Review

Chemical Priming of Plants Against Multiple Abiotic Stresses: Mission Possible?

Andreas Savvides,^{1,2} Shawkat Ali,³ Mark Tester,³ and Vasileios Fotopoulos^{1,*}

Crop plants are subjected to multiple abiotic stresses during their lifespan that greatly reduce productivity and threaten global food security. Recent research suggests that plants can be primed by chemical compounds to better tolerate different abiotic stresses. Chemical priming is a promising field in plant stress physiology and crop stress management. We review here promising chemical agents such as sodium nitroprusside, hydrogen peroxide, sodium hydrosulfide, melatonin, and polyamines that can potentially confer enhanced tolerance when plants are exposed to multiple abiotic stresses. The challenges and opportunities of chemical priming are addressed, with the aim to boost future research towards effective application in crop stress management.

Exploring and Exploiting a Physiological Phenomenon

Abiotic stresses such as salinity, drought, flooding, heat, cold, freezing, excess light, UV radiation, and heavy metal toxicity have a significant impact on plant growth and crop yield worldwide. Anthropogenic contributions due to industrialization and urbanization [1] and climate change [2] continue to exacerbate the detrimental effects of these stresses on crop yield, thereby threatening global food security [3]. Plants grown under field conditions may well be exposed during their lifespan to a range of different abiotic stresses that occur sequentially or simultaneously. A combination of different abiotic stresses may act synergistically or additively in terms of impact on plant growth. Stress phenomena that occur simultaneously, such as salinity and heat [4], drought and heat [5], and heavy metals and heat [6], have been shown to be more detrimental to plant growth than each of these stresses individually. Consequently, considerable attention is now directed towards enhancing plant tolerance to multiple biotic stresses [7].

Different methodologies have been employed aiming at enhancing multiple stress tolerance; some are particularly time-consuming (e.g., conventional breeding) and others are currently unacceptable in many countries around the world (e.g., plant genetic modification). As an alternative, plants can be 'prepared' to more successfully tolerate future biotic and abiotic stress conditions through priming. Plant priming (also known as sensitization or **hardening**; see Glossary) can be initiated naturally in response to an environmental stress event that acts as a cue indicating an increased probability of facing that specific stress factor in the future [8]. Following perception of the cue, plants enter the **primed state** (PS) in which activation of protection responses is faster, stronger, or both when a stress pressure is encountered [9–11]. Compared with non-primed plants, the diminished impact of stress exposure on the physiology and growth of primed plants can be remarkable (Figure 1A). Interestingly, plants can also enter the PS by chemical priming, which involves exposure to a priming agent such as a natural or

Trends

Plant priming using chemical agents such as sodium nitroprusside, hydrogen peroxide, sodium hydrosulfide, melatonin, and polyamines enhances plant tolerance to different abiotic stresses, improving cellular homeostasis and plant growth under stress conditions.

Commonly employed chemical priming agents share components in their modes of action.

When used against different abiotic stresses, the modes of action of a chemical agent show similarities but also distinct specificities.

Chemical priming through using selected chemical agents is a promising tool against various individual or combined abiotic stresses.

The efficiency of chemical priming depends highly on the mode of application.

¹Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603 Lemesos, Cyprus

²Agrisearch Innovations Ltd, 2108 Nicosia, Cyprus

³Center for Desert Agriculture, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia

*Correspondence: vasilis.fotopoulos@cut.ac.cy (V. Fotopoulos).

synthetic chemical compound. Chemical priming presents opportunities for more effective use of plant priming in plant stress physiology studies and crop stress management.

Use of chemical compounds as priming agents has been found to improve plant tolerance significantly in various crop and non-crop species against a range of different individually applied abiotic stresses (Table S1 in the supplemental information online). Although few studies have employed chemical agents against combined abiotic stresses, these studies are yielding promising results [12,13]. Previous reviews have mainly focused on priming against biotic stresses [9,14] or on the use of individual chemical agents against different abiotic and/or biotic stresses [15,16]. However, not much attention has been paid yet to the use of chemical priming against different, sole and combined, abiotic stresses and its effective application in crop stress management. We discuss key findings from the latest research on chemical priming agents that suggest they could be used to reduce the effects of abiotic stresses in commercial crops. The ability of these agents to enhance tolerance to different abiotic stresses, and the specific aspects of their mode of action summarized in this review, suggest great potential for their use against multiple abiotic stresses. We conclude that further research towards fully elucidating their mode of action would be of great significance for plant stress physiology research. In addition, focusing on specific challenges and opportunities related to this technology, such as the mode of application, new methodologies (e.g., seed priming), and the potential impacts of chemical priming on the environment, would result in the optimum and rapid establishment of this technology in crop stress management.

Promising Chemicals for Enhancing Multi-Stress Tolerance

Many types of molecules have the potential to act under specific conditions as a priming agent against a range of different abiotic stresses [14]. A review of the relevant literature reveals a vast range, including amino acids (e.g., proline [17]), hormones (e.g., salicylic acid [18]), **reactive oxygen–nitrogen–sulfur species** (RONSS [19,20]), and even water (i.e., hydropriming [21]). Some of these agents are effective in inducing plant tolerance to various individually applied abiotic stresses (Table S1).

RONSS

Reactive species have long attracted attention in plant science on the basis of both their protective as well as damaging effects. The priming effect of these molecules is exerted largely through their cellular signaling function, which has been linked to the regulation of transcriptional as well as post-translational phenomena. A multitude of reports demonstrate the priming effect against various abiotic stress factors of reactive oxygen species (ROS), particularly hydrogen peroxide (H_2O_2), when applied in low concentrations. Similar observations have been made for reactive nitrogen species (RNS), nitric oxide (NO) being the most commonly studied representative of this group of compounds. NO is donated indirectly by chemical donors such as **sodium nitroprusside** (SNP). However, most attention is focused on hydrogen sulfide (H_2S), attested by the existence of numerous reports on the potential use of H_2S as a priming agent against virtually any type of abiotic stress [16]. Particularly interesting was the establishment of the existence of a complex interaction between ROS and RNS, in which both reactive species are used by plants as signal transduction molecules during basic biological and cellular processes [22]; it has recently been suggested that reactive sulfur species (RSS) may also play a role in this interaction [23]. Interestingly, it should be noted that RNS and RSS applied at low concentrations have shown growth-promoting properties under non-stress conditions [16,24].

