Opinion

Rapid Responses to Abiotic Stress: Priming the Landscape for the Signal Transduction Network

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Plants grow and reproduce within a highly dynamic environment that can see abrupt changes in conditions, such as light intensity, temperature, humidity, or interactions with biotic agents. Recent studies revealed that plants can respond within seconds to some of these conditions, engaging many different metabolic and molecular networks, as well as rapidly altering their stomatal aperture. Some of these rapid responses were further shown to propagate throughout the entire plant via waves of reactive oxygen species (ROS) and Ca^{2+} that are possibly mediated through the plant vascular system. Here, we propose that the integration of these signals is mediated through pulses of gene expression that are coordinated throughout the plant in a systemic manner by the ROS/Ca^{+2} waves.

The Dynamic Environment of Plants

While growing within their natural habitat, or in a manmade field environment, plants are subjected to many different changes in their physical and biological surroundings. These can be gradual, such as the slow decrease in soil water content over time during summer, or rapid, such as changes in light intensity occurring as a result of sun flecks during a cloudy day [1–9] (Figure 1, Key Figure). Some of these rapid changes can occur simultaneously, or on the background of other more persistent stress conditions, such as a prolonged drought or a heat wave, essentially representing a state of stress combination [4,5,10]. In addition, due to the placement of the plant within its environment, for example its position within a row of plants or plot in the field, or its position in nature in close proximity to a tree cover, not all parts or tissues of the plant may experience the rapid change in environmental conditions simultaneously [4,11]. Under such conditions, the **rapid responses** (see Glossary) at the affected tissue could trigger systemic signaling pathways, such as the ROS [12] and calcium waves [13], electric signals [14], and/or hydraulic waves [15]. The occurrence of rapid changes in the physical and/or biological environment of the plant, coupled with the discovery of rapid systemic signaling pathways activated by many of these conditions [12–15], and the discovery of rapid transcriptional and metabolic responses to stress [16,17], support a hypothesis that plants evolved sensing and acclimation mechanisms that may function within the seconds to minute timescale and are important for the overall fitness of the plant and its ability to survive rapid changes within its environment (Figure 1). Here, we examine the molecular networks, and physiological and metabolic changes that occur in plants during rapid responses to stress, and propose that the integration of these responses is mediated through pulses of gene expression that are coordinated throughout the plant in a systemic manner by the ROS/Ca^{+2} waves.

Highlights

Recent studies reveal that plants respond within the seconds to minutes time-scale to different biotic and/or abiotic stimuli.

The rapid response of plants to different stimuli spans many metabolic, molecular, and biochemical pathways, and involves genes essential for plant acclimation or defense.

Stomata can respond within minutes to different stimuli, such as ozone, pathogen infection, or light stress, and this response can be propagated from the affected leaf to different systemic leaves by the ROS/Ca^{2+} wave.

Different transcriptional regulators display a triphasic expression pattern with transient peaks of expression at the seconds, minutes and hours time-scale. These regulators may shape the acclimation and/or defense response of plants in pulses of gene expression that orchestrate the gene response network.

Rapid responses and the rapid systemic signaling pathways they activate could coordinate the systemic response of plants to a combination of different environmental conditions, enhancing the overall acclimation and ability of plants to withstand the rapidly changing conditions within their environment.

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From Physical to Molecular: Sensing Rapid Responses in Environmental Conditions

Rapid changes in external physical parameters, such as temperature, light, or relative air humidity, can be rapidly translated into several different biochemical and metabolic reactions within cells [16–24]. For example, changes in temperature or mechanical stress can rapidly alter membrane fluidity or integrity, modifying membrane function and/or changing the structure of different membrane proteins. Such changes could affect the function of different receptors or channels and initiate signal transduction reactions via phosphorylation and/or altered calcium fluxes. Given that many reactions in cells are coupled across different cellular compartments (e.g., photorespiration), the effects of rapid changes in abiotic conditions on membrane function could be translated into rapid changes in internal metabolic responses and fluxes, potentially affecting different metabolic regulators, such as SNF1-related kinase 1 (SnRK1), and triggering multiple signal transduction reactions [22,24]. Similar alterations in metabolite levels and fluxes could also occur because different enzymes within the same pathway in the plant differentially respond to rapid alterations in conditions, such as temperature or redox potential, causing metabolic imbalances [16–19]. Altered fluxes though the Calvin cycle and altered redox changes can also trigger the chloroplast–nuclei retrograde signaling pathway, driving rapid changes in gene expression [22,25,26]. The retrograde signaling pathway was also shown to mediate rapid stomatal responses in response to drought [27].

Sensing of rapid changes in environmental conditions could trigger rapid changes in gene expression in several ways. For example, stalled RNA polymerases that are 'parked' on the promoters of genes encoding heat shock response proteins were shown to respond to changes in temperature within seconds in fruit fly [28]. Rapid alterations in the steady-state level of many transcripts in cells could also be mediated by changes in mRNA stability [6]. In addition, the association of pre-existing mRNA with polysomes could be altered, resulting in the rapid synthesis of new proteins [29]. Ubiquitination, degradation, and other post-translational modifications of existing proteins, as well as cytoskeleton modifications and carbohydrate adjustments that do not require *de novo* transcription, could also be involved in this response [30–32]. Therefore, a complex set of molecular and metabolic reactions can translate rapid changes in environmental conditions into rapid changes in protein, mRNA, and/or metabolite levels, and lead to rapid acclimation (Figure 1).

