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Towards a grammar of plant stress: modular signalling conveys meaning

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Abstract Stress resilience is central for plant survival. The appropriate adaptive response not only depends on the type of stress, but also on the context with other stresses, the developmental state of the plant, and the history of preceding stress experiences. The response to stress combinations cannot be a mere addition of the responses to the individual factors. For instance, heat stress requires stomatal opening to cool the leaf by increased transpiration, while drought stress needs stomatal closure to reduce water loss by transpiration. However, heat and drought are often coming in concert, such that the plant needs to reach a prioritised decision. Thus, the response to stress combinations constitutes a new quality transcending the addition of individual stress components. In other words: to survive under combined stress, plants need to render real decisions. We propose a model, where different stress inputs share one or more transducing elements, that can be recruited for different downstream pathways. Competition for these shared elements allows for such qualitative decisions, depending on the relative activities in upstream signalling of the individual stress components. Using different types of osmotic stress as paradigm I demonstrates, how signal modularity and differences in temporal sequence can generate qualitatively different outputs. Thus, plant-stress signalling makes use of a limited set of molecular players to generate, by specific rules for their combination and sequence, different "meanings". This can be compared to human language, where information-bearing elements (words) are combined according to grammatical rules to generate a semantic space. (249 words)

Keywords Drought stress · Stress signalling · Modularity · Semantics of plant signalling

1 Introduction

1.1 How plants recognise stress combinations: a conceptual question

Life is not easy. This is especially true, if you are a plant that is doomed to sessility and cannot run away, as animals often do, when they are encountering adverse conditions. Plants have no other choice, rather than sustaining internal homeostasis under the challenge of external fluctuations is a necessary condition for being alive. Thus, all organisms are endowed with means to sense such fluctuations and respond by counteracting processes. The discrepancy between external conditions and internal homeostasis can be conceptualised as stress. If the stressed organism is able to perceive the perturbation and actively respond to this by countermeasures, it will

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re-establish homeostasis and survive. Thus, stress adaptation is an active process and requires resources. If an organism fails to sense and respond swiftly enough, or if it has no command of the resources needed for these responses, it will progressively loose homeostasis and experience irreversible damage or, in extreme cases, even death, a situation that has been termed as "distress" (Selye 1973). It should be kept in mind that homeostasis in living systems, even under normal conditions, is never static, but a dynamic equilibrium of stress and ongoing adaptation. The oscillation between adaptation and stress is even needed for the well-being of an organism, a phenomenon that has been referred to as "eu-stress" (Selye 1973).

From a cybernetic point of view, stress can be described as discrepancy between an internal setpoint representing physiological homeostasis and the actual state deviating from this setpoint due to the challenges of external fluctuations. To ensure survival, an organism under stress needs to reduce or even eliminate this discrepancy.

This means that the stringency of a given stress condition is strongly depending on the recipient plant. The very same cold stress can be deathly to a genotype sensitive to chilling, while a second genotype that either is endowed with a genetically hardwired adaptation to cope or managed to increase its resilience in consequence to previous, less severe, stress conditions, might even not display any symptoms of being challenged. Because stress is a relative phenomenon, depending on two factors—the environmental situation and the physiology of the recipient plant—there are several outcomes for the encounter:

One approach would be to shift the setpoint towards the actual conditions, which means to give up homeostasis. This approach works only, when life activity is reduced to the utmost minimum, a strategy that has been termed as ana or cryptobiosis (Keilin 1959). Plants regularly resort to this strategy, when they set seeds that are able to survive adverse conditions during spread, before they actively launch germination, once they have reached favourable circumstances.

However, when the organism is to sustain life activity, there are two principal ways to do so: Run away or adapt. Animals can run away, which spares them the labour to adapt, in many cases, stress avoidance is their main strategy. Plants cannot run away. Therefore, they have to adapt. Hence, stress resilience is the core strategy for plant survival. The appropriate adaptive response depends not only on the type of stress, but also on the context with other stresses, the developmental state of the plant and the history of preceding stress experiences.

Thus, specificity and versatility are cardinal features of the plant-stress response. How are they achieved? Principally, there exist two possibilities that might act in concert:

1.1.1 Parallel signalling

Each stress input would be sensed by a separate signal transduction chain, activating the appropriate adaptive responses. For stress combinations, the overall response would be composed of the partial responses to the individual stress components. For instance, salinity stress would elicit a response that would sum up from the response to the osmotic and the response to the ionic component of salinity.

1.1.2 Combinatorial signalling

Different stress inputs would share one or more transducing elements, that can be recruited for different downstream pathways. Stress combinations would then result in mutual interaction between signalling chains that can be both, synergistic and antagonistic, depending on the effect of the shared component. With such a mechanism, the plant response to a stress combination would not be the mere addition of the responses seen for the individual stress components, but it would be qualitatively different.

The majority of studies in the field of stress physiology target on single stress factors, but those addressing stress combinations frequently find that combinations of individual stresses lead to a comprehensive response of a new quality. For instance, the responses to osmotic stress would differ when the osmotic component is accompanied by ionic stress (as it is the case in salinity) compared to the osmotic stress acting alone. For instance, a recent meta-study summarising 30 individual studies comparing combined salinity and drought stress with individual stress situations revealed a clear synergy with respect to redox challenges or growth inhibition (Angon et al. 2022). These were also of a qualitative nature. For instance, the combined stress led to an over-proportional inhibition of root growth and, thus, to a different pattern

of biomass allocation, as compared to the individual stress components. Likewise, a comparative study of the combination of heat and drought (Rizhsky et al. 2002) showed that transcripts for adaptive genes that were activated in response to the individual stress component were turned off, when this component was accompanied by a second type of stress.

2 Why this conceptual question is not esoteric, but of practical relevance

Laboratory work on plant stress usually follows a reductionist approach, focussing on single stress components as to handle complexity. To transfer the conclusions from such experiments to a real-world situation is in most cases difficult, because in the real world, mishaps come seldom alone, but in concert. For instance, heat stress (requiring stomatal opening to promote transpiration and cool the leaf) is often accompanied with drought stress (requiring stomatal closure to reduce transpiration), which means that the plant needs to prioritise responses or to deploy a completely different strategy for survival.

This gap between lab and field is reflected in a gap between analytical plant sciences and agronomical and breeding research that is difficult to bridge. Therefore, the two communities rarely communicate. The fact that most laboratory work is conducted with *Arabidopsis thaliana*, which is a great model for functional genomics, but of rather limited impact for food security, does not really help to ameliorate the situation. To reach reproducibility, molecular stress physiology is often working with systems that are simplified in order to be standardised. For instance, hydroponic systems allow to modulate a stress-factor of interest while keeping the remaining conditions constant.

However, this artificial situation can lead to results that are reproducible in the lab, but do not reflect the situation in the real world, where plants grow in soils, such that they need to respond to combinations of stresses. A very drastic example for the discrepancy is given in a comparative study on salt tolerance in barley, once in a hydroponic system, once in a field study (Tavakkoli et al. 2012). The tolerance inferred from the laboratory experiment, was even negatively correlated with the findings from the field patch, which was attributed to differences in oxygenation of the root system, but also to the presence of additional stresses, such as soil compaction or water scarcity. The reduction of experimental systems to single-factor designs is motivated by the attempt to infer causalities between the stress factor of interest and the response of the plant. However, the attempt to shut out any experimental noise can lead to situations, where minute perturbations can shift the system into a different state as illustrated by a study, where physiological parameters of *Arabidopsis* in different labs were compared and found to differ to an extent as usually imposed by stress treatments (Massonnet et al. 2010).