Naturally-Occurring Metabolites

Another category of priming agents currently attracting attention comprises naturally occurring metabolites, including vitamins (e.g., ascorbate [25]) and hormones. The priming function of these molecules may be exerted through indirect mechanisms, such as via osmoprotection (e.

Glossary

Hardening: or cold hardening, the process whereby exposure to low but non-lethal temperatures increases plant tolerance (or the capacity to survive) to subsequent low or freezing temperatures that would be fatal without the hardening treatment.

Heat-shock proteins (HSPs):

molecular chaperones that play a role in preventing protein aggregation by assisting refolding, import, and translocation, and are involved in signal transduction and transcriptional activation [96].

Melatonin (*N*-acetyl-5-

methoxytryptamine; Mel): has many physiological roles in animals and plants. In plants, it acts as a growth regulator, an antioxidant, and has the capacity to fortify plants against abiotic stresses [33].

Polyamines (PAs): nitrogen-containing compounds of low molecular weight. Spermine (Spm), putrescine (Put), and spermidine (Spd) are considered as the most abundant PAs, and these can also be found in plants. They have important roles not only in plant growth and development but also in plant stress responses [97].

Primed state (PS): the state during which the plants can show enhanced tolerance to a biotic or abiotic stress.

Reactive oxygen–nitrogen–sulfur species (RONSS): chemically reactive molecules containing oxygen [e.g., superoxide radicals ($O^{\bullet-}_2$), hydrogen peroxide (H_2O_2)], nitrogen [e.g., nitric oxide (NO)], or sulfur [e.g., hydrogen sulfide (H_2S)]. They are natural byproducts of the normal metabolism of oxygen, nitrogen, and sulfur and have important roles in cell signaling and homeostasis. Under stress, their concentrations increase dramatically, which may result in damage to cell structures and eventually cell death.

Sodium nitroprusside (SNP): an inorganic compound $Na_2[Fe(CN)_5NO]$ that is used as an NO donor (i.e., NO releasing compound) not only for medical uses but also for plant stress physiology research.

Trends in Plant Science

Figure 1. Priming as a Means to Induce Stress Tolerance. (A) Pretreatment using a priming-inducing stimulus (e.g., chemical compound) results in enhanced cell tolerance and amelioration of stress-induced plant growth inhibition. (B) Chemical agents applied at organ level (e.g., roots) activate signaling pathways potentially resulting in the systemic accumulation of dormant tolerance signals. On exposure to stress, primed plants show enhanced tolerance-related responses [e.g., reactive oxygen species (ROS) detoxification, osmoprotection, protein stabilization, and ion homeostasis] that are at least partly regulated by different molecular mechanisms (e.g., transcriptional regulation, post-translational modifications); enhanced plant tolerance improves physiological homeostasis and plant growth. Abbreviations: CBF, C-repeat binding factor; DREB, dehydration-responsive element binding; MYB, myeloblastosis oncogene.

g., trehalose [26]), or even through direct antioxidant properties (e.g., glutathione [27]). A great body of evidence exists, however, on the putative protective functions of polyamines (PAs) under various stress conditions; this is possibly due to the fact that PAs bind strongly to many types of negatively charged proteins, including numerous defense enzymes and, in doing so, directly modulate the activities of these proteins [28]. Recent evidence suggests that PAs and NO have

overlapping physiological functions in plants [29], while it was demonstrated that enhanced NO bioavailability leads to altered PA homeostasis [30]. In addition, the role of PAs in NO production has been indicated [31]. Furthermore, it has long been shown that PAs are linked to ROS homeostasis through their catabolic pathway [32]. These observations suggest a clear link between PAs and nitro-oxidative signaling which could regulate stress responses in plants. Another natural metabolite which is being evaluated by numerous researchers as a biostimulant or growth-promoting molecule is the indoleamine molecule **melatonin** (Mel), which is also involved in multiple physiological processes in plants [33]. The priming potential of Mel as an exogenously applied agent is the result of its dual mode of action as both a direct antioxidant molecule and also a trigger of antioxidant responses in plants [34]; the evidence indicates that environmental stress can increase the level of endogenous Mel in plants as a protective response [15].

Synthetic Agents

Synthetic chemistry can also be employed to produce potent priming agents. An interesting example is that of the strobilurins, which belong to a group of agrochemical fungicides that block electron transport in complex III of the mitochondrial respiratory chain, and often stimulate the fungal mitochondrial alternative oxidase during respiration [35]. Strobilurins also trigger a positive effect on plant growth and function. Although the exact mechanism remains unknown, this is likely to be through an interaction with electron transfer in plant mitochondria [36]. It was demonstrated recently that the synthetic fungicide kresoxim-methyl, a modification of the naturally occurring compound strobilurin A, acts as an effective priming agent in the prevention of salt and drought stress in *Medicago truncatula* plants; this ameliorative effect is achieved through suppressing proteolysis, among other pathways [37]. Synthetic priming agents can also be in the form of simultaneous donors of compounds with recorded priming effects. An example is that of NOSH-aspirin (NBS-1120), a novel nitric oxide- and hydrogen sulfide-releasing hybrid, which was initially formulated as an anticancer drug but also displays ameliorative effects against abiotic stress conditions in plants [38,39].

Promising Chemicals: Assessment of Common Components of the Mode of Action of Agents

Plants pretreated at different developmental stages (e.g., as germinating seed, or in the vegetative or reproductive stage) with SNP (NO donor), H₂O₂, NaHS (H₂S donor), Mel, or PAs show enhanced systemic acquired tolerance, and exposure to various abiotic stresses has less impact on their physiology and growth than on non-pretreated plants (Table S1 and Figure 1). The mode of action of these compounds as priming agents remains unclear; however, the evidence regarding common tolerance activation sites and signaling pathways that appear related to enhanced tolerance against different abiotic stresses strongly supports their potential for enhanced multi-stress tolerance.

NO, H₂O₂, H₂S, Mel, and PAs (or their functional analogs) are all endogenous plant molecules with regulatory functions in plant abiotic stress tolerance [22,23,31,33]. Exogenous application of low concentrations of these molecules initially causes an increase in their endogenous concentrations, but without subsequent inhibition of plant growth [40–44]. Increased endogenous concentrations of these compounds have also been indicated in non-primed plants that have been exposed to different abiotic stresses but, in this case, inhibition of plant growth does follow [20,43–46]. On this basis, it could be concluded that the pretreatment of plants with chemical agents can initiate a mild stress cue, similar to an acclimation response that leads eventually to enhanced tolerance when the plant is exposed to an abiotic challenge.