Early Studies of Rapid Responses to the Environment in Plants: Laying the Foundations

Examples of early work addressing rapid molecular, physiological, and metabolic responses of plants to changes within their environment include the identification of **touch genes** in arabidopsis (*Arabidopsis thaliana*) that increase their expression up to 100-fold as quickly as 10 min following stimuli such as wind, touch, wounding, rain, or transition of plants from light into darkness [33,34]. A notable member of this gene family (termed TCH for touch-induced) is a calmodulin homolog that could be involved in calcium signaling [1]. Several different rapid metabolic responses were also previously studied in response to bacterial or fungal infection and included a burst of ROS production mediated by RESPIRATORY BURST OXIDIZE HOMOLOGS (RBOHs [35]), and rapid metabolic reactions that deposit callose on cell walls [36]. These metabolic responses were triggered as early as minutes following pathogen recognition and played a key role in blocking pathogen spread as well as triggering downstream defense pathways. Early work also demonstrated that some of those rapid responses, triggered by pathogen infection, were also triggered in response to ozone [37], demonstrating an overlap between rapid biotic and abiotic responses in plants and highlighting the key role

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that ROS might have in mediating and coordinating these responses. In addition, although traditional studies of stomatal responses were not aimed at identifying rapid changes in stomatal aperture, it has been known for many years that stomata are capable of much faster (rapid) responses [22,38,39].

Rapid Responses to Abiotic Stress: 'Omics, Physiological, and Molecular Studies

More recent studies of rapid responses to abiotic stress can be divided into: (i) responses that occurred minutes after stress application or recovery from stress, (e.g., [40–44]); and (ii) responses that occurred seconds after stress application (e.g., [16,17,25]). These studies identified several different transcription factors (TFs) that were involved in the rapid response of plants to different stresses, including ethylene response factors (ERFs), WRKY, heat shock factors (HSFs), and MYB [40,41,44] (S.I. Zandalinas et al., unpublished). A combined transcriptomic, methylomic, and RNA degradation study also identified rapid changes in transcript levels occurring 3–5 min after the start of the recovery process in arabidopsis [6,42]. In addition, a dual-phase response, in which certain transcripts peak on a minute timescale and then again on a hour timescale, was identified following chemical activation of retrograde signaling and was linked to the repressive function of two WRKY TFs (AtWRKY15 and AtWRKY40) [44]. Rapid transcriptomic and metabolomic responses of plants to light stress on the seconds timescale (0, 20, 60, or 90 s) range identified over 700 transcripts and 111 metabolites as responding to light stress within 0–90 s, as well as identified a role for glutathione and the plant hormones nitric oxide (NO) and abscisic acid (ABA) in this response [16,17]. Furthermore, at least five of the ultrafast transcripts were shown to be encoded by genes that are required for light stress acclimation in arabidopsis [16].

Rapid Responses to the Environment: Priming the Landscape of Plant Signaling

The occurrence of rapid responses within the seconds–minutes timescale in plants suggests that these responses have an important role in plant acclimation to stress. Supporting this hypothesis is the finding that some of the rapid response transcripts elevated within seconds during excess light stress are encoded by genes that are essential for light stress acclimation [16]. When considering the role that rapid responses may have in stress acclimation, at least two possible options come to mind: (i) rapid responses could have an immediate role in preventing irreversible damage to the plant during the early phases of stress by making appropriate metabolic, molecular, and physiological changes, such as regulating stomatal aperture and altering photosynthesis rates; or (ii) rapid responses could be setting the stage for an appropriate and successful acclimation response by altering different molecular networks, activating different TFs, releasing certain plant hormones from their conjugated forms, and turning on (or off) particular signal transduction networks. For setting the stage to enable an efficient, appropriate, and successful acclimation response, some overlap should occur between rapid response transcripts and transcripts activated during later stages of the plant response to stress. Indeed, as shown in Figure 2A, such overlap exists between transcripts upregulated in plants 20 s and 60 s following light stress [16], and transcripts upregulated in plants 2–8 min following light stress (S.I. Zandalinas et al., unpublished). Furthermore, some regulators of transcript expression, such as WRKY40 and Zat12, show a **triphasic transient expression pattern** during the response of plants to stress. Thus, they peak on a seconds timescale [16], then a second time on a minutes timescale [44,45], and then a third time on an hours timescale [45,46]. Such a triphasic transient expression pattern suggests that the plant transcriptomic landscape is being altered or shaped by pulses of regulatory gene expression

Glossary

Network pulse model: a hypothetical model developed based on the triphasic transient expression pattern of different TFs. It hypothesizes that, in response to a given stimulus, a network of response and acclimation and/or defense genes is activated, and that this network is orchestrated and gradually constructed by pulses of TF gene expression (e.g., triphasic expression pattern).

Rapid responses: physiological, biochemical, metabolic, and molecular responses that occur within a seconds–minutes timescale (≤ 10 min) in plants in response to different stimuli.

ROS wave: an autopropagating systemic signal that spreads from the site of stimuli to the entire plant and is characterized by enhanced production of ROS by cells along its path.

