



Research review paper

Molecular priming as an approach to induce tolerance against abiotic and oxidative stresses in crop plants

Pavel Kerchev^{a,b}, Tom van der Meer^{c,d}, Neerakkal Sujeeth^e, Arno Verlee^f, Christian V. Stevens^f, Frank Van Breusegem^{c,d}, Tsanko Gechev^{g,h,*}

^a Department of Molecular Biology and Radiobiology, Faculty of AgriSciences, Mendel University in Brno, 613 00 Brno, Czech Republic

^b Phytophthora Research Centre, Faculty of AgriSciences, Mendel University in Brno, 613 00 Brno, Czech Republic

^c Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052 Ghent, Belgium

^d Centre for Plant Systems Biology, VIB, 9052 Ghent, Belgium

^e BioAtlantis Ltd., Tralee, Co., Kerry V92RWV5, Ireland

^f Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, 9000 Ghent, Belgium

^g Department of Molecular Stress Physiology, Center of Plant Systems Biology and Biotechnology, Plovdiv 4000, Bulgaria

^h Department of Plant Physiology and Molecular Biology, University of Plovdiv, Plovdiv 4000, Bulgaria

ARTICLE INFO

Keywords:

Biostimulants
Hydrogen peroxide
Molecular priming

ABSTRACT

Abiotic stresses, including drought, salinity, extreme temperature, and pollutants, are the main cause of crop losses worldwide. Novel climate-adapted crops and stress tolerance-enhancing compounds are increasingly needed to counteract the negative effects of unfavorable stressful environments. A number of natural products and synthetic chemicals can protect model and crop plants against abiotic stresses through induction of molecular and physiological defense mechanisms, a process known as molecular priming. In addition to their stress-protective effect, some of these compounds can also stimulate plant growth. Here, we provide an overview of the known physiological and molecular mechanisms that induce molecular priming, together with a survey of the approaches aimed to discover and functionally study new stress-alleviating chemicals.

1. Introduction

Abiotic stresses importantly reduce plant growth, yield, and the marketable produce quality, with annually considerable economic losses as a consequence. Most of the abiotic stresses, including drought, salinity, extreme temperature, high light, and pollutants, such as heavy metals or pesticides, result in oxidative stress, i.e., an increase in the cellular levels of reactive oxygen species (ROS). Abiotic and oxidative stresses not only retard plant growth and diminish yield in cases of crop plants, but also severe stress can trigger programmed cell death (PCD) (Gadjev et al., 2008; Gechev and Hille, 2005; Petrov et al., 2015). However, ROS are not just toxic molecules that adversely affect plant growth and provoke cell death. In the past decades, ROS have been evidenced to be signals that regulate various developmental processes and responses to the environment (Carol et al., 2005; Foreman et al., 2003; Mhamdi and Van Breusegem, 2018; Mühlenbock et al., 2007; Rentel et al., 2004; Takeda et al., 2008; Torres et al., 2005; Tsukagoshi et al., 2010). The increase in ROS levels is often transient and linked

with the subsequent induction of defence mechanisms, such as up-regulation of stress-protective genes and accumulation of defence proteins and metabolites, that collectively provide stress protection. Hydrogen peroxide (H₂O₂) in particular emerges as a prominent signal that modulates stress responses and PCD. Pretreatment with low concentrations of H₂O₂ can induce protection against subsequent severe oxidative or abiotic stresses, a phenomenon known as priming (Chao et al., 2009; Dias de Azevedo Neto et al., 2005; Gechev et al., 2002).

ROS are known to interact with other signals, such as reactive nitrogen species, phytohormones, and lipid messengers (Gechev et al., 2006; Petrov and Van Breusegem, 2012; Scheler et al., 2013). Some plant hormones themselves can act as priming agents alone. Other naturally occurring compounds, such as strobilurins or seaweed-derived biostimulants, have also been shown to prime various model and crop plants (Filippou et al., 2016; Nair et al., 2012). This induction of inherent plant defense systems by various organic or inorganic molecules and biostimulants, resulting in protection against subsequent stresses, is known as molecular priming (the concept presented in Figure 1).

* Corresponding author at: Department of Molecular Stress Physiology, Center of Plant Systems Biology and Biotechnology, 139 Ruski Blvd., Plovdiv 4000, Bulgaria.

E-mail address: tsangech@uni-plovdiv.bg (T. Gechev).

<https://doi.org/10.1016/j.biotechadv.2019.107503>

Received 15 June 2019; Received in revised form 20 November 2019; Accepted 30 December 2019

Available online 31 December 2019

0734-9750/ © 2019 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

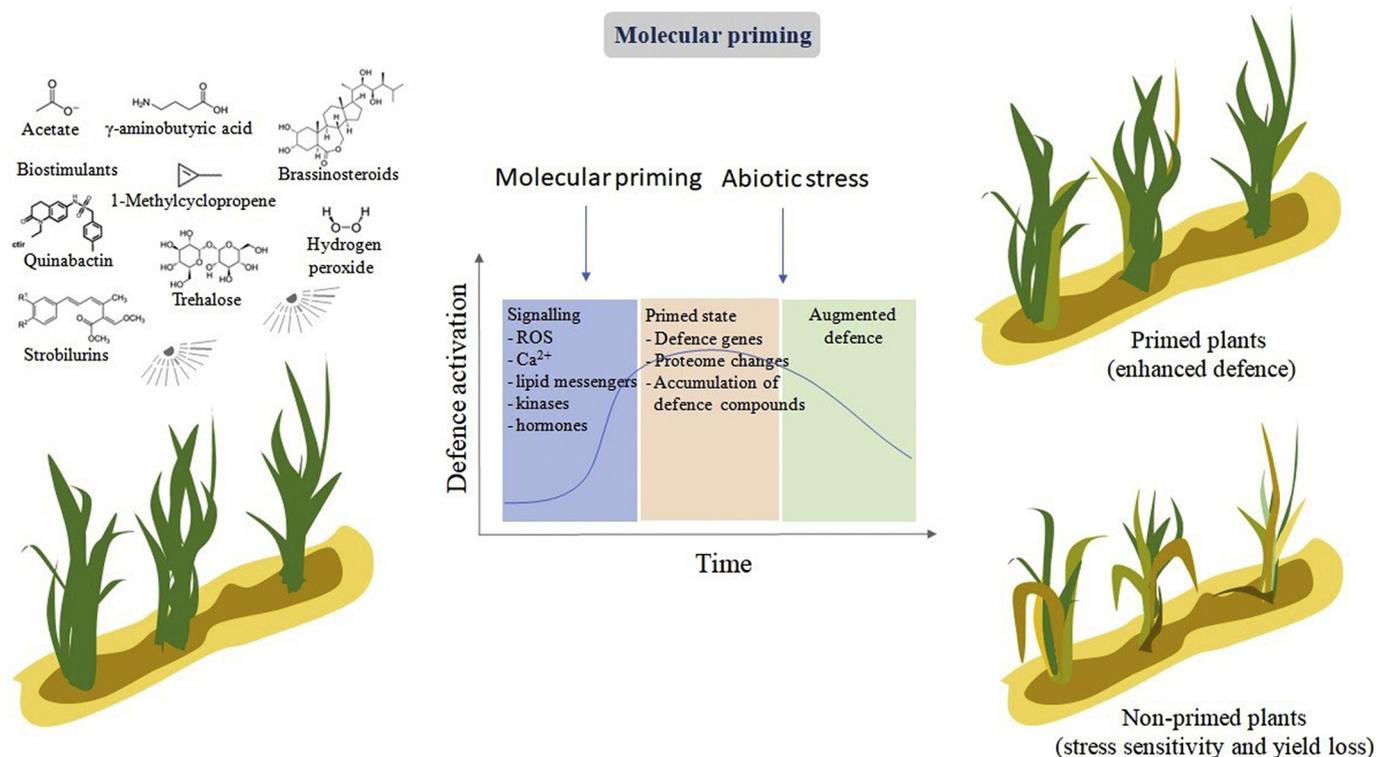


Fig. 1. Principle of plant defense activation by priming. Treatment with priming agents (left) induces endogenous plant defense mechanisms (middle), leading to subsequent stress tolerance in the primed plants. Agrochemicals are predominantly applied as a uniform spray solution (broadcast application) that can easily result in chemical waste, excessive carryovers, and damage to the vegetation. In contrast, directed application targets individual plants. Technological advances, such as computer vision and machine learning, make directed application more efficient than ever. Specific spraying of individual plants allows reduction of the dosage that can eliminate up to 90% of the used chemical and mitigate its environmental impact. Another exciting technology that will soon be available to the farmers combines agrochemicals with nanobodies as formulation agents that specifically target leaves or pests, improving retention and limiting the input of chemicals in the environment.

Many of the priming agents used to trigger natural defence mechanisms are not toxic to plants and mammals at the low concentrations applied; hence they can be used for stress mitigation and to increase crop yield and quality without any negative effects for the crop plants and the environment.

Here we review the existing molecular chemical priming compounds (Supplemental Tables S1 and S2), the knowledge on their action mode, and the strategies to discover new priming agents with stress-protective roles.

2. Molecular priming-inducing compounds

2.1. Plant hormone agonists and antagonists

Given the pivotal role of plant hormone homeostasis in adaptation to environmental changes, it is not surprising that application of various hormone agonists and antagonists prime plants against a range of stress conditions. Gibberellic acid (GA) and its biosynthetic inhibitors, such as paclobutrazol and trinexapac-ethyl (Supplementary Table S1), confer tolerance to drought, chilling, and heat stress in various plant species by reducing stomatal density and conductance (Berova et al., 2002; Bian et al., 2009; Gilley and Fletcher, 1998; Muthukumarasamy and Panneerselvam, 1997; Plaza-Wüthrich et al., 2016;). Also by modifying the abscisic acid (ABA)-dependent stomatal conductance, the ethylene signaling inhibitor 1-methylcyclopropene (1-MCP) promotes drought tolerance in wheat (*Triticum aestivum*) and reduces leaf senescence in heat-stressed cotton (*Gossypium hirsutum*) (Chen et al., 2013, 2015). Interestingly, impairing ethylene and GA levels by genetic means are both proven strategies to enhance stress performance that can be replicated by chemical priming (Habben et al., 2014; Lo et al., 2017;

Waltz, 2014).