On exposure to abiotic stresses, primed plants show enhanced tolerance-related responses that are either common across a range of stresses or are stress-specific. Oxidative stress accompanies almost all abiotic and biotic stresses in plants, and develops as a result of ROS

accumulation [47]. ROS accumulation damages proteins, lipids, carbohydrates, and DNA, leading eventually to membrane damage and cell death [48]. Combination of synergistically or additively acting abiotic stresses results in even more severe oxidative stress [6,49]. Consequently, the control of ROS cellular homeostasis is considered to be a key element underpinning enhanced multi-stress tolerance [50]. When exposed to a range of individual and combined abiotic stresses, plants pretreated with the chemical agents discussed above have been shown to have lower ROS (H_2O_2 , $\text{O}_2^{\bullet-}$) accumulation (Table S1). The mitigation of oxidative stress as a result of reduced ROS accumulation following chemical priming has been related either to the enhanced capacity of the antioxidant machinery or to the direct scavenging of ROS by the agents themselves (e.g., Mel [51]). The capacity of the antioxidant machinery depends on enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaiacol peroxidase, GOPX and glutathione-S-transferase, GST) and non-enzymatic antioxidant systems (ascorbic acid, ASH; glutathione, GSH) that work in concert to control ROS [48]. When exposed to different abiotic stresses, whether applied individually or in combination, primed plants show enhanced enzymatic (activity) and/or non-enzymatic capacity (ASH and GSH redox state) compared to non-primed plants (Table S1).

The priming effects on antioxidant capacity have been found to correlate with increased transcript levels of enzymatic antioxidants [20,41,52–54] and/or ascorbate and glutathione biosynthesis components [52,54,55], indicating complex transcriptional regulation of the antioxidant machinery. Furthermore, some reports have focused on proteomic approaches whereby post-translational modification (PTM) phenomena such as tyrosine nitration, carbonylation, and S-nitrosylation events were also identified in response to chemical priming [56,57]. Interestingly, it was shown that application of H_2O_2 or application of SNP led to RNS-linked PTMs (i.e., S-nitrosylation) or ROS-linked PTMs (i.e., carbonylation), respectively; this observation provides further support for the interaction between these molecules [56]. The reduced ROS accumulation arising from the enhanced plant antioxidant capacity in primed plants was further accompanied by reduced malondialdehyde (MDA) content and electrolyte leakage (EL); these observations indicate lower levels of lipid peroxidation and the preservation of membrane integrity (Table S1).

Exposure of seeds to abiotic stresses, such as salinity, drought, heavy metals, and chilling, results in lower seed viability, reduced germination, and poor seedling establishment [53,58–61]. In addition to enhanced antioxidant capacity, primed seeds show rapid enhancement of α -amylase and/or β -amylase activities, this leads to enhanced starch degradation and sugar accumulation, which in turn results in higher respiration rates, seed viability, germination rates, and seedling establishment than for non-primed seeds [53,58–61]. Recent evidence suggests that, under conditions of water stress, chemical agents such as PAs may not only enhance the activities of α -amylase and/or β -amylase but may also induce the *de novo* biosynthesis of β -amylase through elevating β -amylase gene expression at the early stages of seed germination [61].

Drought, salinity, chilling, and freezing result in osmotic stress that can lead to turgor loss and severe inhibition of growth. Plants sustain their osmotic homeostasis partly through accumulation of osmoregulatory compounds (e.g., free proline and other amino acids, soluble carbohydrates, soluble proteins) [62]. Osmoregulation leads to maintenance of cell and plant turgor, stomatal opening, and thus enhanced photosynthesis and growth. Primed plants have been shown to have higher tissue levels of free proline and/or soluble carbohydrates and/or soluble protein than non-primed plants when exposed to drought [43,63], salinity [43], chilling [51,64,65], or freezing [43,66]. Upon exposure to drought stress, soybean plants pretreated with H_2O_2 contained higher levels than non-primed plants of myo-inositol and galactinol, two oligosaccharides that are considered to be osmoprotectants and ROS scavengers. The enhanced level of these two oligosaccharides was correlated with an induction in the

mRNA levels of D-myo-inositol 3-phosphate synthase 2 (*GmMIPS2*) and galactinol synthase (*GalS*), which encode key enzymes for the biosynthesis of the oligosaccharides [67]. Rice plants pretreated with H₂O₂ or SNP showed higher expression than non-primed plants of transcripts for Δ^1 -pyrroline-5-carboxylate synthase (*P5CS*), an enzyme involved in proline biosynthesis [68,69].

When exposed to different abiotic stresses, chemically primed plants have also shown enhanced tolerance responses that are stress-specific. Salinity imposes ionic stress (salt toxicity) in addition to osmotic stress [70]. Ionic stress results from Na⁺ accumulation in the cytosol and/or disruption of K⁺ homeostasis that results in an imbalance in Na⁺/K⁺ [71]. Compared with non-primed plants, primed seeds or plants showed either lower Na⁺ and/or higher K⁺ contents resulting in a lower Na⁺/K⁺ when exposed to salinity [42,58,72,73], suggesting that priming increased the regulation of ion uptake. Another study showed that pretreatment of *Malus hupehensis* with Mel results in lower Na⁺ and higher K⁺ content in the leaves after exposure to salinity stress [74]. The observed alterations in Na⁺ and K⁺ content were accompanied by increased transcript levels of *MdNHX1* (a vacuolar Na⁺/H⁺ antiporter) and *MdAKT1* (an inward-rectifying channel that catalyzes K⁺ influx upon plasma membrane hyperpolarization). This suggests increased sequestration of Na⁺ in vacuoles and increased accumulation of K⁺ in the cytosol, resulting in a lower cytoplasmic Na⁺/K⁺ ratio. Consequently, more than one salinity tolerance activation mechanisms may be upregulated by chemical priming.

Chemically primed plants have also been shown to have enhanced tolerance to the over-accumulation of heavy metals (e.g., Al, Cd, Cr, and Cu) [41,60,75–79]. In addition to enhanced antioxidant capacity, primed plants showed reduced uptake of heavy metals [41], altered distribution of metals within the plant [75], or increased sequestration into cell vacuoles [76] compared to non-primed plants.