It might be a more robust strategy to increase the variability of the system by introducing multiple stresses, but trying to monitor those stresses as reliably as possible, such that commonalities and differences can be distilled out. To reach relevant results, extension of the system level should become mandatory (for a well-written plea for a new type of stress physiology see Plessis 2023).

In the following, I will spell out the concept of contextual stress signalling using water homeostasis as paradigm.

3 Water homeostasis as central driver of land-plant evolution

The transition to terrestrial habitats represents a key event of plant evolution requiring several achievements in combination. As for other evolutionary innovations, terrestrialisation appears to be a case of irreducible complexity, since exit from the water not only requires systems to transport water and nutrients that no longer can access the plant from all directions, but also systems of mechanical support to compensate for the loss of buoyancy, as well as protective pigments, since water no longer shields off destructive UV irradiation. Interestingly, this crucial transition did not occur only once, but several times in parallel, albeit often ending in an evolutionary dead end, because only some of the tasks mentioned above had been solved. It is worth to consider the stresses acting during terrestrialisation, because the respective adaptations were most likely passed on and, thus, are part of the adaptive repertory which can be deployed when land plants face abiotic stress.

Methodological progress has allowed to extend genomics beyond the classical model organisms, such that hitherto neglected plants could be studied. As a consequence, the transition from water to land became amenable to molecular analysis. It is now generally accepted that land plants derive from a group of freshwater algae, the Streptophytes (for review see de Vries and Archibald 2018). The lineage leading to land plants is not the only path towards terrestrialisation, though: several Streptophyte taxa have successfully acquired an amphibian lifestyle, where phases of water abundance are interspersed by phases, where the water potential of the environment is more negative than in the interior of the cells, and where, in addition, photosynthesis has to cope with abundant light, usually accompanied by the presence of UV–B, as well as more pronounced fluctuations of temperature.

In fact, the genes used in land plants to cope with these stress factors, can be found in Streptophyte algae (Fürst-Jansen et al. 2020). Whether they are integrated in the same functional context, is not fully demonstrated, yet. They might as well be convergent developments. However, what is interpreted as convergence can, upon molecular analysis, turn out to be a homology, as demonstrated by the case of the NAC transcription factors. These were known as key regulators for the development of vasculature in higher plants, but later also shown to regulate the differentiation of hydroids, water-conductive tissues in several leaf mosses and liverworts (Xu et al. 2014). Thus, although hydroids and vascular bundles seem to be convergent structures, at least at first glance, they share details of their molecular regulation.

If stress signalling is modular and generates specificity by combination, it is well conceivable that functional contexts present in the common ancestor of extant Streptophyte algae and land plants have been passed on as entire building blocks, but rewired differently. Using this rational, it is worth considering how Streptophytes can cope with the stress factors linked to a terrestrial habitat.

Since the step on land was undertaken from freshwater, salinity was obviously not a primary factor shaping this step. Instead, the transition from an isotonic, marine environment to freshwater represents a stress that had to be addressed first. Freshwater protists need to invest considerable energy to cope with their hypotonic environment, because the intracellular water potential is more negative than the ion-depleted exterior. Thus, water will enter the cell and make it swell. However, the extensibility of the plasma membrane is rather limited, less than 2% (Wolfe et al. 1986), such that the cell will burst, if it is not able to remove the excess water. Contractile vacuoles that can extrude intracellular water, has been one innovation that allowed the colonisation of freshwater habitats. The impressive complexity and diversity of water-expulsion vacuoles in different protists suggests that this innovation was achieved numerous times independently (for a classic review see Patterson 1980).

The alternative way to cope with the hypotonic challenge was the invention of an elastic cell wall that would dissipate the expansive force resulting from the expanding protoplast, such that the combination of osmotic potential and wall pressure would equal the water potential in the environment establishing the equilibrium of water influx and efflux. In fact, the transition from a motile stage requiring a contractile vacuole towards a so-called palmella, where cells are protected by a protective sheath is a common response to osmotic stress in Chlorophytes. For instance, in *Chlamydomonas* it can be triggered by signals modulating calcium channels (Bai et al. 2017). It is well conceivable that a de-regulation of this response, followed by chemical modifications of this protective sheath enabled the takeover of freshwater habitats for the streptophytes. In support of this hypothesis, the cell wall of *Penium margaritaceum*, a unicellular streptophyte has been shown to undergo specific and substantial remodelling in response to osmotic stress (Domozych et al. 2021).

Thus, the ability to adjust the extracellular matrix in response to osmotic fluctuations was likely a crucial factor for the conquest of freshwater habitats, and, subsequently, the establishment of terrestrial life forms. By secretion of polysaccharides that join with mineralic components of the surrounding into a crust-like protective layer, algae can survive even drought and heat (reviewed in Holzinger and Karsten 2013). The formation of phenolic compounds shielding against UV radiation, further supported survival—the genes required to synthetise protective phenylpropanoids have been meanwhile discovered in different lineages of streptophyte algae (de Vries et al. 2021). The first committed step for this pathway, phenyl ammonium lyase might have been acquired

by horizontal gene transfer from fungi, such that pre-existing, but silent metabolic potencies would emerge and not only allow for the synthesis of UV–absorbing flavonoids and antioxidant tannins, but also mechanically supporting and water-insulating lignins, thus, giving rise to important key players of subsequent terrestrialisation.

While these adaptations helped to sustain homeostasis under the challenge of stresses that come along with water-related stress (drought, UV–B, and high-light stress), they are costly in terms of resources and will not be sustainable when the stress continues over a long period. Especially electron transport across the inner membranes of mitochondria and chloroplasts represents the Achilles' Heel of energy metabolism. Any imbalance in the electron flow towards or from the mitochondrial complex III will result in the accumulation of free electrons that can be transferred to molecular oxygen giving rise to the reactive and, therefore, dangerous superoxide ion (Muller et al. 2004). In case of chloroplasts, it is the triplet state of chlorophyll that can have destructive consequences, if the dissipation through the plastoquinol system becomes limiting (Khorobrykh et al. 2015). In a situation, where redox balance can no longer be sustained, either because the stringency of the stress is overwhelming, or because the resources of the stressed plant become limiting, there exists an escape route: turn off energy metabolism and shift to anabiosis (Keilin 1959). In land plants, this transition is orchestrated by the phytohormone abscisic acid (ABA). The transduction machinery used to convey the ABA signal in land plants has been recently discovered in the Zygnematophyceae, an algal sister clade. This signalling acts in desiccation tolerance, but does not require ABA as ligand to become active (Sun et al. 2019). Thus, the regulatory circuits underlying drought-induced anabiosis, were already developed in some of the Streptophyte algae, and were only later shifted under control of ABA as signal, which allowed to activate this last resort in an anticipative manner, before desiccation had developed to such a degree that cellular damage ensued.

In summary, the novel findings from the Streptophyte sister clades of land plants suggest that the adaptive repertory needed for terrestrialisation was already present and used to cope with the challenges of an amphibic freshwater niche. These acquisitions must be interpreted as exaptations (in *sensu* Gould and Vrba, 1982) for a terrestrial lifestyle. It was their combination (possibly supported by horizontal gene transfer) and their shift under signal control which enabled the evolutionary breakthrough of true terrestrialisation. The adaptive repertory of true land plants has, thus, evolved from individual components that, in the algal ancestors of land plants, must have been fully functional as autonomous units. Terrestrialisation was enabled by combining these autonomous units by shared master regulators. This common legacy of land plants should have left footprints in the way, how plants perceive, process, and evaluate the different forms of water-related stress.

4 Water-related stress comes in many colours

Water homeostasis of land plants is a complex task—water scarcity (drought) does not come alone, but is often accompanied by ionic stress (salinity), or nutrient depletion (alkalinity). Survival will depend on the ability to encounter each of these stress components with a specific adaptive response.