Touch genes: a group of genes that respond to an external stimuli, such as mechanical stress, within seconds to minutes. These genes were originally identified by Braam and Davis [33].
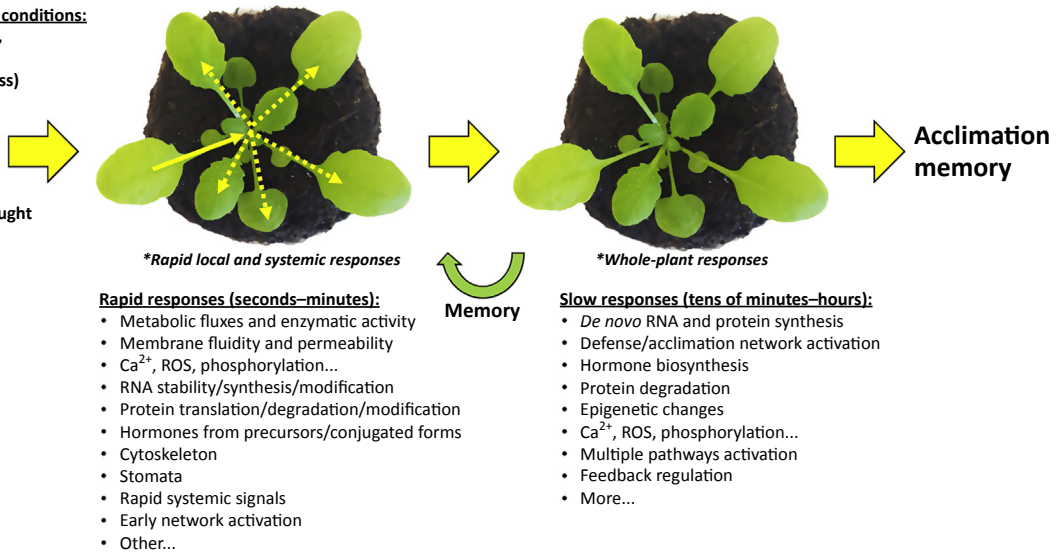
Triphasic transient expression pattern: an expression pattern identified for certain transcriptional regulators (e.g., Zat and WRKY TFs) that display three transient peaks of expression in response to a stimuli, at the seconds, minutes, and hours timescale.

Key Figure

Rapid and Long-Term Responses of Plants to Their Dynamic Environment.


Rapid changes in environmental conditions:
- **Mechanical (raindrops, insect, wind...)**
- **Flash flooding (anaerobic stress)**
- **Ambient ozone**
- **Relative humidity**
- **Light stress (sun flecks)**
- **Recovery from light stress**
- **Air temperature**
- **Recovery from prolonged drought (irrigation or rain)**
- **Osmotic/salt stress (following irrigation or rain)**
- **Herbivores**
- **Other...**

Rapid local and systemic responses

Rapid responses (seconds–minutes):
- Metabolic fluxes and enzymatic activity
- Membrane fluidity and permeability
- Ca^{2+}, ROS, phosphorylation...
- RNA stability/synthesis/modification
- Protein translation/degradation/modification
- Hormones from precursors/conjugated forms
- Cytoskeleton
- Stomata
- Rapid systemic signals
- Early network activation
- Other...

Memory

Whole-plant responses

Slow responses (tens of minutes–hours):
- *De novo* RNA and protein synthesis
- Defense/acclimation network activation
- Hormone biosynthesis
- Protein degradation
- Epigenetic changes
- Ca^{2+}, ROS, phosphorylation...
- Multiple pathways activation
- Feedback regulation
- More...

Acclimation memory

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Figure 1. Biotic and abiotic conditions within the plant environment may change within seconds and require rapid acclimation responses. Plants have evolved sensing and acclimation mechanisms that function within the seconds–minutes timescale, enabling them to respond to these changes. Rapid responses could provide protection from damage to the plant that may occur due to altered environmental conditions, as well as play a key role in the priming and modulation of slower responses (tens of minutes–hours) that follow, and the overall acclimation of the plant to its environment.