Heat stress results in protein denaturation and aggregation, increased membrane fluidity, inactivation of enzymes, inhibition of protein synthesis, and oxidative stress [80]. Plants pretreated with chemical agents have been shown to have enhanced heat tolerance compared with non-primed plants [20,40,69,81–84]. In strawberry plants pretreated with NaHS, enhanced thermotolerance was attributed to prolonged upregulation of **heat-shock proteins** (HSPs; *HSP70*, *HSP80*, *HSP90*) transcripts and enhanced antioxidant capacity [20].

Pretreatment of plants with selected chemical agents at any developmental stage improves tolerance on exposure to almost any abiotic challenge. The hyperactivation of tolerance mechanisms, such as detoxification, osmoprotection, and protein and ionic homeostasis (Figure 1B), is accompanied by significant amelioration of stress impacts on plant physiology and growth (Table S1). As a result, chemical priming improves agronomic traits such as yield. For instance, pretreatment of a salt-sensitive and a salt-tolerant rice cultivar with PAs resulted in 62% and 16% increases in grain yield, respectively, under salt stress [73]. The actual events resulting in this remarkable effect and the sequence in which they take place during the PS (that is, between the pretreatment with a chemical agent and exposure to stress) remain unclear and require further investigation. For instance, significant interest is directed towards the study of the molecular mechanisms where chemically primed plants accumulate dormant tolerance signals that could act as memory imprints during the establishment of priming (Figure 1B). Use of state of the art systems-biology approaches will contribute greatly to full deciphering of the protective mode of action of these compounds.

Challenges and Opportunities

Recent research has revealed challenges as well as opportunities for the employment of chemical priming as a useful tool in plant stress physiology and as a technology applicable

in crop stress management. Chemical agents can be effective at very low concentrations, which would suggest low costs of application, but can be deleterious at higher concentrations [40,83,85–87]. For example, NO and H₂S have inhibiting effects on the mitochondrial electron transport chain when applied at high concentrations [88]. The hazardous effects of these chemical agents at higher concentrations indicate a need for development of smart application technologies enabling application of the most appropriate concentration (Box 1).

Pretreatment with specific chemical agents results in enhanced systemic tolerance even when the agent is applied only on a particular organ. For instance, several studies have shown that root pretreatment will enhance vegetative tissue tolerance when plants are exposed to various abiotic stresses (Table S1). This systemic improvement of plant tolerance implies that there is considerable flexibility in terms of the method of application of the chemical agent, which can be tailored to other crop management practices, such as irrigation.

A discrepancy exists in the findings of different studies on the duration of the PS. In most studies plants were exposed to the abiotic stress immediately following pretreatment with a chemical agent (Table S1). This does not permit quantification of the duration of the PS, which would be of great significance for crop stress management (see Outstanding Questions). Optimally, the PS should last throughout the whole cultivation period, or at least during periods with a high frequency of abiotic stresses. Recent studies revealed that strawberry plants treated by root incubation with either SNP, H₂O₂, or NaHS for 2 days, and then immediately subjected to stress, have enhanced tolerance, whereas pretreated plants subjected to salinity or drought stress 7 days (for SNP and H₂O₂) and 3 days (for NaHS) after pretreatment were found to have no or only moderately enhanced tolerance, respectively [52,54]. Another study demonstrated that tomato plants pretreated with H₂O₂ (within the nutrient solution) showed enhanced tolerance when exposed to chilling stress 4 days after pretreatment [65]. In addition, soybean plants pretreated (using foliar spraying) with the **polyamine** spermine showed enhanced tolerance when exposed to drought 14 days after pretreatment [89]. It appears, therefore, that a plant has the capacity to be maintained in PS after treatment with a chemical agent for no more than few days (or perhaps longer), but this depends on parameters that are currently unknown. Further research needs to be directed at the mechanisms underpinning the PS and its duration.

It has been proposed that dormant signaling molecules, or the proteins involved in their synthesis, are accumulated during priming by pre-exposure to an abiotic or biotic stress factor; these molecules are then recruited upon subsequent stress exposure, resulting in a faster and stronger defense response compared to non-primed plants [90]. Mechanisms underlying a more persistent and dynamic stress- or priming-induced memory are epigenetic modifications. Recently, it has been suggested that epigenetic modifications are involved in priming phenomena against abiotic challenges [10]. Such modifications may lead to long-lasting memory and potentially even transgenerational enhanced protection against abiotic stresses, possibly

Box 1. Mode of Application

An important element to consider in priming approaches is the optimal mode of application. There are often contradictory findings in such studies, which can be attributed at least in part to the great variability in the method of treatment with various compounds. A recent study demonstrated that NO donation efficiency with SNP in *Medicago truncatula* plants depended significantly on the tissue of application and the method used; among other approaches, hydroponics, leaf spraying, root watering, and vacuum infiltration were evaluated [98]. The findings indicated that maximum NO levels were induced in leaves by vacuum infiltration, whereas hydroponic application resulted in maximum NO levels for roots, the latter approach being optimal for a whole-plant study in which leaf tissue was also analyzed. The choice of optimal mode of application is linked to the systemic protective effects often observed with priming agents, and to whether the stress factor is imposed distal to the tissue examined (e.g., the response of leaves to a salinity stress imposed on the roots). Thus, preliminary optimization is crucial before adopting a mode of priming because this may be compound-, tissue-, or even plant-specific.

through modification of histones, including the shortening and fractionation of H3K27me3 (histone H3 trimethylated on lysine 27) islands, and DNA methylation [90]. The study of epigenetics is a new direction in chemical priming research that should be pursued with more vigor.

Innovative application technologies such as seed priming (Box 2) have recently attracted much attention [91]. Seed pretreatment, either by coating (Mel [55]) or pre-soaking (polyamines [72]), was found to maintain enhanced tolerance when plants were exposed to stress conditions 7 and 17 days later, respectively. Application of chemical priming at this stage would seem to be desirable because it would reduce the costs associated with later priming treatments in the field (e.g., by spraying or irrigation) and thus increase the benefits for farmers. Further research will be necessary to assess the duration in which a plant can be maintained in its PS after seed priming.

The use of chemical priming agents to achieve enhanced plant tolerance delivers a more consistent and less variable priming response, which renders the approach accessible to molecular and genetic studies [90]. Pharmacological studies, such as the use of inhibitors, are also crucial to confirm the protective action of specific priming agents. This is particularly important when different protective compounds are applied simultaneously, which requires elucidation of any synergistic/additive/inhibitory combined effects (e.g., NOSH-A [40]), and also in the case where donors are known to induce other side effects, such as the release of cyanide by the NO donor, SNP [92].