Application of brassinosteroids (Supplementary Table 1) to various plant species increases tolerance to drought, salinity, extreme temperature, high light, and flooding (Vardhini and Anjum, 2015). For instance, priming of tomato (*Solanum esculentum*) seedlings with 24-epibrassinolide one day before the onset of the heat treatment enhanced the activities of photosystem II and antioxidant enzymes that improved shoot growth (Ogwenio et al., 2008). Similarly, the biomass of pepper (*Capsicum annuum*) seedlings pretreated with 24-epibrassinolide prior to drought stress exposure was higher in comparison to nontreated seedlings (Hu et al., 2013a, 2013b).

Pretreatment with natural and synthetic auxins alleviates the effects of abiotic stresses (Supplementary Table 2). For example, application of the naturally occurring indole-3-acetic acid (IAA) increased overall growth and upregulated antioxidant enzyme activities during cadmium stress in fenugreek (*Trigonella foenum*) seedlings (Bashri and Prasad, 2016). The synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) reduced the paraquat-derived H_2O_2 levels in alfalfa (*Medicago sativa*) protoplasts, possibly due to the increased activity of the H_2O_2 -scavenging ascorbate peroxidase (APX) (Pasternak et al., 2007).

Following the discovery of the ABA receptors by means of chemical biology approaches (Park et al., 2009), a dihydroquinoline sulfonamide (quinabactin) was characterized as an ABA agonist with a preference for dimeric receptors *in vitro* that activated ABA transcriptional responses in vegetative tissues (Okamoto et al., 2013). Treatment with quinabactin delayed wilting in *Arabidopsis thaliana* and soybean (*Glycine max*) exposed to water stress and restricted water loss from detached barley (*Hordeum vulgare*) and maize (*Zea mays*) leaves (Okamoto et al., 2013).

2.2. Reactive oxygen species

Various ROS are constantly produced inside plant cells and their balance is maintained by a robust and redundant ROS gene network (Gechev et al., 2006). Transient or/and more prominent increase in endogenous ROS levels can serve as a signal that switches on various defense-related genes, in turn, resulting in the accumulation of stress-protective enzymes, proteins, and metabolites (Petrov and Van Breusegem, 2012; Gechev et al., 2006), a mechanism that can be mimicked by exogenous H₂O₂. H₂O₂-induced protection has been documented against severe oxidative stress induced by the catalase (CAT) inhibitor 3-amino-1,2,4-triazole (AT) and by high light in tobacco (*Nicotiana tabacum*) (Gechev et al., 2002) as well as against chilling, salinity, heavy metals, pathogens, and other stresses in a number of model and crop species (mustard [*Brassica juncea*], maize, Manila grass [*Zoysia matrella*], pea [*Pisum sativum*], potato [*Solanum tuberosum*], rice [*Oryza sativa*], tobacco, and wheat), hinting at a general phenomenon that could be used as a strategy for crop improvement (Chao et al., 2009; Dias de Azevedo Neto et al., 2005; Gondim et al., 2012; Hafez et al., 2012; Hossain and Fujita, 2013; Kumar et al., 2010; Martínez-Gutiérrez et al., 2012; Moskova et al., 2009; Romero-Romero and López-Delgado, 2009; Xu et al., 2011).

Although the exact molecular mechanisms of ROS priming are far from understood, antioxidants, such as glutathione, and enhanced antioxidant enzyme activities are probably involved in the organization of an effective defense (Bhattacharjee, 2012; Chao et al., 2009; Gechev et al., 2002; İşeri et al., 2013; Gondim et al., 2012; Hafez et al., 2012; Kumar et al., 2010). For instance, treatment of tomato and rice with H₂O₂ prior to cold stress increased the activities of APX, CAT, superoxide dismutase (SOD) and glutathione reductase (GR) (Bhattacharjee, 2012; İşeri et al., 2013). Furthermore, glutathione was reported to play a role in the H₂O₂-induced tolerance against cadmium in rice and against high light stress in tobacco (Chao et al., 2009; Gechev et al., 2002). H₂O₂ drives the redox metabolism through redox-sensing proteins often undergoing reversible cysteine thiol oxidation upon a H₂O₂ outbreak (Waszczak et al., 2015; Huang et al., 2018). A comprehensive transcriptional network governs the downstream responses triggered by exogenous H₂O₂. Crucial players in the H₂O₂ regulatory network, such as the transcription factors NAC017 and CRF6, have been genetically validated (Ng et al., 2013; Zwack et al., 2016).

2.3. Chemicals used in agriculture

Soon after the strobilurin fungicides had been introduced on the market in the 1990s, they attracted attention due to their positive effects on plant yield and vigor in the absence of fungal pressure. Strobilurins (Supplementary Table S1) delay senescence, improve gas exchange, and alleviate drought and salt stresses. Their positive impact has been observed in various plant species, such as wheat, barley, rapeseed (*Brassica napus*), and potato (Overthrow, 2001; Ijaz and Honermeier, 2012). In contrast to their well-established fungicidal mode of action, which involves blocking of fungal respiration by targeting the quinone binding site on cytochrome *b* (Bartlett et al., 2002), the molecular mechanisms underlying their priming effect remain unknown. Transient inhibition of plant mitochondria and NO signaling has been suggested as a putative mechanism triggered upon treatment with strobilurin (Grossmann et al., 1999; Nason et al., 2007).

The neonicotinoid class of insecticides that activate acetylcholine receptors in the nervous system of insects resulting in paralysis and death, positively affect plant vigor and protect plants against various environmental stresses. Currently, neonicotinoids are banned in the European Union because of environmental concerns linking their application to bee population decline. Thiamethoxam, for example, stimulates root growth and improves plant survival under abiotic stresses (Macedo and de Camargo e Castro, 2011; Stamm et al., 2014). It is metabolized to clothianidin, which is also used as an insecticide (Nauen

et al., 2003). Interestingly, application of clothianidin induces salicylic acid (SA) signaling by promoting SA biosynthesis (Ford et al., 2010). SA signaling has been implicated in abiotic stress tolerance (Rivas-San Vicente and Plasencia, 2011) and its activation probably governs the stress protective neonicotinoid effects. The link between SA signaling and stress tolerance can be further exemplified by the improved heat and drought tolerances triggered by the fungicide acibenzolar-S-methyl, which is a widely used SA agonist (Jespersen et al., 2017; Tripathi et al., 2010).

2.4. Biostimulants

In recent years, eco-friendly approaches are increasingly considered to support agricultural sustainability. Biostimulants are organic molecules or plant extracts known to stimulate plant growth (Chiaiese et al., 2018; du Jardin, 2015). The plant biostimulant sector that is growing worldwide is expected to have a value of \$2.91 billion by 2021 and biostimulants to be applied over an area of 24.9 million hectares (Yakhin et al., 2017). Most commercially available biostimulant products are mixtures of multiple bioactive molecules and these extracts elicit a great diversity of responses in various crop species (Yakhin et al., 2017). Recently, several biostimulants have been shown to enhance broad-spectrum defense systems in plants (Shukla et al., 2019), namely they can increase shoot and root growths, enhance yield, improve fruit quality at harvest and during storage, and reduce stresses in crops. The biostimulant applications influence several plant signaling cascades and metabolic processes, stimulating photosynthesis, altering senescence, improving ion transport and nutrient uptake, modulating phytohormones, increasing C and N assimilation, and enhancing self-defence mechanisms (Backer et al., 2018; Colla et al., 2017; Craigie, 2011; Ertani et al., 2009, 2018; Khan et al., 2009; Paul et al., 2019; Polo and Mata, 2018; Posmyk and Szafranska, 2016; Roupael and Colla, 2018; Santaniello et al., 2017; Soppelsa et al., 2018; Ugena et al., 2018). Stress tolerance is perhaps the most important and consistent benefit achieved by treatment of plants with biostimulants (du Jardin, 2015).

Pretreatment of crops with the fungus *Trichoderma* has been shown to control disease and pests, while also alleviating abiotic stresses (Brotman et al., 2013; Mastouri et al., 2012; Shores et al., 2010). Root colonization by *Trichoderma* can result in elicitation of plant defense responses and induction of antimicrobial compounds through the phenylpropanoid pathway, a process that can limit pathogen ingress. Stimulation of pathways in this manner can also enhance plant tolerance to abiotic stresses (Brotman et al., 2013; Mastouri et al., 2010). Tomato plants pretreated with *Trichoderma harzianum* T22 were modified at the epigenetic level. Abiotic stress protection in plants could be mediated by stimulating memory responses at the epigenetic level (De Palma et al., 2019) but could also be improved by pretreatment with several nonpathogenic bacterial strains. Thus, the belowground interactions between these beneficial bacterial strains and plants have the capacity to prime and activate defense responses (Mhlongo et al., 2018). For example, the beneficial interactions between plant growth-promoting rhizobacteria (PGPR) and roots activate induced systemic acquired resistance (ISR), which is crucial for enhancing tolerance to both biotic and abiotic stresses (Dimkpa et al., 2009).