that drive and control the plant response to stress (Figure 2B; **network pulse model**). Depending on the early sensing of a particular biotic or abiotic stress by rapid responses, the plant would therefore shape an appropriate response (e.g., specific to light, injury, heat, cold, etc.) that would lead to successful acclimation. This model would apply to the initiation of a new type of stress (e.g., [16]), or the initiation of a recovery phase from stress (e.g., a shading period following light stress, or irrigation of drought-stressed plants [6,42]). The rapid response phase of plants to stress could therefore be viewed as an intermediary stage during which rapid physiological, molecular, and metabolic changes occur to immediately counter the damaging effects of the stress on the plant, prevent irreversible damage, and tune and trigger the downstream molecular, metabolic, and physiological responses that occur during the later phases of stress acclimation. Given that plants have a type of memory that enables them to withstand repeated events of stress and relief cycles [42], it is likely that a feedback mechanism from the late to the early response of plants to stress could modify or alter the early response of plants during reoccurring stress events (Figure 1). The comparison between early (20 or 60 s) to late (2–8 min) transcriptomic responses to light stress, shown in Figure 2A, further demonstrates that some early responses (20 or 60 s) are unique to this phase and do not occur during a later stage of stress response [16]. In addition to regulating metabolic, stress-response, and acclimation and/or defense pathways, the different TFs responding in a triphasic manner could also mediate stomatal responses and trigger long-distance systemic signaling pathways, conveying specificity to these responses.
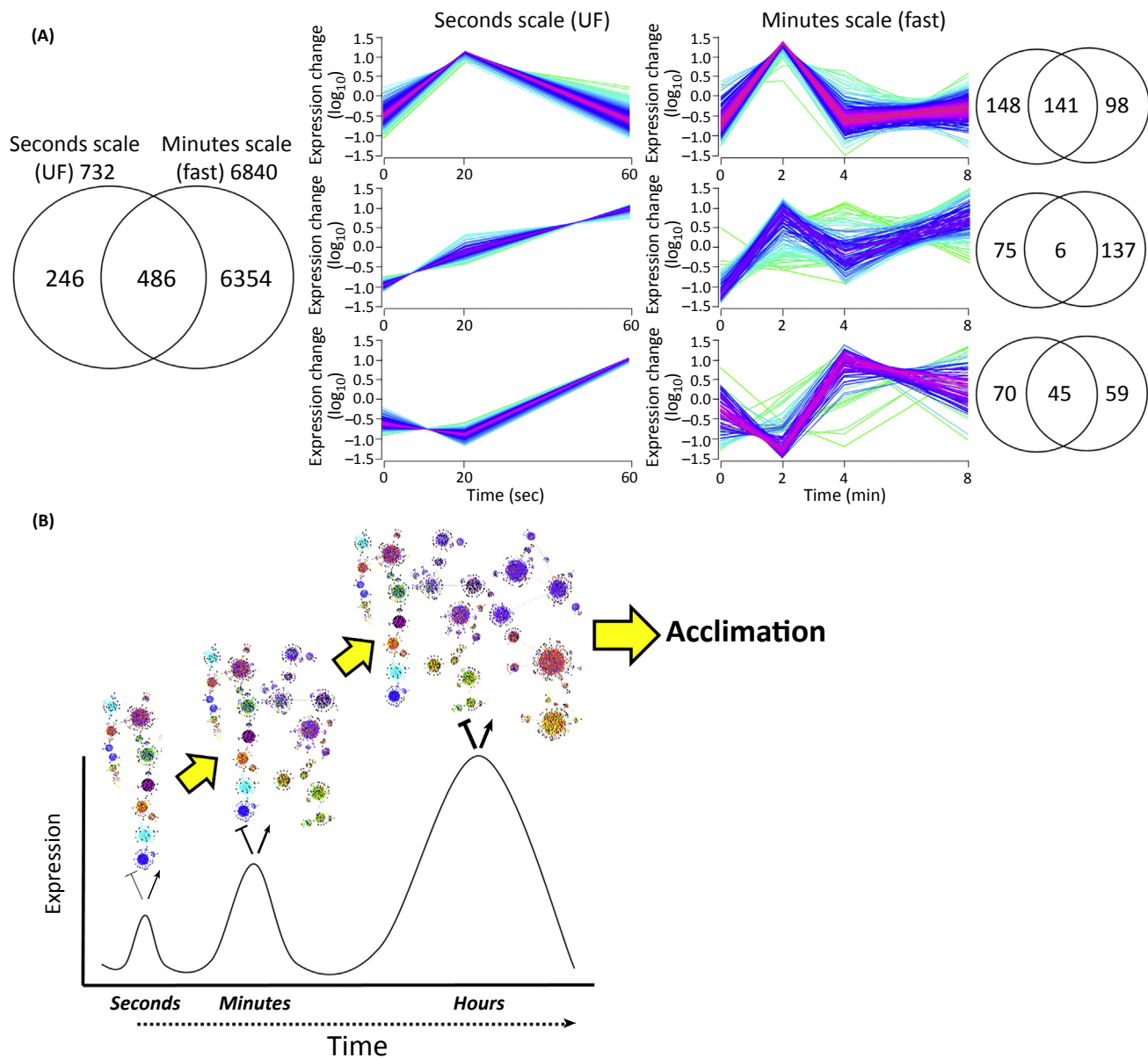
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Figure 2. How Could Early Transcriptional Responses Shape the Landscape of Plant Signaling? (A) Overlap between rapid (seconds-scale) and late (minutes-scale) transcript accumulation during responses to light stress. The kinetics of the different responses is visualized via cluster analysis and the overlap between rapid and late responses is compared between the different clusters. This meta-analysis shows that the majority of overlapping transcripts belong to the transiently accumulating transcripts that peak and then decrease in expression (to almost baseline level). Data from [16] (S.I. Zandalinas *et al.*, unpublished). (B) A triphasic expression pattern was found for several different transcriptional regulators (e.g., WRKY40 and Zat12). This pattern of expression suggests that the plant transcriptomic landscape is shaped by pulses of regulatory gene expression that configure and tune the overall response of plants to stress. The activated response network is therefore being expanded at each phase by the different regulators until acclimation is achieved. We term this regulatory behavior the 'network pulse model'.

Rapid Stomatal Responses

One of the most rapid physical reactions that land plants can use to alter their interaction with the environment is the opening or closing of stomatal pores, which is initiated within 1–2 min and completed within 5 min or more (Figure 3). Guard cells have evolved different mechanisms to sense and integrate various environmental as well as internal signals to optimize the balance between CO_2 exchange for photosynthesis and water loss via transpiration. Maintaining this balance is essential for maximizing CO_2 intake for photosynthesis while simultaneously preventing water loss. In addition to balancing CO_2 with water loss, rapid stomatal closure can prevent the entry of pathogens or air pollutants, such as ozone, into the leaf [22,47]. Rapid stomatal responses are also triggered by changes in ozone-induced extracellular ROS and ROS-induced processes, since ozone decomposes into various ROS in the apoplastic space [48].

The targets of the signaling systems that control stomatal movements are ion channels and transporters in guard cell membranes that mediate the accumulation and release of osmotically active ions, and the subsequent uptake and release of water that drives the opening and closing of stomatal pores. Stimuli-induced stomatal movements occur with a time lag, which differs between different stresses, reflecting the different signal recognition and transduction pathways responsible for initiating stomatal closure during responses to different stresses. However, these pathways converge on common components. Arguably, the most important components are the guard cell plasma membrane anion channels SLAC1, SLAH3, and QUAC1, and potassium channels that drive membrane depolarization and water efflux [49–52]. In some studies, the timing of activation of these channels has been reported. For example, the activation of anion channels, 2 min following the application of ABA, was shown to induce negative currents, which reached a maximum at 5 min [53]. This timing correlated with results from time-resolved experiments, which showed that stomatal closure has an approximately 5-min lag time after the application of ABA [54–56]. Given that anion channels are the presumed end-point in the signaling pathway initiating stomatal closure, it stands that ABA uptake into the cells, and its subsequent perception, as well as all other downstream signaling components, have already played their part within 2 min of ABA application.