Drought will impact cell turgidity, culminating in growth inhibition and stomatal closure which, in turn, will increase the partial pressure of oxygen and decrease the partial pressure of carbon dioxide, leading to increased photorespiration. However, stomatal closure will not only impair assimilation, but also lead to higher levels of reactive oxygen species, because the molecular oxygen from the water-splitting activity of photosystem II will accumulate and act as acceptor for free electrons that are not efficiently dissipated by the electron transport across the thylakoid membrane (for review see Bauwe et al. 2010).

Salinity can be considered as a complex stress, because, in addition to the loss of turgidity, sodium ions penetrate through Non-Selective Cation Channels in the plasma membrane and perturb ionic balance (for a classic review see Munns and Tester 2008). It should be kept in mind that salinity is not necessarily an issue of marine plants, but can also result from salt deposits in the soil, as well as from long-term irrigation leaving ionic residues due to evaporation.

The same holds true for alkalinity, a problem affecting around 1 billion hectares of agricultural land worldwide (Rao et al. 2008). In this stress type, osmotic and ionic stress are even accentuated

by a third component, the loss of the apoplastic pH homeostasis. Under physiological conditions, the apoplast is actively acidified by proton ATPases, and this activity is necessary to sustain growth. When this acidification is impaired, for instance by mutations affecting the activity of these proton ATPases (Haruta et al. 2010), plant growth will come to a halt. Therefore, alkalinity impacts plant growth more severely as compared to equivalent salinity stress (Wang et al. 2011b).

These primary water-related stresses are often accompanied by secondary water-related stresses. For instance, flooding is usually accompanied by oxygen depletion. On the other side, depletion of water from the rhizosphere, due to drought, will also impair temperature buffering provided by a more humid environment. These associate stresses are often of a complex quality. That was shown exemplarily for soil compaction, which can be experimentally dissected into mechanic, hydric, and anoxic components (de Moraes et al. 2019).

Each individual stress component requires specific adaptive responses. For instance, compatible osmolytes such as sugars, sugar alcohols, glycine betaine, or proline can help reducing the intracellular water potential (shaped by both, cytoplasm, and vacuole), sustaining turgescence and, thus, growth. However, this comes with high costs, impacting overall fitness of the plant (for a critical review see Serraj and Sinclair 2002). Thus, these cellular responses need to be supported by systemic responses, such as a rapid closure of stomata, preventing further water loss (Xoconostle-Cázares et al. 2011). Both of these responses allow to evade the impact of the stress to a certain extent. In addition, the cells can deploy truly adaptive responses maintaining functionality of proteins and membranes by protective layers, such as the Late Embryogenesis Abundant Proteins (for review see Tunnacliffe and Wise 2007).

What happens, when two stresses are combined? Will this deploy an adaptive response summing up from the adaptive responses to the individual components, or will the stress combination evoke a new quality of response? This question can be paradigmatically addressed by a comparison of drought and salinity stress. Both stress types share an osmotic component, and, in fact, certain responses seem to be similar, including stomatal closure, the induction of osmolytes, or the accumulation of Late Embryogenesis Abundant Proteins (reviewed in Hasegawa et al. 2000; Munns and Tester 2008).

However, other salinity responses are specific because they target the ionic component. These include the extrusion of sodium from the cytoplasm by the Salt Overly Sensitive 1 (SOS1) exporter, or the sequestration of sodium in the vacuole through the Na^+-H^+ Exchanger 1 (NHX1) system (Munns and Tester 2008). Vacuolar sequestration has the advantage that it lowers the intracellular water potential, such that the cell remains turgescent and sustains growth. During a comparative study between two genotypes of *Sorghum bicolor* contrasting with respect to salinity tolerance, we could show that a more efficient vacuolar sequestration of sodium in the distal elongation zone of the root not only allowed for better root elongation, but also slowed down the translocation of sodium into the transpirational stream, which allowed the leaves to anticipate the advent of sodium by metabolic buffering of photosynthesis (Abuslima et al. 2022).

Thus, the adaptive response to salinity might be understood as addition of the adaptive responses to osmotic with those to ionic adjustment. However, this conclusion is challenged by a recent meta-study comparing the effect of combined drought and salinity stress over the effects caused by the single stresses alone (Angon et al. 2022). Here, several deviations from additivity were noted, for instance, roots were affected more heavily by salinity as compared to drought, while shoots displayed comparable levels of susceptibility. On the other hand, photosynthesis was impacted more by salinity as compared to drought, while ionic homeostasis expectedly responded inversely. While these findings indicate a plant response that is qualitatively different depending on the type of stress, they remain somewhat elusive, because different plants, different conditions, and different developmental situations are compared, where it remains unclear to what extent this reduction is valid.

To pinpoint the comparison between different types of osmotic stress, it is important to adjust the individual components with respect to stringency. This aspect is rarely considered, such that the number of studies leading to a viable conclusion on additivity is rather limited. In order to create a meaningful comparison between the stress imposed by hyperosmolarity, salinity, and alkalinity, the water potential

difference (Dy) imposed by the respective stress type must be kept constant, such that the effect of the additional stressor (ionic stress in case of salinity, ionic stress and pH in case of alkalinity) can be assessed. Administering this experimental design to rice, we comprehensively monitored the physiological, biochemical and molecular responses and found that the combination of individual stress factors was not additive (Hazman et al. 2016). For some responses, the combinations (salinity, alkalinity) produced even a mitigation, if compared to the single stressors alone. Thus, the combination of the individual stress components was perceived by the plant as a stress of a new quality warranting a response that was qualitatively different and not predictable as addition of the responses elicited by the individual components.

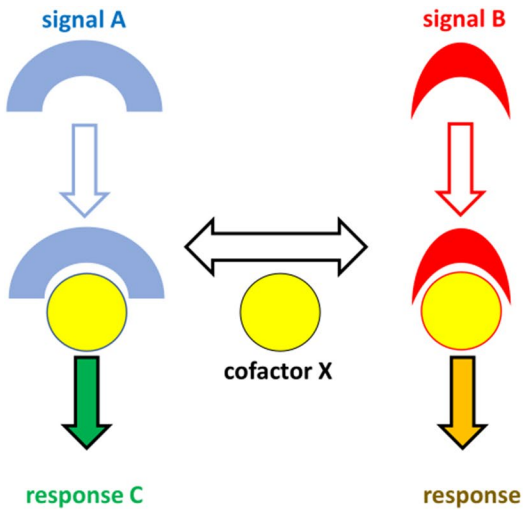
5 If complex stresses are a new quality of stress, stress signalling must render decisions

When the response to a complex stress represents a new quality, this holistic feature should be reflected in qualitative differences in signalling. This requires transitions, where gradual fluctuations of input signals are translated into all-or-none type outputs. In other words, we need to search for molecular correlates of decisions.

Formally, a decision connects two (or more) alternatives and selects one of them after comparing all of them. A decision is, thus, a highly non-linear process, where gradual inputs are translated into an all-or-none output. Translated to the realm of the molecular world, connecting two inputs means the formation of a complex (often transiently, for instance, in case of kinase cascades).

The formation of such complexes is fundamentally shaped by mathematics (Fig. 1). Signalling components are not bulk molecules, but low in abundance. Abundant molecules are not suited to be used as signals, because their information content is low. In other words: if a given signal molecule can join different complexes, these complexes compete for a limited supply of this signalling molecule, establishing a kind zero-sum game. Recruitment of the signal molecule to one complex will automatically cause sequestration of this molecule from the alternative complex, such that these complexes become mutually exclusive.