Pretreatment of crops with extracts and protein hydrolysates has the potential to improve plant growth and performance under environmental constraints. Seed priming with an extract from *Rosmarinus officinalis* L. has been demonstrated to increase germination and to be effective in inducing salt tolerance in maize seedlings (Panuccio et al., 2018). Another type of priming agents, chitin from crustacean shells and its deacetylated form chitosan, are known as elicitors that can prime and induce plant self-defense against plant pathogens (Lopez-Moya et al., 2017; Siddaiah et al., 2018). These compounds will also stimulate plant growth and abiotic stress tolerance. Seed priming with chitosan improved seed germination and was beneficial for seedling

growth under low temperature stress (Guan et al., 2009). Another biostimulant based on enzymatic hydrolysis of mammalian proteins effectively improved yield in cherry (*Prunus avium*) and tomato under the ambient low temperature growing conditions (Polo and Mata, 2018). Biostimulant extracts derived from the brown seaweed *Ascophyllum nodosum* are used extensively in agriculture worldwide and have been proven consistently to reduce abiotic stresses in treated plants (Goñi et al., 2018; Guinan et al., 2013; Nair et al., 2012; Shukla et al., 2019). Pretreatment of *Arabidopsis* plants with *A. nodosum* extracts activates partial closure of stomata and modifies ABA signalling and antioxidant systems. This preactivation results in enhanced protection when a secondary drought stress stimulus is detected (Santaniello et al., 2017). *Arabidopsis*, tomato, and pepper plants primed with an extract from *A. nodosum* are completely protected against paraquat-induced oxidative stress (M.A. Omidbakhshfar, N. Staykov, S. Neerakkal, and T. Gechev, own unpublished results). At the molecular level, the seaweed extract treatment can modulate micro RNAs (miRNAs), which are key regulators of gene expression, and enhance tolerance to salt stress (Shukla et al., 2018).

Biostimulants have the advantage to be naturally derived compounds, to be nontoxic at low concentrations, and not only to mitigate stress, but also to promote plant growth. An in-depth understanding is still required at the molecular and physiological levels to better value and develop these biostimulants as priming agents to counteract abiotic stresses. Such in-depth molecular studies will aid improve the efficacy of these naturally derived extracts and will help optimize their applications in agriculture.

2.5. Small organic molecules

Different structurally unrelated endogenous and synthetic organic molecules have been shown to prime plants against stress. The external application of acetic acid, for example, improved drought tolerance in *Arabidopsis*, rapeseed, maize, rice and wheat plants. This evolutionarily conserved priming mechanism relies on the promotion of jasmonic acid (JA) synthesis and enrichment of histone H4 acetylation that serves as an epigenetic switch by which plants adapt to drought (Kim et al., 2017).

The osmolytes glycine betaine (GB) and proline rapidly accumulate during salt and drought stresses and are crucial for the maintenance of the osmotic balance. Foliar application of GB significantly increased the photosynthetic pigment and biomass levels in salt-stressed cowpea (*Vigna unguiculata*) (Manaf, 2016). GB was also found to enhance photosynthetic rates in salt-stressed maize seedlings (Yang and Lu, 2005). Interestingly, proline levels increase upon GB application possibly by addition to the protective effects of GB (Hasanuzzaman et al., 2014).

Trehalose and trehalose-6-phosphate have been reported to protect several crop species, including rice, tobacco, and potato, against drought and salt stresses (Iordachescu and Imai, 2008). For instance, after spraying with trehalose, drought-stressed wheat had an enhanced membrane stability and elevated peroxidase and ascorbate levels (Aldesuquy and Ghanem, 2015).

The non-protein amino acid γ -aminobutyric acid (GABA) and its synthetic isomer β -aminobutyric acid (BABA) have been described as potent priming agents against abiotic stresses. Several application methods, including foliar spraying and seed soaking have been proven effective in reducing the effects of salinity, drought, temperature, and osmolarity stresses. Tomato seedlings treated with GABA displayed an elevated CAT activity and a reduced H_2O_2 content (Malekzadeh et al., 2014). Similarly, the enzymatic activities of CAT, SOD, and APX were higher in BABA-treated drought-stressed rapeseed than those in non-treated controls (Rajaei and Mohamadi, 2013).

Polyamines are nitrogen-containing basic compounds that play an important role in plant growth and development and are regarded as stress messengers due to their accumulation during environmental

stresses (Gill and Tuteja, 2010). Increase in polyamine content by genetic means or by application can successfully alleviate a wide range of abiotic stresses (Groppa and Benavides, 2007; Liu et al., 2007). In rice, spermidine and spermine reduce salt stress-induced symptoms, such as chlorophyll loss, photosynthesis inhibition, and electrolyte leakage (Chattopadhyay et al., 2002). The potential of polyamines to counteract chilling injury has been demonstrated in cucumber (*Cucumis sativus*) (He et al., 2002).

Initially discovered and extensively characterized as an important molecule in mammals, melatonin is also present in plants. Various studies have revealed the multifaceted bioactivity of melatonin in plants, ranging from plant growth regulation to protection against abiotic stresses (Arnao and Hernández-Ruiz, 2014). Interestingly, melatonin and auxin display similar growth-promoting effects and their biosynthetic pathways share the common precursor tryptophan (Wang et al., 2016). Nevertheless, these effects seem to be uncoupled from the induction of auxin-responsive gene expression (Pelagio-Flores et al., 2012). The antioxidant capacity of melatonin has been attributed to its ability to decrease environmental stresses, such as drought, heat, cold, and salinity (Debnath et al., 2019). This protection is accompanied by induction of stress signaling genes in melatonin-treated plants. In *Arabidopsis*, exogenous melatonin upregulates transcription factors involved in freezing and drought tolerances and in activation of ROS-related antioxidant genes (Bajwa et al., 2014).

Menadione or vitamin K_3 is another potent priming agent that induces tolerance to chilling and salt stresses (Borges et al., 2014). Soaking *Arabidopsis* seeds with menadione decreased the detrimental effect of salt stress that was accompanied with proline accumulation and induction of transcription factors that orchestrate ROS-dependent signaling networks (Jiménez-Arias et al., 2015).

2.6. Inorganic compounds

Besides their role in plant mineral nutrition, certain inorganic compounds have been shown to modulate stress tolerance. For example, potassium (K) applied as K_2CO_3 to hydroponically grown winter wheat alleviated the effects of polyethylene glycol-imposed osmotic stress (Wei et al., 2013), whereas foliar application of KCl to water-limited Kentucky bluegrass (*Poa pratensis*) stimulated stomata re-opening and photosynthetic recovery upon rehydration (Hu et al., 2013a, 2013b). Similarly, application of NH_4^+ counteracted the effect of salinity on sorghum (*Sorghum bicolor*) (de Souza Miranda et al., 2016). Moreover, NH_4^+ induced mild stress that primed *Citrus* rootstocks and conferred protection against subsequent salt stress (Fernández-Crespo et al., 2012). Calcium treatment has also been found to mitigate drought, cold, and heat stresses (Nayyar, 2003; Tan et al., 2011). Priming of maize seeds with a $CaCl_2$ solution beneficially affected the germination rate and seedling biomass and attenuated the negative effects of temperature stress in maize seedlings (Farooq et al., 2008).

Silicium (Si) has attracted a lot of interest in agriculture due to its positive impact on abiotic and biotic stress tolerances (Farooq and Dietz, 2015). For example, sodium silicate (Na_2SiO_3) improved the water status of soil-grown wheat during drought (Gong et al., 2005), and K_2SiO_3 alleviated salt stress in cucumber (Zhu et al., 2004). Similarly to Si, iodine (I) has been reported to modulate stress responses (Medrano-Macías et al., 2016) and applied as iodate (IO_3^-), to counteract the harmful effects of salinity stress in lettuce (*Lactuca sativa*) (Leyva et al., 2011). Another element with positive effects against a range of abiotic stresses is selenium (Se), which is an essential micronutrient for mammals and microorganisms, but not for higher plants. Whereas Se is toxic to plants at high concentrations, at low levels it alleviates the negative effects of drought, cold, and metal stresses (Gupta and Gupta, 2017; Kaur et al., 2014). Moreover, foliar spray of sodium selenite (Na_2SeO_3) increased the yield in pumpkin (*Cucurbita pepo*) (Germ et al., 2005).