Ozone is an abiotic stress that induces stomatal closure within 2 min of application, followed by reopening, whereas application of repeated ozone pulses during the reopening period does not have any further effect (Figure 3A [22]). This finding suggests that, following the initial sensing of ozone, the signaling systems become desensitized, possibly to allow transcriptional activation and reprogramming of cellular metabolism that leads to acclimation. Similarly, application of light stress-triggered stomatal closure within 1 min and was accompanied by increased expression of Zat12, a marker of systemic signaling and the activation of the ROS/Ca^{2+} wave [38]. Rapid ozone or light stress stomatal responses did not occur in plant lines that carry mutations in major stomatal regulators, such as SLAC1 [38] or OST1 [22,50], indicating that this response is not passive but dependent on active regulatory systems. CALCIUM-DEPENDENT PROTEIN KINASES (CDPKs) and the membrane-localized LRR receptor-like protein GHR1 [57] can also activate SLAC1. Activity of many kinases shown to activate SLAC1 is controlled by ABA-induced sequestration of type 2C protein phosphatases (PP2Cs) [21,58]. Key regulators of this signaling pathway did not display ozone, ROS, or light-stress-induced stomatal responses, suggesting that ABA is involved in rapid ROS-induced stomatal regulation (Figure 3B,C [22,59]). Ozone treatment triggered elevation of Ca^{2+} signals in arabidopsis seedlings within 30 s (Figure 3B [60]), which would support the importance of Ca^{2+}-dependent activation of RBOH and SLAC1 as the mechanism of rapid SLAC1 activation by ozone and/or ROS [38,52,60–64]; however, time-resolved ozone-induced Ca^{2+} elevation studies in guard cells remain to be addressed.
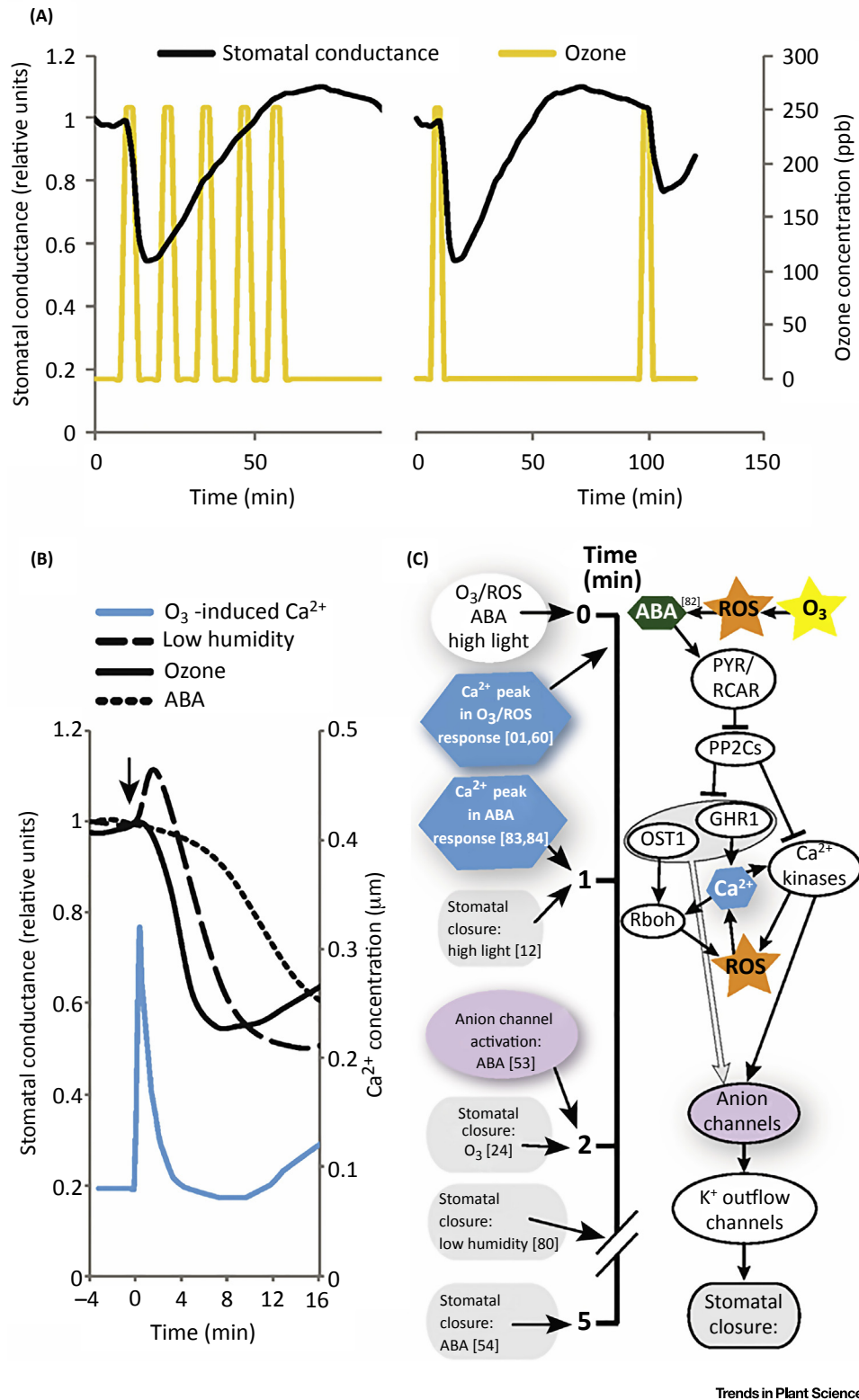
Figure 3. Regulation of Rapid Stomatal Responses in *Arabidopsis thaliana*. (A) Stomatal closure in response to a short pulse of ozone (O_3) is rapid and transient and can be reactivated only after full recovery. (B) The initiation and kinetics