The outcome of this mutual exclusion depends on the effect, this recruitment has for the signalling activity of the respective complex (Fig. 1). For instance, if the recruited molecule is necessary for activation of both complexes, the stimulation of one pathway will lead to the inhibition of the alternative pathway leading to antagonistic signalling. However, mutual



	X positive for B	X negative for B
X positive for A	A ⇒ ¬ D B ⇒ ¬ C	A ⇒ C D B ⇒ ¬ C ¬ D
X negative for A	A ⇒ ¬ C ¬ D B ⇒ C D	A ⇒ ¬ C D B ⇒ C ¬ D

Fig. 1 Decision by competition. When two signal pathways require a common cofactor that is limited in abundance, they will interact. The type of cross-talk will depend on the role of the cofactor in the respective signalling complex. The logical matrix indicates the principle possible readouts of such an interaction. The stringency of the decision depends on the stoichiometry of the components in the complex and on the degree of modulation upon binding the cofactor

inhibition is not the only possibility. For instance, if the recruited signal acts as inhibitor in the context of complex A, while being a positive regulator in the context of complex B, initiation of complex A would block both pathways, while initiation of complex B would stimulate both pathways, leading to a synergistic interaction.

Can we find such "molecular decisions" reflected in stress signalling? While this is rarely thematised explicitly, a short browse through the literature undertaken with this, admittedly, particular viewpoint yields numerous examples, albeit the experimental designs are usually not optimal to make this point. In the following, we try to get this point crisp and clear, briefly describing four cases from our own research, where "molecular decisions" were an explicit target of the experiment.

5.1 Decide the best strategies against pathogen attack

Plant immunity is innate and, thus, principally different from the adaptive immunity found in the vertebrates (for a conceptual review see Jones and Dangl 2006). The first tier of immunity is broad-band and deploys anti-microbial compounds (called phytoalexins), but also the deposition of callose at the penetration site. These responses are targeted to protect the attacked cell and, thus, to hinder the spread of the pathogen. It is most efficient in case of necrotrophic pathogens that pursue the strategy to kill the host cell and then to extract the resources from the dead cell. This first tier of plant innate immunity is activated when conserved molecular patterns of the pathogen are recognised by host receptors localised in the plasma membrane, and is therefore called PAMP (for Pathogen-Associated Molecular Pattern) triggered immunity. Such PAMPs are usually molecular structures on the surface of the pathogen that are essential for the lifecycle. Typical examples are flagellin, the building block of bacterial flagella, or chitin, the building block of fungal cell walls. Evolutionary change towards elimination of these PAMPs to evade recognition by the host is, thus, impossible. In a second round of evolutionary interaction, some pathogens have evolved, therefore, so-called effectors that can enter the host cell and disrupt PAMP-triggered immunity, such that the pathogen can enter the host cell and extract resources, while the infected cell is still alive. This biotrophic lifestyle can only be intercepted when the host is able to recognise these effectors and deploy a second round of defence. However, these defence responses are of a different nature—they culminate in the controlled suicide of the infected cell as most efficient way to kill the pathogen that has hijacked this cell already. This Hypersensitive Reaction will stop the spread of the pathogen and, thus, protect the neighbouring cells. This means that the cell has to decide between two modes of defence—basal immunity that will mobilise responses to contain or kill the pathogen, while sustaining the viability of the attacked cell and cell-death related immunity, where the attacked cell will sacrifice itself for the sake of the other host cells. One might expect that a decision as vital or mortal as this should differ fundamentally with respect to its signalling. However, this seems not to be the case. A comparative study in grapevine cells, where both modes of immunity can be deployed by different bacterial elicitors (a conserved flagellin peptide, flg22, activating PAMP-triggered immunity, while the elicitor from a phytopathogenic bacterium activating cell-death related immunity), revealed that the early signals are the same—influx of calcium from the apoplast, generation of reactive oxygen species in the apoplast by the NADPH oxidase Respiratory burst oxidase Homologue, and the activation of a MAPK cascade followed by the activation of defence genes (Chang and Nick 2012). Only the downstream events, such as the activation of the jasmonate pathway, seen only for PAMP-triggered immunity (Chang et al. 2017), or the accumulation oxidative stilbene dimers, the viniferins, seen only for cell-death related immunity (Chang and Nick 2012), differ between these defence modes. How can the same early signals culminate in outputs of completely opposite quality? The key seems to be the timing: A rapid influx of calcium, which is followed by apoplastic burst is linked with PAMP-triggered immunity. In contrast, an early apoplastic burst that only secondarily activates calcium influx seems to herald subsequent cell-death related immunity. Thus, the decision over the resulting defence strategy depends on the temporal signature of the early signal inputs.

5.2 Decide over death and life—the actin-ROS oscillator

When the integrity of a cell is irreversibly compromised, programmed cell death can sometimes be the best option, not for the cell itself, but for the organism as an entity. But what is integrity in a situation,

where the plasma membrane is vigorously recycled with lifetimes of only a few hours and continuous addition and emission of vesicles (Steer 1988). Thus, the plasma membrane is to be considered as an activity rather than a clear border that separates inside from outside. The discovery that exosomes also occur in plant cells (for review see Cui et al. 2020), shows that the inside can even dissipate in the environment. When the membrane is not a contiguous structure, but a dynamic activity, integrity cannot be a static concept either, but must be a dynamic activity as well. Growth of plant cells depends on the polar transport of auxin and this polar flow of auxin depends on the structure of actin filaments (Maisch and Nick 2007; Kusaka et al. 2009; Nick et al. 2009) establishing a self-referring functional circuit that cannot only sustain growth, but oscillate. These oscillations can be tuned by polar auxin flow, establishing a system able to synchronise neighbouring cells and, thus, establish self-organisation (for a review on this oscillator and its implications for development see Nick 2010). When a plant cell senses a pathogen attack disrupting its membrane integrity, it must turn off this actin-auxin oscillator and initiate programmed cell death, which allows to mobilise the resources otherwise recruited for growth to sustain the surviving cells. It is possible to mimic this situation by cell-penetrating peptides such that the response of the actin filaments can be investigated (Eggenberger et al. 2017). The compromised integrity of the plasma membrane is followed by a rapid remodelling of the subtending cortical actin filaments that lose their dynamics and become depleted, accompanied by a bundling of transvacuolar actin cables and a contraction towards the nucleus. Interestingly, this actin response can be suppressed by diphenylene iodonium, an inhibitor of the membrane located NADPH oxidase Respiratory burst oxidase Homologue (RboH), which generates apoplastic superoxide, a Reactive Oxygen Species (ROS). Since the actin bundling (indirectly, through modulation of phosphorylated membrane lipids) feeds back on the activity of the NADPH oxidase, again a self-referring circuit is established. Unlike the auxin-actin oscillator, which is a driver of growth, this ROS-actin oscillator is a driver of programmed cell death. Interestingly, the actin remodelling can be quelled by additional auxin. The reason seems to be that auxin signalling requires superoxide to sustain growth and, therefore, suppresses the actin remodelling heralding programmed cell death. When integrity is impaired, the stimulation of the NADPH oxidase will generate excessive ROS that can trigger actin remodelling. Again, we have here a classical decision, where two antagonistic functional circuits (the one driven by auxin, the other driven by ROS) compete for a common factor, superoxide.