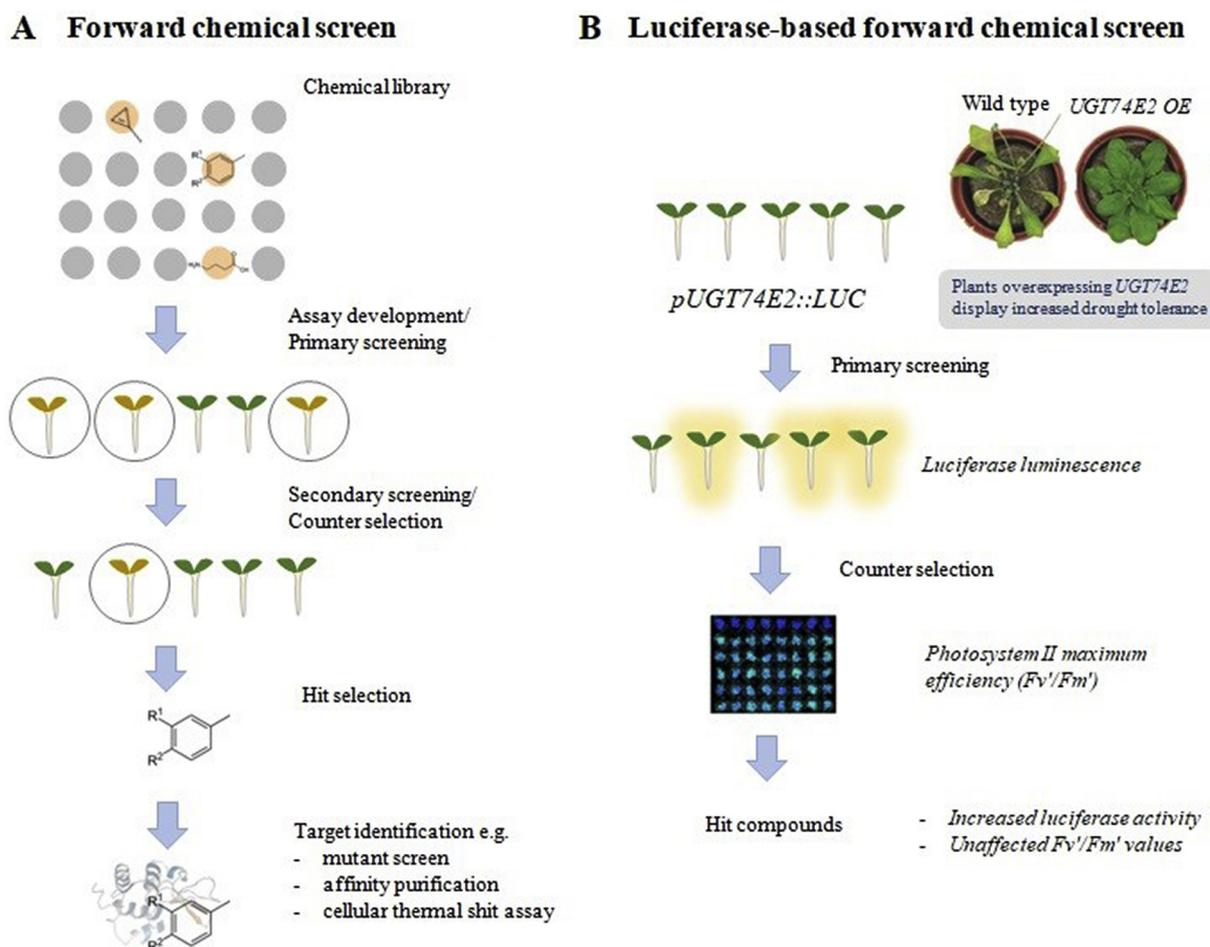


Fig. 2. Schematic depiction of general forward chemical screening approaches and their application to discovery of stress-alleviating small molecules. A. Chemical library used in a high-throughput assay to identify small molecules that trigger a phenotype of interest. A second screening is used to validate and verify the primary hits by means either of the same or of orthogonal assay. The aim of the target identification is the explanation of the mode of action of the hit compounds by identification of potential protein targets. B. Identification of stress alleviating small molecules using a luciferase-based reporter assay. The *UGT74E2* promoter that confers drought tolerance when overexpressed in *Arabidopsis* was fused to a luciferase reporter. Nine-day-old *Arabidopsis* seedlings carrying the corresponding construct (*pUGT74E2::LUC*) were screened in a 96-well format against a chemical library containing 12,000 small molecules. The luciferase activity was quantified 24 hours after the chemical treatment. To eliminate compounds with general toxicity that do not selectively activate the *UGT74E2* promoter, a counterselection was carried out with the chlorophyll fluorescence parameter maximum efficiency of photosystem II (F_v'/F_m') as a stress readout. Compounds that did not affect the F_v'/F_m' ratio and induced the *UGT74E2* promoter were considered hits.

Copper (Cu) salts and Cu fungicides have been used in agriculture for hundreds of years. In addition to its established role as an antifungal agent, Cu as nanoparticles has been reported to positively affect the accumulation of bioactive compounds in tomato fruits and improve their firmness (López-Vargas et al., 2018). Foliar spraying of Cu nanoparticles on tomato further increases the activity of antioxidant enzymes and alleviates salt stress-induced damage (Pérez-Labrada et al., 2019).

3. High-throughput screening strategies to discover abiotic stress-reducing small molecules

The advent of combinatorial chemistry has heralded a new era of opportunities for discovering novel bioactive small molecules. Various commercially available libraries that cover a vast chemical space can now be easily screened in a high-throughput manner. Facilities that accommodate chemical screening libraries and offer support during screen optimization and execution are now readily available. Moreover, international collaborative efforts, such as EU-OPENSREEN, offer the academic community specialized expertise and access to hundreds of thousands of compounds in order to accelerate the detection of biologically active substances (Horvath et al., 2014; Meiners et al., 2014).

These technical advances have contributed to the emergence of the chemical genomics field that uses small molecules to study gene and protein functions (Zheng and Chan, 2002). Small molecules have been employed to dissect endomembrane trafficking and plant hormonal signaling pathways in forward chemical screens with phenotypic readouts (e.g., lateral root formation, hypocotyl elongation, leaf bleaching, gravitropism-stimulated root bending, and germination inhibition) or reporter-based assays (e.g., β -glucuronidase, green fluorescent protein, and luciferase) (Drakakaki et al., 2011; von Kleist et al., 2011; Dejonghe et al., 2014; Mishev et al., 2018; Serrano et al., 2015).

Chemical genomics approaches have been used to discover the ABA receptor family proteins, which were subsequently employed to isolate drought resistance-modulating small molecules (Park et al., 2009). Despite the predominant use of chemical genomics to unravel fundamental biological questions, the isolation of compounds able to activate different ABA receptors (PYR1, PYL1, PYL2, PYL3, and PYL4) by means of yeast two-hybrid strains is an excellent example of a successful strategy to discover stress-protective small molecules (Okamoto et al., 2013).

The use of reporter genes that play crucial roles in abiotic stress responses allows the identification of small molecules that elicit the accumulation of these transcripts and ideally trigger downstream

responses that result in enhanced stress tolerance. For example, *UGT74E2*, encoding UDP-glucosyltransferase with a glycosylation activity toward indole butyric acid, rapidly accumulates under various stress conditions, including osmotic and high salt stresses, implying a conserved role in stress responses (Tognetti et al., 2010). *Arabidopsis* plants overexpressing *UGT74E2* display an enhanced tolerance toward drought and salt stresses, suggesting that chemical induction of *UGT74E2* might be able to counteract the abiotic stress effects. To identify small molecules able to induce *UGT74E2*, the 1500-bp region upstream of the *UGT74E2* start codon was fused to a luciferase reporter and the resulting *pUGT74E2:LUC* plants were used in a 96-well-based chemical screen (Kerchev et al., 2014). To discard hit compounds with general toxicity that nonselectively activate the *UGT74E2* promoter, a quantitative counterselection was done with the chlorophyll fluorescence parameter maximum efficiency of photosystem II (F_v'/F_m) as a stress readout. Tests with the hit compounds under a variety of stress conditions are expected to prioritize the most promising compounds for further structure activity relationship (SAR) analysis, mode of action elucidation, and lead optimization.

An alternative strategy to identify stress-alleviating small molecules utilized the photorespiratory phenotype of peroxisomal catalase (*cat2*)-lacking mutants in *Arabidopsis* that under photorespiration-promoting conditions display a rapid decrease in photosynthetic activity and ultimately cell death (Kerchev et al., 2015). Crucial in this case was the design of a high-throughput screening assay that allows efficient manipulation of photorespiration. To this end, 96-well plates were sealed with parafilm in order to deplete CO₂ levels, resulting in an enhanced metabolic flux through the photorespiratory pathway. Small molecules that alleviated the negative effects of photorespiration and attenuated cell death in *cat2* mutants upon photorespiratory stress were selected as hits (Fig. 2). One of the 34 hit compounds was a pro-auxin that contained a 2,4-D-type moiety, implying a role for auxin signaling in stress tolerance.

4. Conclusion

Molecular priming is evolving as an efficient technology to counteract the adverse effects of abiotic stresses. It is environmentally friendly and applicable to a wide variety of species. The priming agents can be applied only at critical times, for instance just before an upcoming stress, such as chilling or drought, and at discrete developmental stages susceptible to stress (such as fruit set), to maximize their efficiency. In contrast, this flexibility poses certain challenges. An accurate weather forecast and readiness to apply priming agents as environmental conditions change would be crucial for the exploitation of their full potential. The possibility to predict the magnitude of the upcoming stress and, hence, adapt the dosage would similarly be important for the chemical priming toolbox.

The release of chemicals into the environment is a subject of increasingly negative attitudes fueled by ever more restrictive environmental regulations (such as Directive 2009/128/EC for the sustainable use of pesticides; [http://www.europarl.europa.eu/RegData/etudes/STUD/2018/627113/EPRS_STU\(2018\)627113_EN.pdf](http://www.europarl.europa.eu/RegData/etudes/STUD/2018/627113/EPRS_STU(2018)627113_EN.pdf)). This adapting landscape creates additional hurdles for the agriculture that is expected to meet the demands of a growing population with a constantly decreasing number of approved pesticides. New agrochemicals with limited environmental impact and novel modes of action are thus on the immediate wish list of farmers and regulators. In order to be readily adopted by the farmers, any future priming agents needs to have a restricted environmental impact. Some of the molecular priming agents reviewed here, such as H₂O₂ and plant hormones, are easily biodegradable and pose no risk to the environment. Others, such as biostimulants, are not only safe, but also promote growth, in addition to their stress-protective properties. Thus, molecular priming induced by these compounds can be a safe and efficient strategy to combat unfavorable environmental conditions.

As the various priming agents are meant to be used during unfavorable environmental conditions to protect from stress and to reduce yield loss, their impact on yield in the absence of abiotic stresses has not been explored, with the exception of some biostimulants that are actually sold as growth-promoting and yield-increasing plant strengtheners (Polo and Mata, 2018; Popko et al., 2018; Sestili et al., 2018; Wally et al., 2013).

With the advances of the “-omics” technologies, we are beginning to unravel the molecular action mechanisms induced by the priming agents. With its efficiency against multiple stresses, applicability to many crop species, affordable price, and environmentally safe mode of action, this technology has vast potential in the future.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biotechadv.2019.107503>.