Possible environmental impacts of the use of chemical agents in plant priming have not yet attracted attention. The local plant microenvironment, such as microorganism populations (e.g., plants endophytes, microorganisms inhabiting the rhizosphere) and pollinators, or the

Box 2. Seed Priming

Uniform and efficient seed germination and establishment of early seedlings are crucial components for agricultural crop production under stressful environmental conditions [99]. Abiotic stresses such as salinity and drought adversely affect seed germination and young seedling establishment either by reducing external water potentials or through the accumulation of toxic ions such as Na⁺ and Cl⁻ in the seedling tissues [100]. Chemical seed priming, which is the pre-sowing treatment of seeds with synthetic or natural compounds with the aim of increasing the uniformity and vigor of seedlings and enhancing the tolerance of plants to stress factors, affects plant performance in two different ways. (i) Primed seeds are at a developmentally advanced physiological stage compared to non-primed seeds, and this early start improves germination rates and early crop establishment. (ii) The chemical exposure enhances stress tolerance in a manner similar to the way in which plant priming improves tolerance through memory.

Seed priming improves seed viability by inducing the DNA repair machinery to repair damaged DNA, which occurs during seed aging [101], while it also results in the activation of respiration and ATP production during the imbibition stage [102]. In some seed-priming treatments, seeds absorb water slowly owing to the lower water potential of the priming solution. As a result, the seeds have more time to complete DNA repair processes and also have reduced cellular damage, which often occurs during rapid seed rehydration during germination of unprimed seeds [103]. Chemical compounds that are routinely used for seed priming stimulate the generation of signaling molecules or transcription factors in an inactive form, thus resulting in a faster or stronger activation of defense responses upon exposure to stress [104]. In addition, such agents have been shown to reduce oxidative stresses that are associated with most abiotic stresses [105], in a similar fashion to their mode of action in seedlings and more mature plants. Importantly, the priming effect that occurs when chemical compounds are applied to seeds is prolonged into later plant developmental stages. For example, 7-day old soybean seedlings subjected to drought showed enhanced tolerance when their seeds were previously coated with Mel [55]. Similar results were observed when wheat seeds were treated with H₂O₂ [63]. These studies support the notion that seed priming may be a method of improving tolerance through memory (potentially through epigenetic modifications).

In line with increasing commercial interest, because application of chemical priming at this stage seems desirable given reduced application costs and prolonged potential protection, several germplasm banks and seed companies are using different chemical agents to enhance seed germination and early seedling establishment [106], such as the Seed Conservation Department, Royal Botanic Gardens Kew (UK; www.kew.org), Seed Dynamics Inc. (USA; <http://seeddynamics.com>), Benary (DE; www.benary.com), Brasil Sementes e Tecnologia Ltd (BR; www.brasilesementes.com.br), Germains Seed Technology (UK; <http://germains.com/>), Incotec (NL; www.incotec.com/). However, our understanding of the molecular and cellular mechanisms of this form of priming remains limited.

environment in a broader sense, such as aquatic habitats, may be negatively affected by the use of chemical compounds in agriculture. For example, the cyanide gas released when SNP is applied to plants [92] is considered as a 'priority pollutant', especially in aquatic environments [93]. The study of possible environmental impacts by the use of chemical priming agents is necessary for selecting environment-friendly agents. The mode of application (Box 1) should be carefully considered in such studies. For example, the selection of seed priming (Box 2) over other modes of application (e.g., canopy spraying or root watering) may prevent negative effects of a potentially hazardous chemical compound on the environment.

Concluding Remarks and Future Perspectives

The action of chemical compounds such as NO, H₂O₂, H₂S, Mel, and PAs as abiotic stress signaling molecules and as effective chemical priming agents against different abiotic stresses has previously been established. However, few studies have tested these chemical agents against combined abiotic stresses. Known aspects of the mode of action of these chemical agents suggest strongly that chemical priming can potentially be used against multiple abiotic stress phenomena that occur in the field; however, further research is required in this area, both on the mode of action and on the effects on yield.

Recent research on chemical priming has provided further knowledge of the complex mode of action of specific signaling molecules in plant stress tolerance. Further pharmacological research (e.g., to test the use of chemical agents in combination with RONSS scavengers or agent inhibitors) and time-series studies in combination with proteomics, transcriptomics, and metabolomics studies will be invaluable in further unraveling the complex machinery of chemical priming and systemic acquired tolerance. In addition, feasibility studies on the use of chemical priming agents in practice should be performed. Low cost (e.g., SNP [94]), low concentration chemical compounds [95], in combination with the remarkable yield enhancement already seen to occur [73], would indicate a low cost-benefit ratio; however, other parameters such as the mode of application, the integration of chemical priming in crop management practices, and the potential environmental impacts of such an approach also need to be considered.

The use of chemical priming to enhance plant tolerance against multiple abiotic stresses is highly promising. Further research in the directions proposed in this review (see Outstanding Questions) would greatly support the establishment of this technology in crop stress management in the near future.

Acknowledgments

The support of Cyprus University of Technology (V.F.) and King Abdullah University of Science and Technology (M.T.) is gratefully acknowledged. The authors would like to thank Dr Christina Morris for professional editing of this manuscript.

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at [doi:10.1016/j.tplants.2015.11.003](https://doi.org/10.1016/j.tplants.2015.11.003).

References

- Nagajyoti, P.C. *et al.* (2010) Heavy metals, occurrence and toxicity for plants: a review. *Environ. Chem. Lett.* 8, 199–216
- Lobell, D.B. and Field, C.B. (2007) Global scale climate–crop yield relationships and the impacts of recent warming. *Environ. Res. Lett.* 2, 014002
- Reddy, P.P. (2015) Impacts of climate change on agriculture. In *Climate Resilient Agriculture for Ensuring Food Security*, pp. 43–90, Springer India
- Silva, E. *et al.* (2013) Contrasting physiological responses of *Jatropha curcas* plants to single and combined stresses of salinity and heat. *J. Plant Growth Regul.* 32, 159–169
- Perdomo, J.A. *et al.* (2014) Effects of long-term individual and combined water and temperature stress on the growth of rice, wheat and maize: relationship with morphological and physiological acclimation. *Physiologia Plantarum* 155, 149–165
- Zhao, F.-Y. *et al.* (2009) Different responses of plant growth and antioxidant system to the combination of cadmium and heat stress in transgenic and non-transgenic rice. *J. Integr. Plant Biol.* 51, 942–950
- Suzuki, N. *et al.* (2014) Abiotic and biotic stress combinations. *New Phytol.* 203, 32–43

Outstanding Questions

Can commonly used and/or novel chemical compounds be as effective as priming agents against combined abiotic stresses compared to individual abiotic challenges?