5.3 Decide on antibacterial versus antifungal defence

There is not only a decision between basal immunity and cell-death related immunity, but plant cells need also to decide on different versions of basal immunity, depending on the type of pathogen. Most bacteria can be detected by the flagellin receptor, while for fungal attacks the ability to sense chitin is central. Both PAMPs are essential for the life cycle of the pathogen, such that there are evolutionary constraints on losing them as to evade recognition. In both cases, the binding of the PAMP to the receptor deploys a signal chain. Interestingly, the signal is conveyed by the same molecular events (for review see Boller and He 2009). A rapid inflow of calcium is read out by calcium-binding proteins that convey the signal to a cascade of (Mitogen Activating Phosphorylase) (MAP)-kinases that pass on the signal through a phosphorylation cascade into the nucleus, where a cascade of transcription factors receives the signal and translates it into activation of defence genes. Some of these responses are of a generic nature and act against different pathogens. For instance, in grapevine, both, the bacterial elicitor flg22 (Chang and Nick 2012) and the fungal elicitor chitosan (Sofi et al. 2023) activate genes encoding enzymes converting the amino acid phenylalanine into stilbenes, phenolic defence compounds (phytoalexins) that can kill both, bacteria and fungi. However, some responses are specific for the respective pathogen. For instance, chitinase degrading chitin, the building block of fungal cell walls, will severely impact an attacking fungus, because its cell wall becomes perforated, such that it will die. However, chitinase will not be an efficient way to ward off a bacterial attack. This leads to a tricky question: How does the attacked grapevine "know" whether it is confronted with a pathogenic bacterium or with a fungus? Although, there are different receptors, most of the signals deployed in response to the binding of ligand are identical. How can the cell assure specificity under these circumstances?

A surprising observation helped to solve this enigma: While it is true that both elicitors cause a rapid calcium influx, this calcium influx has a completely different "meaning". This becomes manifest, when the inflow of calcium is inhibited, which can be achieved by Lanthanoid metals, such as Gadolinium. In case of the bacterial flagellin, Gadolinium ions block the induction of phytoalexin synthesis genes. In case of the fungal chitosan, the induction of the same genes is boosted. This appears to be paradox. To solve a paradox, usually requires the introduction of a second factor. This second factor is the co-receptor Chitin Elicitor Receptor Kinase 1 (CERK1) that can shuttle between the flagellin and the chitin receptor (Gong et al. 2019). Since the abundance of a receptor, such as CERK1, is limited, the two receptors compete for this limited factor, establishing a molecular mechanism to render decision. In a complex with the chitin receptor CERK1 deploys a second signalling cascade that will modify the response of downstream recipients of the calcium signal in a modular manner (Fig. 2). Some events that would be activated by a bacterial elicitor, will now be quelled, while others, quelled by a bacterial elicitor will become deregulated. Those events needed for shared responses required for the defence of both pathogen types, will proceed unchanged.

Again, there seems to be a "grammar", where a signal changes its "meaning" depending on the presence of a second signal.

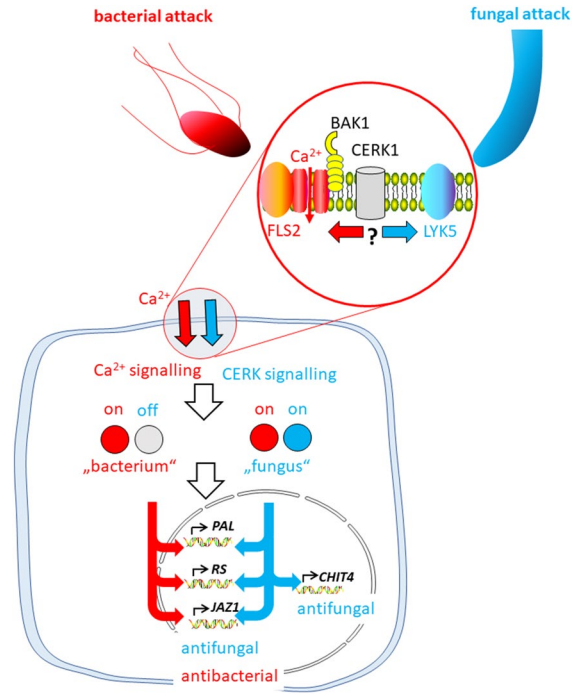


Fig. 2 Decision on the quality of defence against bacterial versus fungal attack by mutual competition of the two receptors FLS2 (binding bacterial flagellin) and LYK5 (binding fungal chitin) for the coreceptor CERK1. The recruitment decides about the "meaning" of calcium influx for defence signalling leading to partially overlapping, partially contrasting gene expression (according to Sofi et al., 2022). FLS2 Flagellin Sensitive 2, LYK Lysine-motif rich receptor-like Kinase, PAL Phenylammonium lyase, RS Resveratrol synthase, JAZ1 Jasmonate ZIM domain protein 1, CERK1 Chitin Elicitor Receptor Kinase 1, BAK1 Brassinosteroid insensitive Associated Kinase 1

5.4 Jasmonate signatures and adaptation to salinity

Salinity is a progressive challenge to agriculture, accentuated by climate change, because rising sea levels cause leakage of salt water into ground water, and because increased evaporation in irrigated fields leaves ions in the soil. The primary organ facing salinity are the roots, but ions reaching the central cylinder will reach the leaves and perturb photosynthetic electron transport leading to considerable oxidative stress. Thus, as to safeguard the upper parts of the plant against the ensuing challenge, signalling of the root to the shoot is crucial. A comparative study on genotypes of *Sorghum bicolor* contrasting with respect to salt tolerance showed that efficient systemic signalling from root to shoot correlates with a more robust redox homeostasis buffering the challenges of ionic stress (Abuslima et al. 2022). On the cellular level, contrasting responses can be observed—in some cases, the cell is trying to adapt, for instance, by sequestering ions in the vacuole through the tonoplast-located transporter NHX1 (Ismail et al. 2012; Abuslima et al. 2022), while in other cases, the challenged cell decides to undergo necrosis. This might appear as a form of unspecific damage, but can be of adaptive nature, for instance, when certain organs sequester sodium and remove the challenge by abscission, while young tissue is protected and can initiate regeneration, once the stress episode has eased off. In fact, the decision between these contrasting responses seems to depend on the temporal signature of jasmonate signalling as concluded from comparative time-course studies in grapevine cells contrasting with respect to their salinity response (Ismail et al.

2012). A swift, but transient activation of jasmonate signalling is a hallmark for cellular adaptation for salinity, while a slugglish, but continuous jasmonate signalling heralds subsequent necrosis. Why should a changed temporal signature of the very same molecules lead to so contrasting outputs? The reason is that, due to the temporal shift, jasmonate signalling cross-talks with different signals (mainly the events deployed by calcium and reactive oxygen species), culminating in different responses (for review see Ismail et al. 2014). In fact, jasmonate signalling is endowed with at least two control mechanisms that ensure a transient activity. On the one hand, the active signal, the isoleucine conjugate of jasmonic acid (JA-Ile), rapidly activates Jasmonate ZIM Domain (JAZ) proteins. These are negative regulators of transcriptional activation through jasmonates and are rapidly degraded proteolytically upon binding of JA-Ile (for review see Wasternack and Song 2017). Activation of jasmonate signalling will, therefore, rapidly deploy the formation of a negative regulator for this signalling leading to a transient signature of activity. However, also the activating signal, JA-Ile as well as its precursors, are subjected to tight regulation, by efficient catabolism (for review see Heitz et al. 2016).

Is it possible to provide functional proof beyond a hypothetical framework and correlative evidence linking different temporal patterns with different readouts? This would require control over temporal signatures of jasmonate signalling, which is far from trivial. As proof of concept, we engineered a rice plant, where the salinity specific JAZ8 was truncated from the domain mediating the proteolytic decay and placed it under control of the salt-inducible Salt Tolerance Zinc Finger 11 (STZ11) promoter (Peethambaran et al. 2018). In wildtype rice, salinity deploys the constitutive accumulation of jasmonates This accumulation of jasmonates is responsible for salt-induced necrosis, as demonstrated by the fact that mutants, where expression of the jasmonate synthesis gene *Allenoxide cyclase* is disrupted, such that jasmonates cannot accumulate, can cope better under salinity (Hazman et al. 2015). The engineered construct described above allows to obtain a persistent activity of the negative regulator JAZ8 (which cannot be degraded proteolytically), such that even under the persistent supply with JA-Ile salt-induced jasmonate responses remain silent. In fact, these transgenic plants perform significantly better under salt stress as compared to the wild type. Since the promoter is specifically induced under salinity, the system remains silent in the absence of salinity minimising side effects.