Acknowledgements

This work was supported by the European Union's Horizon 2020 research and innovation programme, projects PlantaSYST (SGA-CSA No. 739582 under FPA No. 664620) and RESIST (GA No. 823746); the Special Research Funds of Ghent University (BOF); the Research Foundation-Flanders (Excellence of Science project no. 30829584 to F.V.B); and the European Regional Development Fund, Project Phytophthora Research Centre Reg. No. CZ.02.1.01/0.0/0.0/15_003/0000453.

References

- Aldequy, H., Ghanem, H., 2015. Exogenous salicylic acid and trehalose ameliorate short term drought stress in wheat cultivars by up-regulating membrane characteristics and antioxidant defense system. *J. Horticult.* 2, 139.
- Arnao, M.B., Hernández-Ruiz, J., 2014. Melatonin: plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* 19, 789–797.
- Backer, R., Rokem, J.S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., Smith, D.L., 2018. Plant growth-promoting rhizobacteria: Context, mechanisms of action, and roadmap to commercialization of biostimulants for sustainable agriculture. *Front. Plant Sci.* 9, 1473.
- Bajwa, V.S., Shukla, M.R., Sherif, S.M., Murch, S.J., Saxena, P.K., 2014. Role of melatonin in alleviating cold stress in *Arabidopsis thaliana*. *J. Pineal Res.* 56, 238–245.
- Bartlett, D.W., Clough, J.M., Godwin, J.R., Hall, A.A., Hamer, M., Parr-Dobrzanski, B., 2002. The strobilurin fungicides. *Pest Manag. Sci.* 58, 649–662.
- Bashri, G., Prasad, S.M., 2016. Exogenous IAA differentially affects growth, oxidative stress and antioxidants system in Cd stressed *Trigonella foenum-graecum* L. seedlings: Toxicity alleviation by up-regulation of ascorbate-glutathione cycle. *Ecotox. Environ. Safe.* 132, 329–338.
- Berova, M., Zlatev, Z., Stoeva, N., 2002. Effect of paclobutrazol on wheat seedlings under low temperature stress. *Bulg. J. Plant Physiol.* 28, 75–84.
- Bhattacharjee, S., 2012. An inductive pulse of hydrogen peroxide pretreatment restores redox-homeostasis and oxidative membrane damage under extremes of temperature in two rice cultivars. *Plant Growth Regul.* 68, 395–410.
- Bian, X., Merewitz, E., Huang, B., 2009. Effects of trinexapac-ethyl on drought responses in creeping bentgrass associated with water use and osmotic adjustment. *J. Am. Soc. Hortic. Sci.* 134, 505–510.
- Borges, A.A., Jiménez-Arias, D., Expósito-Rodríguez, M., Sandalio, L.M., Pérez, J.A., 2014. Priming crops against biotic and abiotic stresses: MSB as a tool for studying mechanisms. *Front. Plant Sci.* 5, 642.
- Brotman, Y., Landau, U., Cuadros-Inostroza, A., Takayuki, T., Fernie, A.R., Chet, I., Viterbo, A., Willmitzer, L., 2013. *Trichoderma*-plant root colonization: escaping early plant defense responses and activation of the antioxidant machinery for saline stress tolerance. *PLoS Pathog.* 9, e1003221.
- Carol, R.J., Takeda, S., Linstead, P., Durrant, M.C., Kakesova, H., Derbyshire, P., Drea, S., Zarsky, V., Dolan, L., 2005. A RhoGDP dissociation inhibitor spatially regulates growth in root hair cells. *Nature* 438, 1013–1016.
- Chao, Y.-Y., Hsu, Y.T., Kao, C.H., 2009. Involvement of glutathione in heat shock- and hydrogen peroxide-induced cadmium tolerance of rice (*Oryza sativa* L.) seedlings. *Plant Soil* 318, 37–45.
- Chattopadhyay, M.K., Tiwari, B.S., Chattopadhyay, G., Bose, A., Sengupta, D.N., Ghosh, B., 2002. Protective role of exogenous polyamines on salinity-stressed rice (*Oryza sativa*) plants. *Physiol. Plant.* 116, 192–199.
- Chen, L., Dodd, I.C., Davies, W.J., Wilkinson, S., 2013. Ethylene limits abscisic acid- or soil drying-induced stomatal closure in aged wheat leaves. *Plant Cell Environ.* 36, 1850–1859.
- Chen, J., Wang, W.-H., Wu, F.-H., He, E.-M., Liu, X., Shangguan, Z.-P., Zheng, H.-L., 2015. Hydrogen sulfide enhances salt tolerance through nitric oxide-mediated maintenance of ion homeostasis in barley seedling roots. *Sci. Rep.* 5, 12516.
- Chiaiese, P., Corrado, G., Colla, G., Kyriacou, M.C., Roupael, Y., 2018. Renewable sources of plant biostimulation: Microalgae as a sustainable means to improve crop