Which molecular mechanisms determine the duration of primed state and consequently the length of the period during which a seed or a plant maintains its ability for enhanced tolerance?

Are there significant genetic differences in responsiveness to priming? In what species is priming most effective? In what species does priming have the biggest economic impact?

Can priming with chemical compounds lead to transgenerational protection against abiotic stress and, if so, which molecular mechanisms govern this process?

8. Filippou, P. *et al.* (2013) Plant acclimation to environmental stress using priming agents. In *Plant Acclimation to Environmental Stress* (Tuteja, N. and Singh Gill, S., eds), pp. 1–27, Springer
9. Conrath, U. (2009) Priming of induced plant defense responses. In *Advances in Botanical Research* (Loon, L.C.V., ed.), pp. 361–395, Academic Press
10. Sani, E. *et al.* (2013) Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. *Genome Biol.* 14, R59
11. Ellouzi, H. *et al.* (2013) Drought and cadmium may be as effective as salinity in conferring subsequent salt stress tolerance in *Cakile maritima*. *Planta* 237, 1311–1323
12. Fu, X.-Z. *et al.* (2014) Exogenous spermine pretreatment confers tolerance to combined high-temperature and drought stress in vitro in trifoliate orange seedlings via modulation of antioxidative capacity and expression of stress-related genes. *Biotechnol. Biotechnol. Equip.* 28, 192–198
13. Tian, X.R. and Lei, Y.B. (2007) Physiological responses of wheat seedlings to drought and UV-B radiation. Effect of exogenous sodium nitroprusside application. *Russ. J. Plant Physiol.* 54, 676–682
14. Beckers, G.J.M. and Conrath, U. (2007) Priming for stress resistance: from the lab to the field. *Curr. Opin. Plant Biol.* 10, 425–431
15. Paredes, S.D. *et al.* (2009) Phytomelatonin: a review. *J. Exp. Bot.* 60, 57–69
16. Fotopoulos, V. *et al.* (2015) Hydrogen sulphide: a versatile tool for the regulation of growth and defence responses in horticultural crops. *J. Horticultural Sci. Biotechnol.* 90, 227–234
17. Islam, M.M. *et al.* (2009) Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. *J. Plant Physiol.* 166, 1587–1597
18. Li, T. *et al.* (2014) Salicylic acid alleviates the adverse effects of salt stress in *Torreya grandis* cv. merrillii seedlings by activating photosynthesis and enhancing antioxidant systems. *PLoS ONE* 9, e109492
19. Tanou, G. *et al.* (2009) Hydrogen peroxide- and nitric oxide-induced systemic antioxidant prime-like activity under NaCl-stress and stress-free conditions in citrus plants. *J. Plant Physiol.* 166, 1904–1913
20. Christou, A. *et al.* (2014) Sodium hydrosulfide induces systemic thermotolerance to strawberry plants through transcriptional regulation of heat shock proteins and aquaporin. *BMC Plant Biol.* 14, 42
21. Casenave, E.C. and Toselli, M.E. (2007) Hydropriming as a pre-treatment for cotton germination under thermal and water stress conditions. *Seed Sci. Technol.* 35, 88–98
22. Molassiotis, A. and Fotopoulos, V. (2011) Oxidative and nitrosative signaling in plants. *Plant Signal. Behav.* 6, 210–214
23. Calderwood, A. and Kopriva, S. (2014) Hydrogen sulfide in plants: from dissipation of excess sulfur to signaling molecule. *Nitric Oxide* 41, 72–78
24. Gouvêa, C.M.C.P. *et al.* (1997) NO[•]-releasing substances that induce growth elongation in maize root segments. *Plant Growth Regul.* 21, 183–187
25. Athar, H.-u.-R. *et al.* (2008) Exogenously applied ascorbic acid alleviates salt-induced oxidative stress in wheat. *Environ. Exp. Bot.* 63, 224–231
26. Bae, H. *et al.* (2005) Exogenous trehalose alters *Arabidopsis* transcripts involved in cell wall modification, abiotic stress, nitrogen metabolism, and plant defense. *Physiol. Plant.* 125, 114–126
27. Chen, F. *et al.* (2010) Modulation of exogenous glutathione in antioxidant defense system against Cd stress in the two barley genotypes differing in Cd tolerance. *Plant Physiol. Biochem.* 48, 663–672
28. Bouchereau, A. *et al.* (1999) Polyamines and environmental challenges: recent development. *Plant Sci.* 140, 103–125
29. Parra-Lobato, M.C. and Gomez-Jimenez, M.C. (2011) Polyamine-induced modulation of genes involved in ethylene biosynthesis and signalling pathways and nitric oxide production during olive mature fruit abscission. *J. Exp. Bot.* 62, 4447–4465
30. Filippou, P. *et al.* (2013) The nitric oxide donor sodium nitroprusside regulates polyamine and proline metabolism in leaves of *Medicago truncatula* plants. *Free Radic. Biol. Med.* 56, 172–183
31. Minocha, R. *et al.* (2014) Polyamines and abiotic stress in plants: a complex relationship. *Front. Plant Sci.* 5, 175
32. Moschou, P.N. *et al.* (2008) Spermidine exodus and oxidation in the apoplast induced by abiotic stress is responsible for H₂O₂ signatures that direct tolerance responses in tobacco. *Plant Cell Online* 20, 1708–1724
33. Arnao, M.B. and Hernández-Ruiz, J. (2014) Melatonin: plant growth regulator and/or biostimulator during stress? *Trends Plant Sci.* 19, 789–797
34. Zhang, N. *et al.* (2015) Roles of melatonin in abiotic stress resistance in plants. *J. Exp. Bot.* 66, 647–656
35. Sauter, H. (2007) Strobilurins and other complex III inhibitors. *Mod. Crop Prot. Compounds* 2, 457–495
36. Diaz-Espejo, A. *et al.* (2012) The effect of strobilurins on leaf gas exchange, water use efficiency and ABA content in grapevine under field conditions. *J. Plant Physiol.* 169, 379–386
37. Filippou, P. *et al.* (2015) 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.* 66, 516
38. Antoniou, C. *et al.* (2014) P77: Exploring the potential of NOSH-aspirin as a plant priming agent against abiotic stress factors. *Nitric Oxide* 39 (Suppl.), S39
39. Kashfi, K. and Fotopoulos, V. Research Foundation Of The City University Of New York, Cyprus University Of Technology. Method of priming plants against abiotic stress factors and promoting growth, WO2015123273 A1
40. Li, Z.-G. *et al.* (2013) Hydrogen sulphide may be a novel downstream signal molecule in nitric oxide-induced heat tolerance of maize (*Zea mays*[L.] seedlings. *Plant Cell Environ.* 36, 1564–1572
41. Li, L. *et al.* (2012) Roles of hydrogen sulfide and nitric oxide in the alleviation of cadmium-induced oxidative damage in alfalfa seedling roots. *Biometals* 25, 617–631
42. Wahid, A. *et al.* (2007) Pretreatment of seed with H₂O₂ improves salt tolerance of wheat seedlings by alleviation of oxidative damage and expression of stress proteins. *J. Plant Physiol.* 164, 283–294
43. Shi, H. *et al.* (2014) Comparative physiological, metabolomic, and transcriptomic analyses reveal mechanisms of improved abiotic stress resistance in bermudagrass [*Cynodon dactylon* (L.) Pers.] by exogenous melatonin. *J. Exp. Bot.* 66, 681–694
44. Shi, J. *et al.* (2010) Spermine pretreatment confers dehydration tolerance of citrus in vitro plants via modulation of antioxidative capacity and stomatal response. *Tree Physiol.* 30, 914–922
45. Fu, P. *et al.* (2013) Hydrogen sulfide is involved in the chilling stress response in *Vitis vinifera* L. *Acta Soc. Bot. Pol.* 82, 295–302
46. Hu, X. *et al.* (2012) Effect of exogenous spermidine on polyamine content and metabolism in tomato exposed to salinity-alkalinity mixed stress. *Plant Physiol. Biochem.* 57, 200–209
47. Demidchik, V. (2015) Mechanisms of oxidative stress in plants: From classical chemistry to cell biology. *Environ. Exp. Bot.* 109, 212–228
48. Gill, S.S. and Tuteja, N. (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48, 909–930
49. Prasad, S.M. *et al.* (2005) Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. *Photosynthetica* 43, 177–185
50. Atkinson, N.J. and Urwin, P.E. (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. *J. Exp. Bot.* 63, 3523–3543
51. Turk, H. *et al.* (2014) The regulatory effect of melatonin on physiological, biochemical and molecular parameters in cold-stressed wheat seedlings. *Plant Growth Regul.* 74, 139–152

