[187] X. Jin, R.S. Wang, M. Zhu, B.W. Jeon, R. Albert, S. Chen, S.M. Assmann, Abscisic acid-responsive guard cell metabolomes of Arabidopsis wild-type and gpa1 G-protein mutants, Plant Cell 25 (2013) 4789–4811, https://doi.org/10.1105/tpc.113.119800.
[188] H. Li, B.K. Smith, B. Shrestha, L. Márk, A. Vertes, Automated cell-by-cell tissue imaging and single-cell analysis for targeted morphologies by laser ablation electrospray ionization mass spectrometry, Methods Mol. Biol. (Clifton, N.J.) 1203 (2015) 117–127, https://doi.org/10.1007/978-1-4939-1357-2_12.
[189] E.K. Neumann, L.G. Migas, J.L. Allen, R.M. Caprioli, R. Van De, J.M. Spraggins, Spatial metabolomics of the human kidney using MALDI trapped ion mobility imaging mass spectrometry, ChemRxiv (2020) 4–6, https://doi.org/10.26434/chemrxiv.12118644.v1.
[190] J.M. Spraggins, K.V. Djambazova, E.S. Rivera, L.G. Migas, E.K. Neumann, A. Fuetterer, J. Suetering, N. Goedecke, A. Ly, R. Van De Plas, R.M. Caprioli, High-performance molecular imaging with MALDI trapped ion-mobility time-of-flight (timsTOF) mass spectrometry, Anal. Chem. 91 (2019) 14552–14560, https://doi.org/10.1021/acs.analchem.9b03612.
[191] M.E. Ridgeway, M. Lubeck, J. Jordens, M. Mann, M.A. Park, Trapped ion mobility spectrometry: a short review, Int. J. Mass Spectrom. 425 (2018) 22–35, https://doi.org/10.1016/j.ijms.2018.01.006.
[192] M. Dilillo, D. Pellegrini, R. Ait-Belkacem, E.L. De Graaf, M. Caleo, L.A. McDonnell, Mass spectrometry imaging, laser capture microdissection, and LC-MS/MS of the same tissue section, J. Proteome Res. 16 (2017) 2993–3001, https://doi.org/10.1021/acs.jproteome.7b00284.
[193] L. Cui, H. Lu, Y.H. Lee, Challenges and emergent solutions for LC-MS/MS based untargeted metabolomics in diseases, Mass Spectrom. Rev. 37 (2018) 772–792, https://doi.org/10.1002/mas.21562.