- performance. *Front. Plant Sci.* 9, 1782.
- Colla, G., Hoagland, L., Ruzzi, M., Cardarelli, M., Bonini, P., Canaguier, R., Roupael, Y., 2017. Biostimulant action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Front. Plant Sci.* 8, 2202.
- Craigie, J.S., 2011. Seaweed extract stimuli in plant science and agriculture. *J. Appl. Phycol.* 23, 371–393.
- De Palma, M., Salzano, M., Villano, C., Aversano, R., Lorito, M., Ruocco, M., Docimo, T., Piccinelli, A.L., D'Agostino, N., Tucci, M., 2019. Transcriptome reprogramming, epigenetic modifications and alternative splicing orchestrate the tomato root response to the beneficial fungus *Trichoderma harzianum*. *Hortic. Res.* 6, 5.
- Debnath, B., Islam, W., Li, M., Sun, Y., Lu, X., Mitra, S., Hussain, M., Liu, S., Qiu, D., 2019. Melatonin mediates enhancement of stress tolerance in plants. *Int. J. Mol. Sci.* 20, 1040.
- Dejonghe, W., Mishev, K., Russinova, E., 2014. The brassinosteroid chemical toolbox. *Curr. Opin. Plant Biol.* 22, 48–55.
- Dias de Azevedo Neto, A., Prisco, J.T., Enéas-Filho, J., Rolim Medeiros, J.-V., Gomes-Filho, E., 2005. Hydrogen peroxide pre-treatment induces salt-stress acclimation in maize plants. *Journal of Plant Physiology* 162, 1114–1122.
- Dimkpa, C., Weinand, T., Asch, F., 2009. Plant-rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ.* 32, 1682–1694.
- Drakakaki, G., Robert, S., Szatmari, A.-M., Brown, M.Q., Nagawa, S., Van Damme, D., Leonard, M., Yang, Z., Girke, T., Schmid, S.L., Russinova, E., Friml, J., Raikhel, N.V., Hicks, G.R., 2011. Clusters of bioactive compounds target dynamic endomembrane networks in vivo. *Proc. Natl. Acad. Sci. USA* 108, 17850–17855.
- Ertani, A., Cavani, L., Pizzeghello, D., Brandellero, E., Altissimo, A., Ciavatta, C., Nardi, S., 2009. Biostimulant activity of two protein hydrolysates in the growth and nitrogen metabolism of maize seedlings. *J. Plant Nutr. Soil Sci.* 172, 237–244.
- Ertani, A., Francioso, O., Tinti, A., Schiavon, M., Pizzeghello, D., Nardi, S., 2018. Evaluation of seaweed extracts from *Laminaria* and *Ascophyllum nodosum* spp. as biostimulants in *Zea mays* L. using a combination of chemical, biochemical and morphological approaches. *Front. Plant Sci.* 9, 428.
- Farooq, M.A., Dietz, K.-J., 2015. Silicon as versatile player in plant and human biology: overlooked and poorly understood. *Front. Plant Sci.* 6, 994.
- Farooq, M., Aziz, T., Basra, S.M.A., Wahid, A., Khaliq, A., Cheema, M.A., 2008. Exploring the role of calcium to improve chilling tolerance in hybrid maize. *J. Agron. Crop Sci.* 194, 350–359.
- Fernández-Crespo, E., Camañes, G., García-Agustín, P., 2012. Ammonium enhances resistance to salinity stress in citrus plants. *J. Plant Physiol.* 169, 1183–1191.
- Filippou, P., Antoniou, C., Obata, T., Van Der Kelen, K., Harokopos, V., Kanetis, L., Aidinis, V., Van Breusegem, F., Fernie, A.R., Fotopoulos, V., 2016. Kresoxim-methyl primes *Medicago truncatula* plants against abiotic stress factors via altered reactive oxygen and nitrogen species signalling leading to downstream transcriptional and metabolic readjustment. *J. Exp. Bot.* 67, 1259–1274.
- Ford, K.A., Casida, J.E., Chandran, D., Gulevich, A.G., Okrent, R.A., Durkin, K.A., Sarpong, R., Bunnelle, E.M., Wildermuth, M.C., 2010. Neonicotinoid insecticides induce salicylate-associated plant defense responses. *Proc. Natl. Acad. Sci. USA* 107, 17527–17532.
- Foreman, J., Demidchik, V., Bothwell, J.H.F., Mylona, P., Miedema, H., Torres, M.A., Linstead, P., Costa, S., Brownlee, C., Jones, J.D.G., Davies, J.M., Dolan, L., 2003. Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. *Nature* 422, 442–446.
- Gadjev, I., Stone, J.M., Gechev, T.S., 2008. Programmed cell death in plants: new insights into redox regulation and the role of hydrogen peroxide. *Int. Rev. Cell Mol. Biol.* 270, 87–144.
- Gechev, T.S., Hille, J., 2005. Hydrogen peroxide as a signal controlling plant programmed cell death. *J. Cell Biol.* 168, 17–20.
- Gechev, T., Gadjev, I., Van Breusegem, F., Inzé, D., Dukjandjiev, S., Toneva, V., Minkov, I., 2002. Hydrogen peroxide protects tobacco from oxidative stress by inducing a set of antioxidant enzymes. *Cell. Mol. Life Sci.* 59, 708–714.
- Gechev, T.S., Van Breusegem, F., Stone, J.M., Denev, I., Laloi, C., 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. *BioEssays* 28, 1091–1101.
- Germ, M., Krefl, I., Osvald, J., 2005. Influence of UV-B exclusion and selenium treatment on photochemical efficiency of photosystem II, yield and respiratory potential in pumpkins (*Cucurbita pepo* L.). *Plant Physiol. Biochem.* 43, 445–448.
- Gill, S.S., Tuteja, N., 2010. Polyamines and abiotic stress tolerance in plants. *Plant Signal Behav.* 5, 26–33.
- Gilley, A., Fletcher, R.A., 1998. Gibberellin antagonizes paclobutrazol-induced stress protection in wheat seedlings. *J. Plant Physiol.* 153, 200–207.
- Gondim, F.A., Gomes-Filho, E., Costa, J.H., Mendes Alencar, N.L., Prisco, J.T., 2012. Catalase plays a key role in salt stress acclimation induced by hydrogen peroxide pretreatment in maize. *Plant Physiol. Biochem.* 56, 62–71.
- Gong, H., Zhu, X., Chen, K., Wang, S., Zhang, C., 2005. Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Sci.* 169, 313–321.
- Goni, O., Quille, P., O'Connell, S., 2018. *Ascophyllum nodosum* extract biostimulants and their role in enhancing tolerance to drought stress in tomato plants. *Plant Physiol. Biochem.* 126, 63–73.
- Groppa, M.D., Benavides, M.P., 2007. Polyamines and abiotic stress: recent advances. *Amino Acids* 34, 35–45.
- Grossmann, K., Kwiatkowski, J., Caspar, G., 1999. Regulation of phytohormone levels, leaf senescence and transpiration by the strigolactone kresoxim-methyl in wheat (*Triticum aestivum*). *J. Plant Physiol.* 154, 805–808.
- Guan, Y.-j., Hu, J., Wang, X.-j., Shao, C.-x., 2009. Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. *J. Zhejiang Univ. Sci. B* 10, 427–433.
- Guinan, K.J., Sujeeth, N., Copeland, R.B., Jones, P.W., O'Brien, N.M., Sharma, H.S.S., Prouteau, P.F.J., O'Sullivan, J.T., 2013. Discrete roles for extracts of *Ascophyllum nodosum* in enhancing plant growth and tolerance to abiotic and biotic stresses. *Acta Hort.* (1009), 127–135.
- Gupta, M., Gupta, S., 2017. An overview of selenium uptake, metabolism, and toxicity in plants. *Front. Plant Sci.* 7, 2074.
- Habben, J.E., Bao, X., Bate, N.J., DeBruin, J.L., Dolan, D., Hasegawa, D., Helentjaris, T.G., Lafitte, R.H., Lovan, N., Mo, H., Reimann, K., Schussler, J.R., 2014. Transgenic alteration of ethylene biosynthesis increases grain yield in maize under field drought-stress conditions. *Plant Biotechnol. J.* 12, 685–693.
- Hafez, Y.M., Bacsó, R., Király, Z., Künstler, A., Király, L., 2012. Up-regulation of anti-oxidants in tobacco by low concentrations of H₂O₂ suppresses necrotic disease symptoms. *Phytopathology* 102, 848–856.
- Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K., Fujita, M., 2014. Exogenous proline and glycine betaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed Res. Int.* 2014, 757219.
- He, L., Nada, K., Tachibana, S., 2002. Effects of spermidine pretreatment through the roots on growth and photosynthesis of chilled cucumber plants (*Cucumis sativus* L.). *J. Jpn. Soc. Hortic. Sci.* 71, 490–498.
- Horvath, D., Lisurek, M., Rupp, B., Kühne, R., Specker, E., von Kries, J., Rognan, D., Andersson, C.D., Almqvist, F., Elofsson, M., Enqvist, P.-A., Gustavsson, A.-L., Remež, N., Mestres, J., Marcou, G., Varnek, A., Hibert, M., Quintana, J., Frank, R., 2014. Design of a general-purpose European compound screening library for EU-OPENSREEN. *ChemMedChem* 9, 2309–2326.
- Hossain, M.A., Fujita, M., 2013. Hydrogen peroxide priming stimulates drought tolerance in mustard (*Brassica juncea* L.) seedlings. *Plant Gene Trait* 4, 109–123.
- Hu, L., Wang, Z., Huang, B., 2013a. Effects of cytokinin and potassium on stomatal and photosynthetic recovery of Kentucky bluegrass from drought stress. *Crop Sci.* 53, 221–231.
- Hu, W.-h., Yan, X.-h., Xiao, Y.-a., Zeng, J.-j., Qi, H.-j., Ogwen, J.O., 2013b. 24-Epibrassinosteroid alleviate drought-induced inhibition of photosynthesis in *Capsicum annum*. *Sci. Hortic.* 150, 232–237.
- Huang, J., Willems, P., Van Breusegem, F., Messens, J., 2018. Pathways crossing mammalian and plant sulfenomic landscapes. *Free Radic. Biol. Med.* 122, 193–201.
- Ijaz, M., Honermeier, B., 2012. Effect of triazole and strigolactin fungicides on seed yield formation and grain quality of winter rapeseed (*Brassica napus* L.). *Field Crop. Res.* 130, 80–86.
- Iordachescu, M., Imai, R., 2008. Trehalose biosynthesis in response to abiotic stresses. *J. Integr. Plant Biol.* 50, 1223–1229.
- İşeri, Ö.D., Körpe, D.A., Sahin, F.I., Haberal, M., 2013. Hydrogen peroxide pretreatment of roots enhanced oxidative stress response of tomato under cold stress. *Acta Physiol. Plant.* 35, 1905–1913.
- du Jardin, P., 2015. Plant biostimulants: Definition, concept, main categories and regulation. *Sci. Hortic.* 196, 3–14.
- Jespersen, D., Yu, J., Huang, B., 2017. Metabolic effects of acibenzolar-S-methyl for improving heat or drought stress in creeping bentgrass. *Front. Plant Sci.* 8, 1224.
- Jiménez-Arias, D., Pérez, J.A., Luis, J.C., Martín-Rodríguez, V., Valdés-González, F., Borges, A.A., 2015. Treating seeds in menadione sodium bisulphite primes salt tolerance in *Arabidopsis* by inducing an earlier plant adaptation. *Environ. Exp. Bot.* 109, 23–30.
- Kaur, N., Sharma, S., Kaur, S., Nayyar, H., 2014. Selenium in agriculture: a nutrient or contaminant for crops? *Arch. Agron. Soil Sci.* 60, 1593–1624.
- Kerchev, P.I., De Clercq, I., Denecker, J., Mühlenbock, P., Kumpf, R., Nguyen, L., Audenaert, D., Dejonghe, W., Van Breusegem, F., 2014. Mitochondrial perturbation negatively affects auxin signaling. *Mol. Plant* 7, 1138–1150.
- Kerchev, P., Mühlenbock, P., Denecker, J., Morreel, K., Hoerichs, F.A., Van Der Kelen, K., Vandoorpe, M., Nguyen, L., Audenaert, D., Van Breusegem, F., 2015. Activation of auxin signalling counteracts photorespiratory H₂O₂-dependent cell death. *Plant Cell Environ.* 38, 253–265.
- Khan, W., Rayirath, U.P., Subramanian, S., Jithesh, M.N., Rayorath, P., Hodges, D.M., Critchley, A.T., Craigie, J.S., Norrie, J., Prithiviraj, B., 2009. Seaweed extracts as biostimulants of plant growth and development. *J. Plant Growth Regul.* 28, 386–399.
- Kim, J.-M., To, T.K., Matsui, A., Tanoi, K., Kobayashi, N.I., Matsuda, F., Habu, Y., Ogawa, D., Sakamoto, T., Matsunaga, S., Bashir, K., Rasheed, S., Ando, M., Takeda, H., Kawaura, K., Kusano, M., Fukushima, A., Takahashi, A., Kurumori, T., Ishida, J., Morosawa, T., Tanaka, M., Torii, C., Takebayashi, Y., Sakakibara, H., Ogihara, Y., Saito, K., Shinozaki, K., Devoto, A., Seki, M., 2017. Erratum: Acetate-mediated novel survival strategy against drought in plants. *Nat. Plants* 3, 17119.
- von Kleist, L., Stahlschmidt, W., Bulut, H., Gromova, K., Puchkov, D., Robertson, M.J., MacGregor, K.A., Tomilin, N., Pechstein, A., Chau, N., Chircop, M., Sakoff, J., von Kries, J.P., Saenger, W., Kräusslich, H.-G., Shupliakov, O., Robinson, P.J., McCluskey, A., Haucke, V., 2011. Role of the clathrin terminal domain in regulating coated pit dynamics revealed by small molecule inhibition. *Cell* 146, 471–484.
- Kumar, M., Sirhindi, G., Bhardwaj, R., Kumar, S., Jain, G., 2010. Effect of exogenous H₂O₂ on antioxidant enzymes of *Brassica juncea* L. seedlings in relation to 24-epibrassinolide under chilling stress. *Indian J. Biochem. Biophys.* 47, 378–382.
- Leyva, R., Sánchez-Rodríguez, E., Ríos, J.J., Rubio-Wilhelmi, M.M., Romero, L., Ruiz, J.M., Blasco, B., 2011. Beneficial effects of exogenous iodine in lettuce plants subjected to salinity stress. *Plant Sci.* 181, 195–202.
- Liu, J.-H., Kitashiba, H., Wang, J., Ban, Y., Moriguchi, T., 2007. Polyamines and their ability to provide environmental stress tolerance to plants. *Plant Biotechnol.* 24, 117–126.
- Lo, S.-F., Ho, T.-H.D., Liu, Y.-L., Jiang, M.-J., Hsieh, K.-T., Chen, K.-T., Yu, L.-C., Lee, M.-H., Chen, C.-Y., Huang, T.-P., Kojima, M., Sakakibara, H., Chen, L.-J., Yu, S.-M., 2017. Ectopic expression of specific GA2 oxidase mutants promotes yield and stress tolerance in rice. *Plant Biotechnol. J.* 15, 850–864.