52. Christou, A. *et al.* (2014) Systemic mitigation of salt stress by hydrogen peroxide and sodium nitroprusside in strawberry plants via transcriptional regulation of enzymatic and non-enzymatic antioxidants. *Environ. Exp. Bot.* 107, 46–54
53. Li, X. *et al.* (2013) Induction of chilling tolerance in wheat during germination by pre-soaking seed with nitric oxide and gibberellin. *Plant Growth Regul.* 71, 31–40
54. Christou, A. *et al.* (2013) Hydrogen sulfide induces systemic tolerance to salinity and non-ionic osmotic stress in strawberry plants through modification of reactive species biosynthesis and transcriptional regulation of multiple defence pathways. *J. Exp. Bot.* 64, 1953–1966
55. Wei, W. *et al.* (2014) Melatonin enhances plant growth and abiotic stress tolerance in soybean plants. *J. Exp. Bot.* 66, 695–707
56. Tanou, G. *et al.* (2012) Oxidative and nitrosative-based signaling and associated post-translational modifications orchestrate the acclimation of citrus plants to salinity stress. *Plant J.* 72, 585–599
57. Tanou, G. *et al.* (2014) Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress. *Plant Cell Environ.* 37, 864–885
58. Zheng, C. *et al.* (2009) Exogenous nitric oxide improves seed germination in wheat against mitochondrial oxidative damage induced by high salinity. *Environ. Exp. Bot.* 67, 222–227
59. Amooaghaie, R. and Nikzad, K. (2013) The role of nitric oxide in priming-induced low-temperature tolerance in two genotypes of tomato. *Seed Sci. Res.* 23, 123–131
60. Zhang, H. *et al.* (2010) Hydrogen sulfide alleviates aluminum toxicity in germinating wheat seedlings. *J. Integr. Plant Biol.* 52, 556–567
61. Li, Z. *et al.* (2014) Exogenous spermidine improves seed germination of white clover under water stress via involvement in starch metabolism, antioxidant defenses and relevant gene expression. *Molecules* 19, 18003–18024
62. Krasensky, J. and Jonak, C. (2012) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J. Exp. Bot.* 63, 1593–1608
63. He, L. *et al.* (2009) Pretreatment of seed with H₂O₂ enhances drought tolerance of wheat (*Triticum aestivum* L.) seedlings. *Afr. J. Biotechnol.* 8, 6151–6157
64. Liu, X. *et al.* (2011) Alleviating effect of exogenous nitric oxide in cucumber seedling against chilling stress. *Afr. J. Biotechnol.* 10, 4380–4386
65. İşeri, Ö. *et al.* (2013) Hydrogen peroxide pretreatment of roots enhanced oxidative stress response of tomato under cold stress. *Acta Physiol. Plant* 35, 1905–1913
66. Todorova, D. *et al.* (2012) Application of natural and synthetic polyamines as growth regulators to improve the freezing tolerance of winter wheat (*Triticum aestivum* L.). *Acta Agronomica Hung.* 60, 1–10
67. Ishibashi, Y. *et al.* (2011) Hydrogen peroxide spraying alleviates drought stress in soybean plants. *J. Plant Physiol.* 168, 1562–1567
68. Seki, M. *et al.* (2007) Regulatory metabolic networks in drought stress responses. *Curr. Opin. Plant Biol.* 10, 296–302
69. Uchida, A. *et al.* (2002) Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci.* 163, 515–523
70. Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681
71. Bartels, D. and Sunkar, R. (2005) Drought and salt tolerance in plants. *Crit. Rev. Plant Sci.* 24, 23–58
72. Chunthaburee, S. *et al.* (2014) Alleviation of salt stress in seedlings of black glutinous rice by seed priming with spermidine and gibberellic acid. *Not Bot Horti Agrobo* 42, 405–413
73. Saleethong, P. *et al.* (2013) Effects of exogenous spermidine (Spd) on yield, yield-related parameters and mineral composition of rice (*Oryza sativa* L. ssp. *indica*) grains under salt stress. *Aust. J. Crop Sci.* 7, 1293–1301
74. Li, C. *et al.* (2012) The mitigation effects of exogenous melatonin on salinity-induced stress in *Malus hupehensis*. *J. Pineal Res.* 53, 298–306
75. Hu, Y. *et al.* (2009) Cadmium toxicity and translocation in rice seedlings are reduced by hydrogen peroxide pretreatment. *Plant Growth Regul.* 59, 51–61
76. Sun, J. *et al.* (2013) Hydrogen sulfide alleviates cadmium toxicity through regulations of cadmium transport across the plasma and vacuolar membranes in *Populus euphratica* cells. *Plant Physiol. Biochem.* 65, 67–74
77. Posmyk, M.M. *et al.* (2008) Presowing seed treatment with melatonin protects red cabbage seedlings against toxic copper ion concentrations. *J. Pineal Res.* 45, 24–31
78. Groppa, M. *et al.* (2007) Polyamines and heavy metal stress: the antioxidant behavior of spermine in cadmium- and copper-treated wheat leaves. *Biometals* 20, 185–195
79. Yildiz, M. *et al.* (2013) Protective role of hydrogen peroxide pretreatment on defense systems and BnMP1 gene expression in Cr (VI)-stressed canola seedlings. *Ecotoxicology* 22, 1303–1312
80. Tan, W. *et al.* (2011) Photosynthesis is improved by exogenous calcium in heat-stressed tobacco plants. *J. Plant Physiol.* 168, 2063–2071
81. Gao, Y. *et al.* (2010) Hydrogen peroxide pretreatment alters the activity of antioxidant enzymes and protects chloroplast ultrastructure in heat-stressed cucumber leaves. *Sci. Horticulturae* 126, 20–26
82. Tiryaki, I. and Keles, H. (2012) Reversal of the inhibitory effect of light and high temperature on germination of *Phacelia tanacetifolia* seeds by melatonin. *J. Pineal Res.* 52, 332–339
83. Sagor, G.H.M. *et al.* (2013) The polyamine spermine protects *Arabidopsis* from heat stress-induced damage by increasing expression of heat shock-related genes. *Transgenic Res.* 22, 595–605
84. Mostofa, M. *et al.* (2014) Spermidine pretreatment enhances heat tolerance in rice seedlings through modulating antioxidative and glyoxalase systems. *Plant Growth Regul.* 73, 31–44
85. Xu, Y. *et al.* (2010) Protective effect of nitric oxide on light-induced oxidative damage in leaves of tall fescue. *J. Plant Physiol.* 167, 512–518
86. Sathiyaraj, G. *et al.* (2014) Acclimation of hydrogen peroxide enhances salt tolerance by activating defense-related proteins in *Panax ginseng* C.A. Meyer. *Mol. Biol. Rep.* 41, 3761–3771
87. Bajwa, V.S. *et al.* (2014) Role of melatonin in alleviating cold stress in *Arabidopsis thaliana*. *J. Pineal Res.* 56, 238–245
88. Cooper, C. and Brown, G. (2008) The inhibition of mitochondrial cytochrome oxidase by the gases carbon monoxide, nitric oxide, hydrogen cyanide and hydrogen sulfide: chemical mechanism and physiological significance. *J. Bioenerg. Biomembr.* 40, 533–539
89. Radhakrishnan, R. and Lee, I.-J. (2013) Ameliorative effects of spermine against osmotic stress through antioxidants and abscisic acid changes in soybean pods and seeds. *Acta Physiol. Plant* 35, 263–269
90. Pastor, V. *et al.* (2013) Primed plants do not forget. *Environ. Exp. Bot.* 94, 46–56
91. Jisha, K.C. *et al.* (2013) Seed priming for abiotic stress tolerance: an overview. *Acta Physiol. Plant* 35, 1381–1396
92. Bethke, P. *et al.* (2006) Sodium nitroprusside, cyanide, nitrite, and nitrate break *Arabidopsis* seed dormancy in a nitric oxide-dependent manner. *Planta* 223, 805–812
93. Boening, D. and Chew, C. (1999) A critical review: general toxicity and environmental fate of three aqueous cyanide ions and associated ligands. *Water Air Soil Poll.* 109, 67–79
94. Zandonadi, D. *et al.* (2010) Nitric oxide mediates humic acids-induced root development and plasma membrane H⁺-ATPase activation. *Planta* 231, 1025–1036
95. Antoniou, C. *et al.* (2013) Developmental stage- and concentration-specific sodium nitroprusside application results in nitrate reductase regulation and the modification of nitrate metabolism in leaves of *Medicago truncatula* plants. *Plant Signal. Behav.* 8, e25479
96. Wang, W. *et al.* (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* 9, 244–252