These data show that beyond the correlation between transient jasmonate signatures and adaptation, it is possible to obtain adaptation by artificially engineering such transient signatures.

As exemplarily shown in this section, the same molecular players can lead to qualitatively different readouts depending on their temporal patterns (decision between basal immunity versus cell-death related immunity depending on the temporal relationship between calcium influx and apoplastic oxidative burst; decision between salt adaptation versus salt necrosis depending on the temporal signature of jasmonate signalling), or depending on their coincidence with other signals (decision between bacterial and fungal defence depending on meaning of calcium influx caused by mutual competition for the co-receptor CERK1). In the next step, we will apply this concept to water-related stress. This will require to introduce a further concept, the so-called susception.

6 How to sense physical stresses—the susceptor concept

Unlike a pathogen attack that is sensed by receptors binding a pathogen-related molecule and deploying chemical signal cascades, water-related stresses are physical signals and need first to be translated into something that can be transduced by chemical signalling. This translation step has been termed *susception* (Björkman 1988) to distinguish it from receptors that bind ligands. For instance, the sedimenting amyloplasts in gravity sensing are not perceiving anything, but they translate gravity into force that can be sensed by mechanosensitive ion channels. Physical signalling is, thus, bipartite, because the actual perceptive structure must act in concert with a susceptor. Water-related stress is mechanic load on the plasma membrane in the first place, because it always contains an osmotic component. This mechanic load is minute, however, and must be amplified to cross the threshold of thermal noise. This amplification can be achieved by focussing the minute mechanic forces along anisotropic structures endowed with sufficient stiffness. Cortical microtubules qualify as force focussing

structure, since they are fairly rigid (coming close to class in terms of Young's modulus) and can pass on force efficiently as shown by experiments where vibrations were imposed on beads coupled by microtubules using optical tweezers (Koch et al. 2017). These forces open calcium channels. Relevant candidates are cyclic nucleotide-gated channels (reviewed in Dietrich et al. 2020), or REDUCED HYPEROSMOLALITY INDUCED-[Ca^{2+}] INCREASE 1 (OSCA1), shown to import calcium from the apoplast to the cytoplasm in response to osmotic stress (Yuan et al. 2014). Calcium influx from internal stores contributes as well. An example is the release of calcium from the ER into the cytoplasm through the calcium/cation transporter (CCX2) that has been shown to be essential for salinity tolerance using loss-of-function and overexpression lines in *Arabidopsis thaliana* (Corso et al. 2018).

The susceptor concept bears on the resulting signalling. Numerous stresses harbour a mechanic component. This includes not only different forms of water-related stress, but also direct mechanic perturbations, such as soil compaction, wounding, wind, as well as stresses causing changes in the fluidity of membranes, such as cold or heat stress. To conceive separate, parallel signal-transduction chains for these stresses is not compatible with a role of microtubules as common susceptor for these stresses. In case of water-related stresses, microtubules seem to interact with phospholipase D, a central signalling hub. For hyperosmotic stress in durum wheat, a drought tolerant crop, microtubules are transiently eliminated, but subsequently replaced by bundled arrays, so called macrotubules (Komis et al. 2002). This microtubule response is necessary for successful adaptation because persistent elimination of microtubules interferes with osmotic adjustment. Treatment with *n*-butanol, consuming phosphatidic acids generated by phospholipase D can block both, macrotubules and osmotic adaptation (Komis et al. 2006). A similar, transient, eclipse of microtubules, followed by the formation of bundled and persistent arrays, has been shown for the adaptation to cold stress as well (Abdrakhamanova et al. 2003), again depending on phospholipase D (Wang and Nick 2017; Zhang et al. 2022). A specific isotype of this enzyme, PLD1a, interacts with detyrosinated a-tubulin (Zhang et al. 2021). The link between the two stress types might be mechanic load on the plasma membrane. In case of hyperosmotic challenge, the membrane is experiencing a drop in turgor pressure, in case of cold stress, it is the drop in fluidity, which acts as primary input (reviewed in Wang et al. 2020). For both types of stress, microtubules as susceptors interact with phospholipase D as element of perception itself. Due to feedback on microtubules themselves, even minute mechanic stimuli can be amplified efficiently (for a mechanistic spell-out see Wang et al. 2020).

Along similar lines, phosphatidic acid formed in response to salt stress promotes binding of the microtubule-stabilising protein Microtubule Associated Protein 65 (MAP65) to microtubules (Zhang et al. 2012), which allows them to re-organise a stable array subsequent to disassembly caused by salt-dependent detachment and proteolytic decay of the microtubule-stabilising protein SPIRAL1 (Wang et al. 2011b).

In the summary, microtubules as susceptors are shared by several qualities of water-related stress. This susception deploys signalling second messengers such as the phospholipase D system. This signalling feeds back on microtubules, becoming manifest as transient disappearance, followed by more stable, often bundled arrays of microtubules. Different qualities of water-related stress share this susceptor because they also share a mechanic component (changed mechanic load upon the plasma membrane). Thus, the modularity of a stress quality (mechanic load is modular element integral not only to osmotic stress, but also salinity, alkalinity, low temperature, possibly also heat) might be reflected in the modularity of early signalling, here, by making use of a common susceptor-perception system (microtubules and phospholipase D).

7 Combinatorial signalling in water-related stress: let us spell it out

Microtubules act as susceptors for stress qualities that bring changes in mechanic load of the membrane. This holds true for a large variety of stresses. In addition to obvious inputs, such as touch or attachment of pathogens, as well as changes of orientation with respect to gravity, these include also stress qualities where the mechanic component is less obvious. For instance, hyperosmotic stress means a loss of turgor pressure and, thus, leads to a reduction of membrane tension. Likewise, chilling does not appear to be a

straightforward mechanic stress. However, the rigidification of the membrane leads to mechanic stress because the membrane is regionally subdivided into different patches that will rigidify at different pace. The minute forces barely cross the threshold of thermal noise, but microtubules associated with the membrane can, due to their rigidity transmit and focus forces, such that they are able to activate calcium-influx channels (for review see Nick 2011).

Microtubules are not the only susceptor, though. As pointed out above, also actin filaments play a sensory role. Here, it is not mechanic load, but membrane integrity serving as input. The actual signal might be the leaking of reactive oxygen species from the apoplast into the cytoplasm (Eggenberger et al. 2017). While a certain import of superoxide is needed to sustain the dynamic turnover of subcortical actin as driver of growth, excessive superoxide will interfere with actin-associated proteins and also elicit actin modification culminating in a retraction of actin monomers from the cortex subtending the plasma membrane and bundling of perinuclear actin cables. This actin response heralds ensuing programmed cell death, which will remove the cell, whose membrane integrity has been irreversibly compromised, from the tissue after it has mobilised its resources for the sake of the neighbouring cells.