- Lopez-Moya, F., Escudero, N., Zavala-Gonzalez, E.A., Esteve-Bruna, D., Blázquez, M.A., Alabadi, D., Lopez-Llorca, L.V., 2017. Induction of auxin biosynthesis and *WOX5* repression mediate changes in root development in *Arabidopsis* exposed to chitosan. *Sci. Rep.* 7, 16813.
- López-Vargas, E.R., Ortega-Ortiz, H., Cadenas-Pliego, G., de Alba Romenus, K., Cabrera de la Fuente, M., Benavides-Mendoza, A., Juárez-Maldonado, A., 2018. Foliar application of copper nanoparticles increases the fruit quality and the content of bioactive compounds in tomatoes. *Appl. Sci.* 8, 1020.
- Macedo, W.R., de Camargo e Castro, P.R., 2011. Thiamethoxam: molecule moderator of growth, metabolism and production of spring wheat. *Pestic. Biochem. Physiol.* 100, 299–304.
- Malekzadeh, P., Khara, J., Heydari, R., 2014. Alleviating effects of exogenous gamma-aminobutyric acid on tomato seedling under chilling stress. *Physiol. Mol. Biol. Plants* 20, 133–137.
- Manaf, H.H., 2016. Beneficial effects of exogenous selenium, glycine betaine and seaweed extract on salt stressed cowpea plant. *Ann. Agric. Sci.* 61, 41–48.
- Martínez-Gutiérrez, R., Mora-Herrera, M.E., López-Delgado, H.A., 2012. Exogenous H₂O₂ in phytoplasma-infected potato plants promotes antioxidant activity and tuber production under drought conditions. *Am. J. Potato Res.* 89, 53–62.
- Mastouri, F., Björkman, T., Harman, G.E., 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. *Phytopathology* 100, 1213–1221.
- Mastouri, F., Björkman, T., Harman, G.E., 2012. *Trichoderma harzianum* enhances antioxidant defense of tomato seedlings and resistance to water deficit. *Mol. Plant-Microbe Interact.* 25, 1264–1271.
- Medrano-Macias, J., Leija-Martínez, P., González-Morales, S., Juárez-Maldonado, A., Benavides-Mendoza, A., 2016. Use of iodine to biofortify and promote growth and stress tolerance in crops. *Front. Plant Sci.* 7, 1146.
- Meiners, T., Stechmann, B., Frank, R., 2014. EU-OPENSCREEN—chemical tools for the study of plant biology and resistance mechanisms. *J. Chem. Biol.* 7, 113–118.
- Mhamdi, A., Van Breusegem, F., 2018. Reactive oxygen species in plant development. *Development* 145, dev164376.
- Mhlongo, M.I., Piater, L.A., Madala, N.E., Labuschagne, N., Dubery, I.A., 2018. The chemistry of plant-microbe interactions in the rhizosphere and the potential for metabolomics to reveal signaling related to defense priming and induced systemic resistance. *Front. Plant Sci.* 9, 112.
- Mishev, K., Lu, Q., Denoo, B., Peurois, F., Dejonghe, W., Hullaert, J., De Rycke, R., Boeren, S., Bretou, M., De Munck, S., Sharma, I., Goodman, K., Kalinowska, K., Storme, V., Nguyen, L.S.L., Drozdzecki, A., Martins, S., Nerinckx, W., Audenaert, D., Vert, G., Maddar, A., Otegui, M.S., Isono, E., Savvides, S.N., Annaert, W., De Vries, S., Cherifils, J., Winne, J., Russinova, E., 2018. Nonselective chemical inhibition of Sec7 domain-containing ARF GTPase exchange factors. *Plant Cell* 30, 2573–2593.
- Moskova, I., Todorova, D., Alexieva, V., Ivanov, S., Sergiev, I., 2009. Effect of exogenous hydrogen peroxide on enzymatic and nonenzymatic antioxidants in leaves of young pea plants treated with paraquat. *Plant Growth Regul.* 57, 193–202.
- Mühlenböck, P., Plaszczyca, M., Mellerowicz, E., Karpinski, S., 2007. Lysigenous aerenchyma formation in *Arabidopsis* is controlled by *LESION SIMULATING DISEASE1*. *Plant Cell* 19, 3819–3830.
- Muthukumarasamy, M., Panneerselvam, R., 1997. Amelioration of NaCl stress by triadimefon in peanut seedlings. *Plant Growth Regul.* 22, 157–162.
- Nair, P., Kandasamy, S., Zhang, J., Ji, X., Kirby, C., Benkel, B., Hodges, M.D., Critchley, A.T., Hiltz, D., Prithiviraj, B., 2012. Transcriptional and metabolomic analysis of *Ascophyllum nodosum* mediated freezing tolerance in *Arabidopsis thaliana*. *BMC Genomics* 13, 643.
- Nason, M.A., Farrar, J., Bartlett, D., 2007. Strobilurin fungicides induce changes in photosynthetic gas exchange that do not improve water use efficiency of plants grown under conditions of water stress. *Pest Manag. Sci.* 63, 1191–1200.
- Nauen, R., Ebbinghaus-Kintscher, U., Salgado, V.L., Kaussmann, M., 2003. Thiamethoxam is a neonicotinoid precursor converted to clothianidin in insects and plants. *Pestic. Biochem. Physiol.* 76, 55–69.
- Nayyar, H., 2003. Calcium as environmental sensor in plants. *Curr. Sci.* 84, 893–902.
- Ng, S., Ivanova, A., Duncan, O., Law, S.R., Van Aken, O., De Clercq, I., Wang, Y., Carrie, C., Xu, L., Kmiec, B., Walker, H., Van Breusegem, F., Whelan, J., Giraud, E., 2013. A membrane-bound NAC transcription factor, ANAC017, mediates mitochondrial retrograde signaling in *Arabidopsis*. *Plant Cell* 25, 3450–3471.
- Ogwen, J.O., Song, X.S., Shi, K., Hu, W.H., Mao, W.H., Zhou, Y.H., Yu, J.Q., Nogueús, S., 2008. Brassinosteroids alleviate heat-induced inhibition of photosynthesis by increasing carboxylation efficiency and enhancing antioxidant systems in *Lycopersicon esculentum*. *J. Plant Growth Regul.* 27, 49–57.
- Okamoto, M., Peterson, F.C., Defries, A., Park, S.-Y., Endo, A., Nambara, E., Volkman, B.F., Cutler, S.R., 2013. Activation of dimeric ABA receptors elicits guard cell closure, ABA-regulated gene expression, and drought tolerance. *Proc. Natl. Acad. Sci. USA* 110, 12132–12137.
- Overthrow, R.B., 2001. Effect on yield and quality of strobilurin applications to spring malting barley, Project Report 250. Agriculture and Horticulture Development Board, Kenilworth, pp. 1–39.
- Panuccio, M.R., Chaabani, S., Roula, R., Muscolo, A., 2018. Bio-priming mitigates detrimental effects of salinity on maize improving antioxidant defense and preserving photosynthetic efficiency. *Plant Physiol. Biochem.* 132, 465–474.
- Park, S.-Y., Fung, P., Nishimura, N., Jensen, D.R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T.-f., Alfred, S.E., Bonetta, D., Finkelstein, R., Provart, N.J., Desveaux, D., Rodriguez, P.L., McCourt, P., Zhu, J.-K., Schroeder, J.I., Volkman, B.F., Cutler, S.R., 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* 324, 1068–1071.
- Pasternak, T.P., Ötvös, K., Domoki, M., Fehér, A., 2007. Linked activation of cell division and oxidative stress defense in alfalfa leaf protoplast-derived cells is dependent on exogenous auxin. *Plant Growth Regul.* 51, 109–117.
- Paul, K., Sorrentino, M., Lucini, L., Roupael, Y., Cardarelli, M., Bonini, P., Reynaud, H., Canaguier, R., Trtílek, M., Panzarová, K., Colla, G., 2019. Understanding the biostimulant action of vegetal-derived protein hydrolysates by high-throughput plant phenotyping and metabolomics: a case study on tomato. *Front. Plant Sci.* 10, 47.
- Pelagio-Flores, R., Muñoz-Parra, E., Ortiz-Castro, R., López-Bucio, J., 2012. Melatonin regulates *Arabidopsis* root system architecture likely acting independently of auxin signaling. *J. Pineal Res.* 53, 279–288.
- Pérez-Labrada, F., López-Vargas, E.R., Ortega-Ortiz, H., Cadenas-Pliego, G., Benavides-Mendoza, A., Juárez-Maldonado, A., 2019. Responses of tomato plants under saline stress to foliar application of copper nanoparticles. *Plants* 8, 151.
- Petrov, V.D., Van Breusegem, F., 2012. Hydrogen peroxide—a central hub for information flow in plant cells. *AoB Plants* 2012, pls014.
- Petrov, V., Hille, J., Mueller-Roerber, B., Gechev, T.S., 2015. ROS-mediated abiotic stress-induced programmed cell death in plants. *Front. Plant Sci.* 6, 69.
- Plaza-Wüthrich, S., Blösch, R., Rindisbacher, A., Cannarozzi, G., Tadele, Z., 2016. Gibberellin deficiency confers both lodging and drought tolerance in small cereals. *Front. Plant Sci.* 7, 643.
- Polo, J., Mata, P., 2018. Evaluation of a biostimulant (Pepton) based in enzymatic hydrolyzed animal protein in comparison to seaweed extracts on root development, vegetative growth, flowering, and yield of gold cherry tomatoes grown under low stress ambient field conditions. *Front. Plant Sci.* 8, 2261.
- Popko, M., Michalak, I., Wilk, R., Gramza, M., Chojnacka, K., Górecki, H., 2018. Effect of the new plant growth biostimulants based on amino acids on yield and grain quality of winter wheat. *Molecules* 23, 470.
- Posmyk, M.M., Szafrńska, K., 2016. Biostimulators: a new trend towards solving an old problem. *Front. Plant Sci.* 7, 748.
- Rajaei, P., Mohamadi, N., 2013. Effect of beta-aminobutyric acid (BABA) on enzymatic and non-enzymatic antioxidants of *Brassica napus* L. under drought stress. *Int. J. Biosci.* 3, 41–47.
- Rentel, M.C., Lecourieux, D., Ouaked, F., Usher, S.L., Petersen, L., Okamoto, H., Knight, H., Peck, S.C., Grierson, C.S., Hirt, H., Knight, M.R., 2004. OX11 kinase is necessary for oxidative burst-mediated signalling in *Arabidopsis*. *Nature* 427, 858–861.
- Rivas-San Vicente, M., Plasencia, J., 2011. Salicylic acid beyond defence: its role in plant growth and development. *J. Exp. Bot.* 62, 3321–3338.
- Romero-Romero, M.T., López-Delgado, H.A., 2009. Ameliorative effects of hydrogen peroxide, ascorbate and dehydroascorbate in *Solanum tuberosum* infected by phytoplasma. *Am. J. Potato Res.* 86, 218–226.
- Roupael, Y., Colla, G., 2018. Synergistic biostimulatory action: designing the next generation of plant biostimulants for sustainable agriculture. *Front. Plant Sci.* 9, 1655.
- Santaniello, A., Scartazza, A., Gresta, F., Loreti, E., Biasone, A., Di Tommaso, D., Piaggese, A., Perata, P., 2017. *Ascophyllum nodosum* seaweed extract alleviates drought stress in *Arabidopsis* by affecting photosynthetic performance and related gene expression. *Front. Plant Sci.* 8, 1362.
- Scheler, C., Durner, J., Astier, J., 2013. Nitric oxide and reactive oxygen species in plant biotic interactions. *Curr. Opin. Plant Biol.* 16, 534–539.
- Serrano, M., Kombrink, E., Meesters, C., 2015. Considerations for designing chemical screening strategies in plant biology. *Front. Plant Sci.* 6, 131.
- Sestili, F., Roupael, Y., Cardarelli, M., Pucci, A., Bonini, P., Canaguier, R., Colla, G., 2018. Protein hydrolysate stimulates growth in tomato coupled with N-dependent gene expression involved in N assimilation. *Front. Plant Sci.* 9, 1233.
- Shoreh, M., Harman, G.E., Mastouri, F., 2010. Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu. Rev. Phytopathol.* 48, 21–43.
- Shukla, P.S., Borza, T., Critchley, A.T., Hiltz, D., Norrie, J., Prithiviraj, B., 2018. *Ascophyllum nodosum* extract mitigates salinity stress in *Arabidopsis thaliana* by modulating the expression of miRNA involved in stress tolerance and nutrient acquisition. *PLoS ONE* 13, e0206221.
- Shukla, P.S., Mantin, E.G., Adil, M., Bajpai, S., Critchley, A.T., Prithiviraj, B., 2019. *Ascophyllum nodosum*-based biostimulants: sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. *Front. Plant Sci.* 10, 655.
- Siddaiah, C.N., Prasanth, K.V.H., Satyanarayana, N.R., Mudili, V., Gupta, V.K., Kalagatur, N.K., Satyavati, T., Dai, X.-F., Chen, J.-Y., Mocan, A., Singh, B.P., Srivastava, R.K., 2018. Chitosan nanoparticles having higher degree of acetylation induce resistance against pearl millet downy mildew through nitric oxide generation. *Sci. Rep.* 8, 2485.
- Soppelsa, S., Kelderer, M., Casera, C., Bassi, M., Robatscher, P., Andreotti, C., 2018. Use of biostimulants for organic apple production: effects on tree growth, yield, and fruit quality at harvest and during storage. *Front. Plant Sci.* 9, 1342.
- de Souza Miranda, R., Gomes-Filho, E., Prisco, J.T., Alvarez-Pizarro, J.C., 2016. Ammonium improves tolerance to salinity stress in *Sorghum bicolor* plants. *Plant Growth Regul.* 78, 121–131.
- Stamm, M.D., Enders, L.S., Donze-Reiner, T.J., Baxendale, F.P., Siegfried, B.D., Heng-Moss, T.M., 2014. Transcriptional response of soybean to thiamethoxam seed treatment in the presence and absence of drought stress. *BMC Genomics* 15, 1055.
- Takeda, S., Gapper, C., Kaya, H., Bell, E., Kuchitsu, K., Dolan, L., 2008. Local positive feedback regulation determines cell shape in root hair cells. *Science* 319, 1241–1244.
- Tan, W., Meng, Q.W., Brestic, M., Olšovská, K., Yang, X., 2011. Photosynthesis is improved by exogenous calcium in heat-stressed tobacco plants. *J. Plant Physiol.* 168, 2063–2071.
- Tognetti, V.B., Van Aken, O., Morreel, K., Vandenbroucke, K., van de Cotte, B., De Clercq, I., Chiwocha, S., Fenske, R., Prinsen, E., Boerjan, W., Genty, B., Stubbs, K.A., Inzé, D., Van Breusegem, F., 2010. Perturbation in indole-3-butyric acid homeostasis by the UDP-glucosyltransferase *UTG74E2* modulates *Arabidopsis* architecture and water stress tolerance. *Plant Cell* 22, 2660–2679.
- Torres, M.A., Jones, J.D.G., Dangl, J.L., 2005. Pathogen-induced, NADPH oxidase-derived