*(Figure legend continued on the bottom of the next page.)*

Air humidity and temperature can drastically change during the day resulting in transient stomatal closure, (e.g., around midday) to prevent failures within the plant hydraulic system [65,66]. Water content within the intercellular air spaces is close to saturation and a dry atmosphere could give rise to steep water vapor gradients across the stomatal pore. Accordingly, a decrease in atmospheric humidity increases evaporative demand and leads to increased transpiration. As a response, plants trigger rapid stomatal closure to prevent excessive water loss. This response is termed 'passive' when it is caused by a reduction in leaf water content, and 'active' when it is controlled by the signaling systems within guard cells. Low air humidity-induced stomatal closure is evident after 4 min. However, due to the parallel occurrence of the passive response, which initially leads to stomatal opening caused by the larger loss of water from epidermal cells, and the concomitant reduction of their pressure on guard cells, it is challenging to measure the exact timing of active stomatal closure caused by a reduction in air humidity (Figure 3B [67]). In recent years, an active debate regarding the role of ABA and ABA signaling in the regulation of low air humidity-induced active stomatal closure has ensued [67–70]. Although ABA has a prominent role in controlling overall conductance of water through stomatal pores [71] and OST1 is required for rapid stomatal closure by low air humidity, recent studies demonstrated enhanced activation of OST1 in response to osmotic stress in a duodecuple mutant of ABA receptors [72], suggesting that additional mechanisms of ABA-independent activation of OST1 exist, possibly involved in stomatal responses to changes in air humidity. In addition, the retrograde signaling pathway was shown to control stomatal closure in an ABA-independent manner [27].

Several reports have demonstrated interplay between Ca^{2+} and ROS in guard cells regulation and their potential co-amplification effects on each other is a topic of ongoing discussion [73–77]. Recent developments in designing genetically encoded biosensors that follow rapid changes in Ca^{2+}, ROS, pH, and ABA [78,79] provide excellent tools for studying the spatio-temporal roles of these messengers in defining rapid stomatal responses.

Rapid Systemic Responses to Stress

In addition to orchestrating rapid acclimation and/or defense responses at the local stress-exposed level, rapid responses could be important for triggering rapid systemic signaling pathways that coordinate the overall systemic response of plants (Figure 4). For example, it was recently demonstrated that light stress applied to one arabidopsis leaf initiated a stomatal aperture response within 1–2 min in all systemic leaves. This response, which required ABA and was orchestrated by the **ROS wave**, demonstrated that plants can rapidly coordinate their rapid responses in a systemic 'whole-plant' manner [38]. In addition to stomatal responses, metabolic responses were also recently shown to propagate rapidly throughout the plant [80].

of the rapid stomatal closure response differs with respect to the different closing stimuli. Arrow indicates the time point at which the closing stimuli [reduction of relative air humidity from 70% to 30%; application of 250 ppb of O_3; spray with 5 μM abscisic acid (ABA)] were applied. Response curves were drawn based on whole-plant gas-exchange results [22,82]. O_3 induced a biphasic calcium elevation in arabidopsis seedlings, initiating a few seconds following the application of O_3 (drawn based on [60,83]). (C) A simplified timescale of events in rapid stomatal closure in response to O_3. On the left, time-resolved sequence of events [12,24,52,53,79,82,83]. On the right, the signaling components involved in stomatal closure are depicted [81]. Formation of ABA by O_3-induced oxidation of ABA precursors is shown [84]. O_3-derived ROS triggers increase in cytosolic Ca^{2+} that in turn activates Ca^{2+} kinases that activate anion channels followed by activation of potassium channels. SLAC1 can also be activated by GUARD CELL HYDROGEN PEROXIDE-RESISTANT1 (GHR1) and OPEN STOMATA1 (OST1), which, in the absence of ABA, are suppressed by Type 2C protein phosphatases (PP2Cs). ABA activates stomatal closure by inactivating PP2Cs via binding to the PYRABACTIN RESISTANCE/REGULATORY COMPONENTS OF ABA RECEPTORS (PYR/RCAR) family proteins. OST1 and GHR1 can influence production or ROS by RESPIRATORY BURTS OXIDASE HOMOLOGS (RBOHs) and Ca^{2+} elevation. It is hypothesized, that the stomatal response to O_3-derived ROS takes a shortcut via faster elevation of Ca^{2+} levels. See also [1,59].
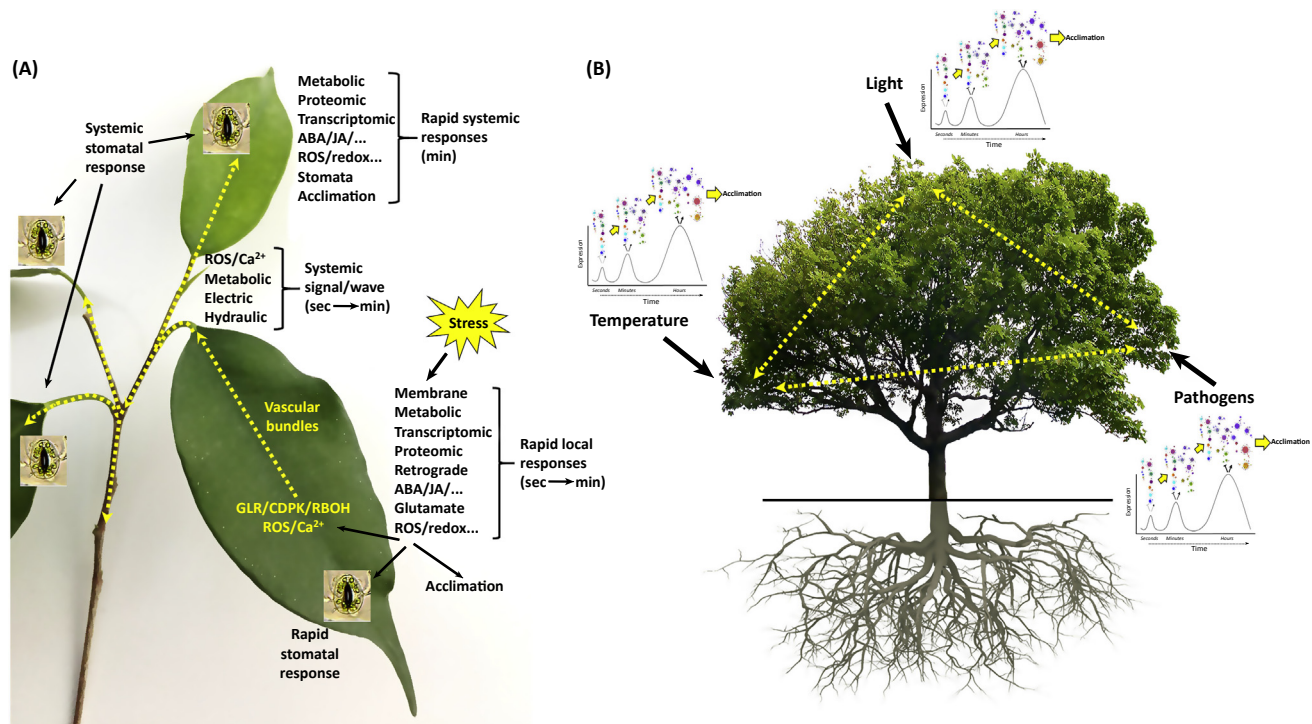
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Figure 4. Rapid Local and Systemic Responses Coordinate Whole-Plant Acclimation to Stress. (A) Rapid responses at the affected tissues and/or leaves are thought to trigger the reactive oxygen species (ROS)/Ca^{2+}/electric/hydraulic wave that rapidly propagates throughout the plant and coordinates whole-plant stomatal responses and acclimation. Key players in this pathway include RESPIRATORY BURST OXIDASE HOMOLOGS (RBOHs), GLUTAMATE RECEPTOR-LIKE (GLR), Ca^{2+}-DEPENDENT KINASE (CDPK), and different metabolic responses along the path of the propagating signal. (B) A key role for rapid responses and rapid systemic signaling pathways could be to coordinate the response of plants to different stresses that affect the plant simultaneously. Local rapid responses, the stress-specific transcription factor (TF) networks they activate, and the systemic signals they send to the other parts of the plant could coordinate and integrate responses among the different parts of the plant to different stresses. Abbreviations: ABA, abscisic acid; JA, jasmonic acid.

