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Integrated Chemical Control of Abiotic Stress Tolerance Using Biostimulants

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Abstract

Given the high sensitivity of plants to environmental stress, the extreme environmental conditions derived from global climate change are now leading to a risk of decreases in crop production. The use of biostimulants, which enhance stress tolerance in plants, in combination with more traditional countermeasures, such as fertilizer application and irrigation, has significant potential to overcome stress-derived impacts on crops. In this review, the reasons for the inherent sensitivity of plants to environmental stress and the effects of biostimulants on enhancing stress tolerance are introduced. The availability of methods of integrated chemical control for improving crop production in the context of environmental stress is also discussed.

Keywords: abiotic stress, biostimulant, chemical control

1. Introduction

Global climate change means that extreme environmental conditions are now being experienced more frequently. The Intergovernmental Panel on Climate Change [1] has suggested that global warming increases the incidence of various natural disasters, such as extreme temperatures, flood, and drought; agriculture is particularly susceptible to the influence of such events, because plants are organisms that show great sensitivity to changes in their environment.

As sessile organisms, plants are constantly exposed to widely varying and unfavorable environmental conditions, such as drought and extreme temperatures, which are major limiting factors in crop production [2]. Plants therefore have an inherently complicated response

mechanism against environmental stresses, including developmental, physiological, and biochemical changes that are regulated by abiotic-related gene expression. In this response process, environmental physical stimuli are perceived and transduced to biochemical processes, resulting in the induction of a series of abiotic stress-related gene expressions. The involvement of chemicals such as phytohormones, abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and ethylene has been shown to be important in the stress signaling process [3]. In addition, recent research suggests that a central role in the various causes of environmental stress is played by oxidized chemicals, which are produced in response to oxidative stress, an unavoidable stress for plants.

Stress-related disturbance of the metabolic balance in oxidative organelles often results in the enhanced production of reactive oxygen species (ROS) [4]. The sensitivity of plants to environmental stress partly arises because the cause of the damage derived from almost all abiotic stressors is related to photosynthesis. In terms of plant energy metabolism, photosynthesis is the process that is most sensitive in the presence of abiotic stress, because any imbalance between energy production in photochemical reactions and energy consumption in the Calvin-Benson cycle is often a result. As shown in **Figure 1**, the rate of photochemical reactions is almost dependent on a linear function with light intensity, because the photochemical reactions are mirrored