- reactive oxygen intermediates suppress spread of cell death in *Arabidopsis thaliana*. *Nat. Genet.* 37, 1130–1134.
- Tripathi, D., Jiang, Y.-L., Kumar, D., 2010. SABP2, a methyl salicylate esterase is required for the systemic acquired resistance induced by acibenzolar-S-methyl in plants. *FEBS Lett.* 584, 3458–3463.
- Tsukagoshi, H., Busch, W., Benfey, P.N., 2010. Transcriptional regulation of ROS controls transition from proliferation to differentiation in the root. *Cell* 143, 606–616.
- Ugena, L., Hýlová, A., Podlešáková, K., Humplik, J.F., Doležal, K., De Diego, N., Spíchal, L., 2018. Characterization of biostimulant mode of action using novel multi-trait high-throughput screening of *Arabidopsis* germination and rosette growth. *Front. Plant Sci.* 9, 1327.
- Vardhini, B.V., Anjum, N.A., 2015. Brassinosteroids make plant life easier under abiotic stresses mainly by modulating major components of antioxidant defense system. *Front. Environ. Sci.* 2, 67.
- Wally, O.S.D., Critchley, A.T., Hiltz, D., Craigie, J.S., Han, X., Zaharia, L.I., Abrams, S.R., Prithiviraj, B., 2013. Regulation of phytohormone biosynthesis and accumulation in *Arabidopsis* following treatment with commercial extract from the marine macroalga *Ascophyllum nodosum*. *J. Plant Growth Regul.* 32, 324–339.
- Waltz, E., 2014. Beating the heat. *Nat. Biotechnol.* 32, 610–613.
- Wang, Q., An, B., Wei, Y., Reiter, R.J., Shi, H., Luo, H., He, C., 2016. Melatonin regulates root meristem by repressing auxin synthesis and polar auxin transport in *Arabidopsis*. *Front. Plant Sci.* 7, 1882.
- Waszczak, C., Akter, S., Jacques, S., Huang, J., Messens, J., Van Breusegem, F., 2015. Oxidative post-translational modifications of cysteine residues in plant signal transduction. *J. Exp. Bot.* 66, 2923–2934.
- Wei, J., Li, C., Li, Y., Jiang, G., Cheng, G., Zheng, Y., 2013. Effects of external potassium (K) supply on drought tolerances of two contrasting winter wheat cultivars. *PLoS One* 8, e69737.
- Xu, F.J., Jin, C.W., Liu, W.J., Zhang, Y.S., Lin, X.Y., 2011. Pretreatment with H₂O₂ alleviates aluminum-induced oxidative stress in wheat seedlings. *J. Integr. Plant Biol.* 53, 44–53.
- Yakhin, O.I., Lubyantsev, A.A., Yakhin, I.A., Brown, P.H., 2017. Biostimulants in plant science: a global perspective. *Front. Plant Sci.* 7, 2049.
- Yang, X., Lu, C., 2005. Photosynthesis is improved by exogenous glycinebetaine in salt-stressed maize plants. *Physiol. Plant.* 124, 343–352.
- Zheng, X.F.S., Chan, T.-F., 2002. Chemical genomics: A systematic approach in biological research and drug discovery. *Curr. Issues Mol. Biol.* 4, 33–43.
- Zhu, Z., Wei, G., Li, J., Qian, Q., Yu, J., 2004. Silicon alleviates salt stress and increases antioxidant enzymes activity in leaves of salt-stressed cucumber (*Cucumis sativus* L.). *Plant Sci.* 167, 527–533.
- Zwack, P.J., De Clercq, I., Howton, T.C., Hallmark, H.T., Hurny, A., Keshishian, E.A., Parish, A.M., Benkova, E., Mukhtar, M.S., Van Breusegem, F., Rashotte, A.M., 2016. Cytokinin Response Factor 6 represses cytokinin-associated genes during oxidative stress. *Plant Physiol.* 172, 1249–1258.