Many of these responses were dependent on the function of the RBOHD protein, which mediates the ROS wave, and could be detected not only at the local and systemic tissues, but also along the systemic signal path in tissues that connect the local to the systemic leaves [80]. One of the metabolites that changed rapidly in response to light stress in a systemic manner was glutamic acid. Accumulation of glutamate at the apoplast was recently shown to trigger the Ca^{2+} wave in response to wounding via the function of GLUTAMATE RECEPTOR-LIKE (GLR) proteins [13]. This finding suggests that different rapid systemic signaling pathways interact through the function of GLRs and RBOHs in response to different abiotic stresses (Figure 4A). The conduit for mediating systemic responses to light stress, wounding, or other stresses has, until recently, been the subject of much debate (e.g., [81]). At least when it comes to wounding, it was recently shown that both the Ca^{2+} wave and electric signals are propagated through the vascular system possibly via phloem and phloem companion cells [13,14]. Given that, in nature or under field conditions, plants experience different stresses, and these stresses may affect different parts or tissues of the plant in different manners, it is plausible to speculate that rapid local responses and the rapid systemic signaling pathways they activate coordinate the overall response of plants to a combination of different environmental conditions (Figure 4B). Rapidly coordinating responses between different parts or tissues of the plant is likely to enhance the

overall acclimation and ability of the plant to withstand the rapidly changing conditions within its environment. In this context it is important to note that the different pulses of gene expression involved in the local response of plant to stress (Figure 2) could be triggering different waves at different times, thereby coordinating responses to different stresses among different parts of

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Box 1. Occurrence of Transcriptomics Studies on Rapid Responses of Plants: Why We Know So Little

Transcriptomics studies that analyzed rapid responses of plants to different stimuli appear to be scarce. To examine how true this notion is, we conducted a literature search of different plant 'omics studies to identify how many of them produced transcriptomics data sets for time points that were less than 10 min following stress or stimuli application (Figure I). We identified 74 transcriptomics studies that performed a time-course analysis of plant responses to biotic, abiotic, or chemical stimuli. Of these, only five included time points that were sampled at less than 10 min following stress or stimuli application (Figure IA). In addition, we surveyed transcriptomics studies that used only one time point and examined how many of them were performed less than 10 min following stress or stimuli application. We identified 56 such studies, with none of them using a sampling point of less than 10 min following stress or treatment application (Figure IB). References for all of the studies included in our analysis can be found in Table S1 in the supplemental information online.