Mechanic load and membrane integrity are common inputs for a wide range of stresses: Membrane rigidification alone can indicate cold stress—in fact, it is possible to evoke a part of cold-induced signalling by DMSO, a membrane rigidifier (Wang and Nick 2017). Loss of membrane integrity by ice crystals is added upon the mechanic load from membrane rigidification and leads to a qualitatively different cellular response as compared to mere chilling stress (Shi et al. 2022). Conversely, heat stress will result in fluidisation of the membrane, which will lead to a microtubular response as well. In fact, benzyl alcohol, a membrane fluidiser, can act as negative regulator of cold-induced signalling (Wang and Nick 2017). Activation of basal immunity goes along with membrane rigidification as well linked with formation of so called nanodomains (Bücherl et al. 2017). In fact, it is possible to elicit several responses of basal immunity by rendering the membrane stiffer with Dimethylsulfoxide (Nick et al. 2021). In case of cell-death related immunity, the mechanic load suscepted by microtubules, is accompanied by a loss of membrane integrity, requiring the activity of the NADPH oxidase Respiratory burst oxidase Homologue in the plasma membrane activating actin remodelling (Chang et al. 2015). Hyperosmotic stress leads to a sudden relaxation of membrane tension followed by microtubular breakdown and reformation of macrotubules (Komis et al. 2002). In case of salinity, this osmotic stress is accompanied by the entrance of sodium ions that will perturb electron transport in mitochondria and plastids causing the accumulation of reactive oxygen species as reporters for impaired membrane integrity (Asfaw et al. 2020), which will trigger retrograde signalling to the nucleus to activate salvage by boosting the expression of antioxidant enzymes such as Superoxide Dismutase, or, alternatively, initiate salt-dependent necrosis. Touch, wind, but also mechanic load due to gravity, can be sensed by microtubules as well (Nick et al. 1991). In case of wounding, mechanic load is, again, accompanied by a loss of membrane integrity.

Summarising, from the viewpoint of a plant cell under stress, different challenges can be perceived by combinations of mechanic challenge and compromised membrane integrity (Fig. 3). The mechanic challenge can be sensed by virtue of the microtubule susceptor, linked with the innate rigidity of microtubules allowing for efficient transmission of mechanic input (Koch et al. 2017). Compromised membrane integrity can be sensed by virtue of the ROS-actin susceptor (Eggenberger et al. 2017). Both susceptive systems are endowed with efficient self-amplification. Microtubule disassembly in response to mechanic input is amplified by the calcium influx amplified by this disassembly (Wang et al. 2020). Actin remodelling in response to impaired membrane integrity is amplified by differential sequestering of actin-associated proteins that becomes shifted in response to remodelling (Eggenberger et al. 2017).

However, when so many different stressors use the same susceptive input for sensing, the issue of specificity remains a pertinent issue. In the following, I will point out that specificity can be achieved by virtue of two factors: timing and combination.

8 How time and context give meaning

The practice to visualise signalling processes in form of static images helps to predict testable implications

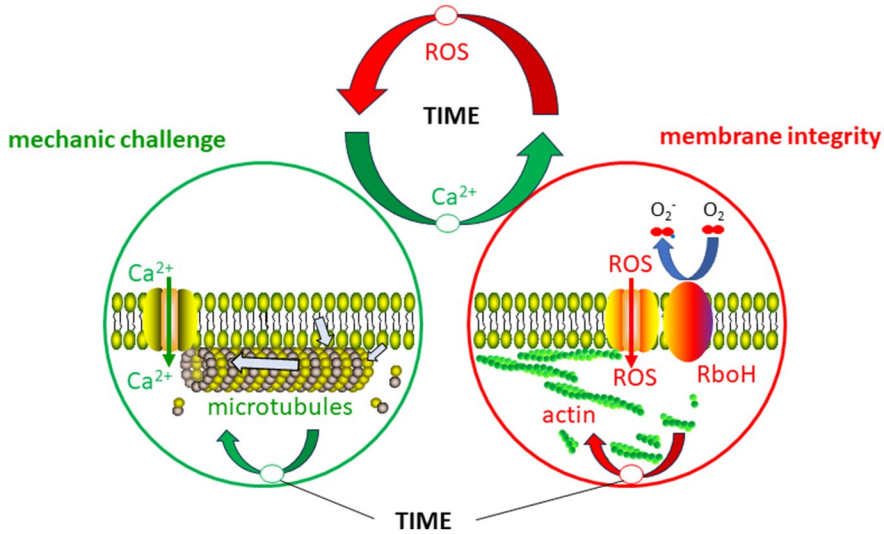


Fig. 3 Modular model of stress signalling based on two susceptor-perception systems. One involves microtubules and calcium-influx channels and can sense mechanic challenge of the membrane, the second is based on cortical actin filaments and the NADPH oxidase Respiratory burst oxidase Homologue (RboH) and can sense compromised membrane integrity. Both systems are interconnected because the activity of RboH depends on calcium, while the activity of calcium channels depends on apoplastic Reactive Oxygen Species (ROS). The susceptive role of microtubules and actin filaments depends on their innate turnover such that the readout of the two systems is strongly depending on time

of the model, but comes with a cost. Signalling is depending on change. The usual graphically depicted network are therefore not even snapshots, but projected compositions of snapshots taken at different time points. Images shape our thoughts, still images inspire static concepts. Keeping this in mind, we should question the modular model depicted above (Fig. 3) by focussing on two central elements of change:

1. The two inputs are interdependent, and this interdependence requires a certain time to proceed. Thus, if one of the two susceptor systems is activated, this will, after a certain delay, modulate the activation of the second susceptor as well, leading to distinct temporal signatures of the resulting signalling output. The activity of a given susceptor will, therefore, depend on the activity of the second susceptor, creating a situation, where transduction of a given signal will depend on the *context* of signalling.
2. The cytoskeleton, as susceptor, is not static either, but continuously turning over by addition of building blocks at the growing end and release of building blocks. This release can happen at the opposite end establishing treadmilling, or also by a phase change at the hitherto growing end itself that will now turn into a shrinking end. Cytoskeletal turnover depends on interaction with steering proteins, binding to organelles, and also on post-translational modification of the building blocks themselves. All three interactions are determined, at least partially, by the degree of cytoskeletal dynamics itself, establishing a feedback loop. Thus, the susceptive function of microtubules and actin filaments integrates not only the actual status of signalling, but also the preceding signalling activities, which is nothing else than the *history* of signalling. In other words, the susceptor has an innate *memory* that will shape, how it responds to a current input.

The impact of *context* and *memory* on stress signalling need to be spelled out for each stress condition individually. This would certainly go beyond the scope of a contribution to this special issue. However, in the following, I will paradigmatically explain some of the central mechanisms at work here:

There is a mutual interdependence of the two input channels: On the one hand calcium influx channels

are activated by apoplastic ROS (for review see Mori and Schroeder 2004). Calcium influx can be monitored by apoplastic alkalinisation caused by co-transport of protons (Felix et al. 1993). While addition of the bacterial elicitor flg22 activates the pH shift immediately, the bacterial elicitor harpin, which activates RboH, activates this phenomenon with a delay of ~5 min (Chang and Nick 2012), consistent with the notion that RboH first needs to generate superoxide, which has to be converted to hydrogen peroxide and reach the calcium channel by diffusion, before alkalinisation becomes detectable. However, activation of calcium will also generate feedback to RboH via activation of phospholipase D (for review see Selvy et al. 2011). The resulting phosphatidic acids can recruit the small GTPase Ras-related C3 botulinum toxin substrate (Rac) for RboH leading to its stimulation (Wong et al. 2007). Again, there is a delay of ~10 min, when the accumulation of intracellular superoxide is followed after direct activation of RboH by harpin as compared to indirect activation through flg22 (Chang and Nick 2012). Thus, if any of the two susceptor-perceptor systems is activated, it will initially (for around 5–10 min) trigger signalling alone, before the second input kicks in. At this time, signalling has already moved into the later tiers of transduction, including ROS-dependent elements of MAPK signaling (for review see Jalmi and Sinha 2015) or calcium-dependent kinases (for review see Ravi et al. 2023). This signalling involves numerous complexes with components that can either accelerate or intercept the signal. As spelled out in Fig. 1, this can easily lead to sign reversals of activity, depending on whether the respective step is active in the early phase (where it is governed by only one of the susceptor-perception inputs) versus the late phase of (where the second input is deployed in addition).