97. Alcázar, R. *et al.* (2010) Polyamines: molecules with regulatory functions in plant abiotic stress tolerance. *Planta* 231, 1237–1249
98. Filippou, P. *et al.* (2012) NO loading: efficiency assessment of five commonly used application methods of sodium nitroprusside in *Medicago truncatula* plants. *Plant Physiol. Biochem.* 60, 115–118
99. Chen, K. and Arora, R. (2013) Priming memory invokes seed stress-tolerance. *Environ. Exp. Bot.* 94, 33–45
100. Khajeh-Hosseini, M. *et al.* (2003) The interaction between salinity stress and seed vigour during germination of soyabean seeds. *Seed Sci. Technol.* 31, 715–725
101. Thornton, J. *et al.* (1993) The effect of aerated hydration on DNA synthesis in embryos of *Brassica oleracea* L. *Seed Sci. Res.* 3, 195–199
102. Weibrecht, K. *et al.* (2011) First off the mark: early seed germination. *J. Exp. Bot.* 62, 3289–3309
103. Balestrazzi, A. *et al.* (2011) Seed imbibition in *Medicago truncatula* Gaertn.: expression profiles of DNA repair genes in relation to PEG-mediated stress. *J. Plant Physiol.* 168, 706–713
104. Bruce, T.J.A. *et al.* (2007) Stressful 'memories' of plants: evidence and possible mechanisms. *Plant Sci.* 173, 603–608
105. Demidchik, V. (2015) Mechanisms of oxidative stress in plants: from classical chemistry to cell biology. *Environ. Exp. Bot.* 109, 212–228
106. Butler, L.H. *et al.* (2009) Priming and re-drying improve the survival of mature seeds of *Digitalis purpurea* during storage. *Ann. Bot.* 103, 1261–1270