Our analysis highlights a need to conduct more studies on the rapid response of plants to different stresses and stimuli. As we argue in this review, these studies could reveal how plants distinguish between different stresses or treatments and how they modulate and coordinate their acclimation or defense responses. We call upon the scientific community to consider addressing these important questions to unravel many of the hidden early aspects of the plant response to stress.
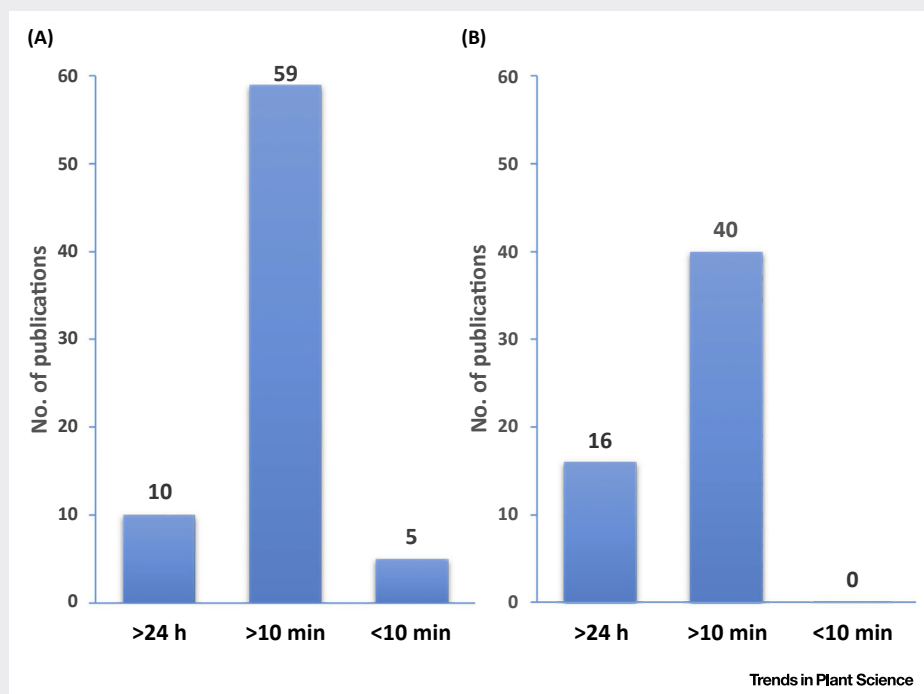

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Figure I. Occurrence of Transcriptomics Data Sets for Rapid Responses of Plants. Transcriptomics studies that used RNA-Seq or different array platforms to study the response of plants to different biotic, abiotic, or different chemical treatments were identified using PubMed searches with keywords such as 'stress; transcriptomics; plant'; or 'stress; microarray; plant'; and then manually curated. (A) Transcriptomics studies that performed a time-course analysis of plant responses to biotic; abiotic; or chemical stimuli. (B) Transcriptomics studies that used only one time point. References for all of studies included in our analysis can be found in Table S1 in the supplemental information online.

the plant. For example, if one leaf is responding to light stress and another to pathogen infection, the different pulses of gene expression triggered in each leaf in a stress-specific manner could interact with each other using waves of ROS, Ca^{2+}, and/or electric signals (Figure 4B).

Concluding Remarks and Prospects

Little is currently known about the nature and function of rapid local and systemic responses to stress in plants. In principle, this situation resulted from the lack of studies that address very early responses and the lack of early time points sampled, analyzed, and deposited in different repository databases (Box 1). We argue that studying rapid local and systemic responses to stress would lead to the identification of new and novel pathways that could be used to enhance the tolerance of crops and other plants to biotic and abiotic stresses. By finding new pathways to trigger, prime, or coordinate responses, as well as by improving the efficiency and speed at which plants respond to stress, we could improve yield and growth under adverse conditions [4]. Deciphering the 'black box' that is early and/or rapid responses to stress and understanding how early responses shape the overall plant acclimation response could also unravel new mechanisms for stress sensing, as well as new modes for regulating and coordinating transcriptional networks. In this respect it should be noted that many different genetically encoded programs and pathways could be triggered by different stimuli, such as wounding, abiotic stress, or pathogen infection, and that fine tuning them to match the exact stress encountered may involve suppressing particular networks while triggering others. For example, a rapid response to mechanical stress could be triggered in reply to a simple mechanical cut, wind pressure, pathogen infection, or attack by herbivorous insects. In addition to the common signaling pathways activated during all of these scenarios, each of them may require a different response encoded by a different set of stress-specific pathways. A decision on what pathway(s) should be activated and what pathway(s) should not, may be needed as early as possible to preserve resources and maximize the efficiency of the defense and/or acclimation response. Such a decision could be made during early stages of the response and controlled by different rapid response pathways.

Given that living organisms have evolved in the presence of fluctuating environmental conditions, it is likely that they acquired the ability to rapidly respond to these changes relatively early during evolution. Rapid responses to stress may therefore be prewired into the metabolome, proteome, transcriptome, and degradome of almost all organisms, and may even involve responses that require *de novo* synthesis of transcripts and proteins (e.g., from stalled RNA polymerases or preloaded polysomes). The study of rapid responses to stress is likely to be highly exciting and rewarding, revealing many currently unknown and unique strategies and mechanisms for stress sensing, network coordination, acclimation, and defense (also see Outstanding Questions).

Supplemental Information

Supplemental information associated with this article can be found online at https://doi.org/10.1016/j.tplants.2018.10. 003.

Outstanding Questions

What is the role of rapid response pathways in optimizing plant acclimation and defense? We know that some of the players in these responses are essential for plant acclimation to abiotic stress, but how do they function?

To what extent do rapid responses orchestrate and coordinate the different pathways activated by a given stimuli? We know that almost any given stimulus activates multiple pathways and that many of these pathways have an overlapping function between different stresses. However, some response pathways could be damaging or redundant under certain conditions and may need to be suppressed. Orchestrating and coordinating these responses at an early stage of stimuli perception would facilitate and optimize the overall defense and/or acclimation response of a plant.

How are early and late responses coordinated, and are there positive and/or negative interactions between the different pathways activated or suppressed by these responses?

How are rapid local and systemic stomatal responses mediated and how do they contribute to the overall acclimation of plants?

How are rapid local and systemic metabolic responses affecting plant acclimation and how are they linked with different molecular events and gene expression?

What was the evolutionary process that shaped rapid response pathways and coordinated between them and the late response pathways to stress or stimuli in plants?

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