The impact of susceptor dynamics can be illustrated using post-translational modification of tubulin as example. The highly conserved tyrosine at the C-terminus of all eukaryotic a-tubulins can be cleaved off and re-ligated enzymatically. The de-tyrosinated tubulin accumulates in stable microtubules, where turnover is low, while dynamic microtubules are rich in the tyrosinated full-length form of a-tubulin. The relative abundance of the two tubulin pools can, therefore, be used as proxy for tubulin turnover. For instance, the dynamic microtubules that need to disassemble in response to cold stress in order to deploy efficient adaptation to cold stress are highly tyrosinated (Abdrakhamanova et al. 2003), while the stable microtubules that replace them at a later stage, are de-tyrosinated. The de-tyrosinated tubulin can bind to phospholipase D (Zhang et al. 2022), whose product, phosphatidic acids, can activate MAP65-1 (Zhang et al. 2012). This microtubule-associated protein is stabilising microtubules and binds to the C-terminus of a-tubulin itself (Wicker-Planquart et al. 2004). This target is altered by the de-tyrosinating enzyme, such that MAP65-1 will render dynamic (tyrosinated) microtubules stable and, thus, prone, to become de-tyrosinated, which will strengthen their interaction with phospholipase D. This circuit will, therefore, replace the initially dynamic, sensory, microtubules by stable, structural microtubules. There is a slower, second circuit that is antagonistic: microtubules rich in de-tyrosinated a-tubulin can increase the stability of transcripts for tubulin, such that the pool of tyrosinated tubulin will be replenished after some time (Zhang et al. 2022). Thus, the susceptor function of microtubules during cold sensing is not a natural constant, but changes over time, with a short-term transient due to post-translational modification, followed by a long-term mitigation due to post-transcriptional regulation of tubulin expression. The fact that a similar transient elimination of microtubules, followed by the formation of stable "macrotubules" is observed also under salinity, indicates that for this stress type, too, similar mechanisms are at work.

9 Conclusions

How to cope with adverse environmental conditions is a central question of plant survival. It is also a central challenge for humans that need to adjust their agriculture to the rapidly deteriorating conditions due to climate change, which is a consequence of their own actions. The molecular mechanisms underlying stress signalling and adaptation have been mostly dissected using highly standardised laboratory conditions, usually in *Arabidopsis thaliana*. It is questionable, to what extent the results can be transferred to the real world, first, because this model plant differs considerably from most crops, second, because stress conditions in the real world come usually as combinations. Using different forms of water-related stress, I show paradigmatically that combined stresses are to

be seen as new qualities of stress that cannot merely derived from the addition of the individual components. Using an evolutionary approach, I propose that stress signalling is modular, whereby different signalling chains interact by competing for shared signalling elements, leading to highly- non-linear outcomes, which represent nothing else than fully-fledged decisions, where adaptation to one stress can be prioritised over those towards a concurrent stress, depending on the context (presence of other stress factors, temporal patterns of stress, developmental state of the plant). The meaning of the individual signals depends on their temporal patterns, as well as on their temporal sequence to other signal components. This concept of modularity and time is then illustrated with examples from abiotic and biotic stress responses. Broken down to their cellular impact, many stresses can be categorised as mechanic load on the membrane and perturbed membrane integrity. These primary inputs can be sensed by the cytoskeleton—the rigid microtubules can perceive minute tensions of the membrane in consequence of physical challenges such as force or temperature-dependent changes of fluidity. The tensile actin filaments subtend the membrane and can re-modell in response to reactive oxygen species generated by the membrane-located NADPH oxidase Respiratory burst oxidase Homologue and leaking into the cytoplasm when membrane integrity is perturbed. I propose a model, where the primary components of stress (mechanic strain of the membrane, impaired membrane integrity) are perceived, integrated in space and time, and transduced to parallel, but interconnected signalling chains that induce immediate compensatory responses that are later stabilised by expression of adaptive genes.

10 Perspective: why deciphering the stress grammar requires new experiments

The advent of high-throughput technology that allows to collect all transcripts, proteins, or metabolites present at a particular time point under a particular condition has allowed unprecedented abundance of molecular data. However, this data foraging has not necessarily led to corresponding insight. The reason may be that concepts do not crystallise from the primordial soup of abundant data by themselves, but need to be pulled out from this soup by asking questions that are guided by working models. If one browses reviews on stress signalling, one will encounter many examples of apparently discrepant data. In some studies, a given hormone promotes resilience, in others it impairs resilience. The problems do not arise from problems with the experiment, or even fabrication of data. They rather arise from lacking or superficial concepts used to interpret this abundance of data. In addition to comparing cases that are not really comparable. For instance, the attempt to extract commonalities between the cold signalling in a species that is freezing tolerant (such as *Arabidopsis thaliana*) with that in a species that is chilling sensitive (such as rice) will rarely promote our insight into mechanisms, but only contribute to confusion, because two stress qualities are compared that are qualitatively different (for a detailed discussion see Wang et al. 2020).

A second drawback, however, is the lack of timing. The methodological sophistication of -omics comes with a price. It is expensive and laborious to construct a transcriptome, a proteome, or a metabolome. Therefore, it is usually done for particular conditions and time points yielding snapshots rather than movies. This is inevitable, but becomes problematic, when one forgets that this is just a snapshot. As I have tried to work out in this review, specificity is less embodied in the molecular nature of molecules, but in the dynamics of their genesis and decay, and in the dynamics of their interactions.

If we want to truly understand stress signalling, we must strive to monitor its temporal signatures. The potential of non-invasive monitoring of stress signals and phenomena is still mostly untapped, but will be crucial to detect signatures. Monitoring is not sufficient, though. As to test implications from models on signature models, we need methodology to impose stress in defined signatures, or to interfere with natural signatures of stress signals. Targets for such approaches might be promoters driving the expression of components involved in activation or deactivation of signals (for instance catabolic enzymes for phytohormones), but also tools to modulate the activity of proteins directly. These tools should be as non-invasive as possible—optogenetics might offer here fascinating options.

In addition to temporal signatures, spatial patterns are relevant. Many stresses can, in addition to local responses of the challenged organ itself, induce

systemic signals that will activate adaptation in other parts of the plants before they are challenged directly. Systemic responses have been studied intensively in the context of pathogen or herbivor attack, but have not acquired the same degree of attention in abiotic stresses. They are of relevance, though. For instance, during a comparative study on salinity responses in *Sorghum bicolor*, we were able to demonstrate that adaptive responses in the leaves of the resilient genotype initiated at a time point, where the actual ionic challenge had not even reached the shoot (Abuslima et al. 2022). To elucidate the molecular or physical nature of the underlying systemic signals represents a rewarding question for future research.

In addition to these methodological and conceptual advances, we need advances in the conceptual framework. Current research in plant stress is dominated by reductionist studies on model organisms mostly based on molecular genetics, for instance using genetically engineered mutants, whose responses are then mapped in great detail by high-throughput approaches. We need a renaissance of physiology, both in our methodology as well as in our thinking. Physiology is the science of timing in the first place. When we are able to bring time back into biology, we should be able to reach a new level of understanding that will also help us to approach the tasks of the real world, such as rendering our agriculture resilient to climate change.

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Conflict of interest The author confirms that there is no conflict of interest whatsoever.

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