Review

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

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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.

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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$_2$O$_2$), 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$_2$S), attested by the existence of numerous reports on the potential use of H$_2$S 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.g., 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$^-$)], hydrogen peroxide (H$_2$O$_2$), nitrogen [e.g., nitric oxide (NO)], or sulfur [e.g., hydrogen sulfide (H$_2$S)]. 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$_3$[Fe(CN)$_6$]NO that is used as an NO donor (i.e., NO releasing compound) not only for medical uses but also for plant stress physiology research.

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].
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

![Diagram](image-url)

**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.

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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 protein synthesis, 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$_2$O$_2$, O$_2$•-) 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; guaicol 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$_2$O$_2$ 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$_2$O$_2$ 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 (GolS), which encode key enzymes for the biosynthesis of the oligosaccharides [67]. Rice plants pretreated with H$_2$O$_2$ or SNP showed higher expression than non-primed plants of transcripts for Δ$_2$-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 antioxidative 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 thermostolerance was attributed to prolonged upregulation of heat-shock proteins (HSPs; HSP70, HSP80, HSP90) transcripts and enhanced antioxidative 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...
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
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.

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**Supplemental Information**

Supplemental information associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2015.11.003.

**References**


**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?


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