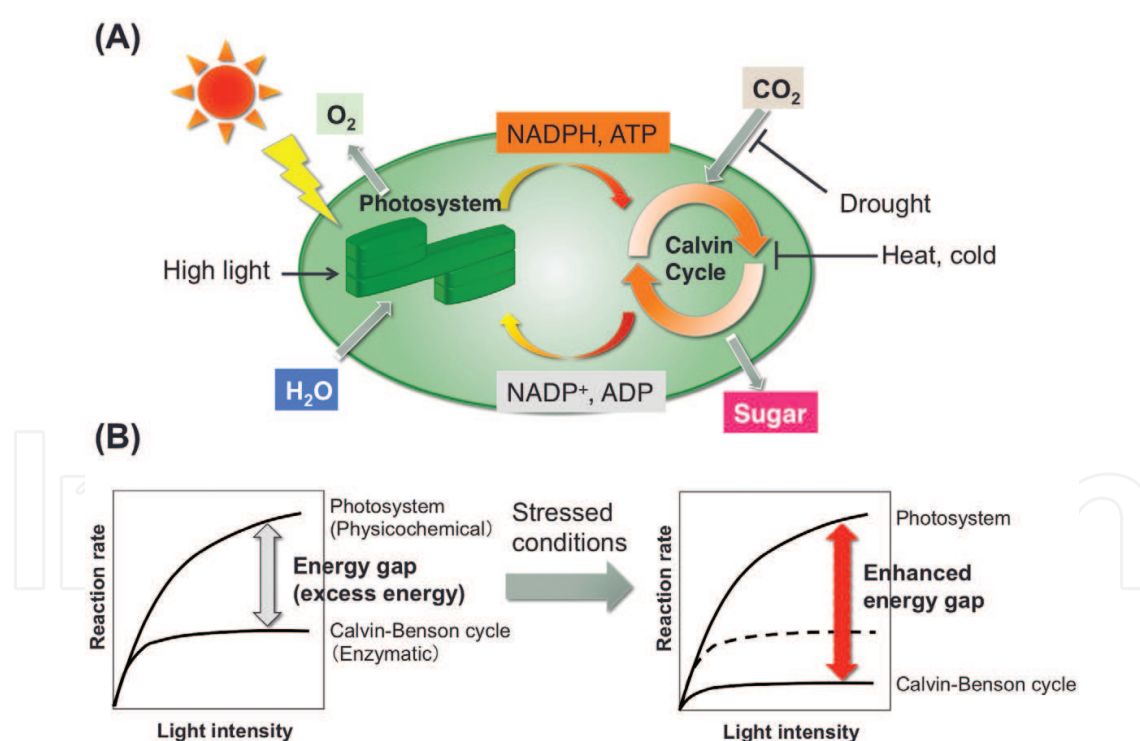


Figure 1. Chloroplasts comprise the most sensitive site in plants in responding to various environmental stresses. (A) Photosynthesis comprises two distinct processes: the photochemical reaction mediated by the photosystem, and CO₂ assimilation mediated by the Calvin-Benson cycle. Under environmental stresses such as high light levels, drought and temperature stresses, the NADPH and ATP supplied by photosystem and their consumption in the Calvin-Benson cycle become imbalanced [4]. (B) The energy gap between the photosystem and Calvin-Benson cycle is normally eliminated by thermal dissipation, but the energy imbalance occurred under environmental stresses enlarges the energy gap, often exceeding the dissipation capacity.

by physicochemical reactions. On the other hand, the Calvin-Benson cycle is a complex process comprising various enzymes; its rate is therefore restricted by enzymatic properties, such as the maximal velocity ($V_{\max}$) value of each enzyme and the rate-limiting steps, and eventually reaches a plateau. Under balanced conditions such as low and moderate light intensity conditions, the quantity of NADPH and ATP supplied via the photosystem is almost equal to the NADP^+ and ADP returned from the Calvin-Benson cycle; this is not, however, the case under conditions of high light intensity, such as sunny weather. High light levels enhance photochemical reactions in the photosystem; in contrast, the Calvin-Benson cycle is inhibited by CO_2 deficiency under drought stress conditions, and its enzyme activity reduces under heat or cold conditions. When facing these stresses, the NADPH and ATP supplied by photosystem and their consumption in the Calvin-Benson cycle become imbalanced. This “energy gap,” that is, the difference between energy supply and consumption, is usually eliminated by thermal energy dissipation. However, CO_2 deficiency due to stomatal closure under conditions of drought or high salinity, as well as enzyme inactivation during heat or cold stress, causes the rate of the Calvin-Benson cycle to lower, thereby increasing the energy gap. When this gap exceeds the capacity required for thermal energy to dissipate, the excess energy causes the production of ROS, potentially damaging many bioprocesses.

Consequently, disturbance of the photochemical reaction leads to the production of ROS, an effect that is further enhanced by conditions limiting CO_2 fixation, such as drought, salinity, heat and cold stress, and a combination of these conditions with high light intensity [4, 5]. ROS are primarily toxic compounds that damage cellular components because of their high reactivity, resulting in a decrease in plant production. Under oxidative stress conditions, ROS attack polyunsaturated fatty acids (PUFAs) in the thylakoid membrane; PUFAs are easily oxidized by ROS, releasing various degraded products. Malondialdehyde, which is representative of these degraded products and is easily produced by the oxidation of PUFAs [6], chemically modifies proteins, especially in conditions of high light intensity and heat stress [7]. Decreases in photosynthetic activity are partly due to the modification of malondialdehyde by reaction center proteins in photosystem II [8]. On the other hand, ROS [9], ROS-related chemicals such as carotenoid oxidation products [10], and lipophilic reactive electrophilic species [11] are recognized as important signal chemicals involved in the responses to environmental stress.

2. Potential chemicals involved in abiotic stress responses and their use as biostimulants

2.1. Reactive short-chain leaf volatiles as potential signaling chemicals

As described above, chloroplasts are the organelles that are most susceptible to damage under conditions of oxidative stress. Therefore, chloroplasts are also potential sensors of environmental stress, assimilating environmental changes, and transmitting information about the changes to other organelles using infochemicals. Recently, we have found evidence to support the premise that chloroplasts produce signal chemicals that induce gene expression and enhance stress tolerance.

Reactive short-chain leaf volatiles (RSLVs) are a group of C4–C9 straight chain carbonyls characterized by an α,β -unsaturated carbonyl bond (**Figure 2**). They are oxylipins and are derived from PUFAs in the thylakoid membrane. Biologically, plants treated with vaporized RSLVs show an enhanced expression of genes involved in responding to environmental stresses, such as high temperatures and oxidative stress [12]. As this response resembles the acquired thermotolerance inherent in plants as a response mechanism for surviving stress caused by elevated temperatures, plants treated with RSLVs show enhanced thermotolerance. As described later, the discovery of this bioactivity has opened the possibility of the chemical control of plants by volatiles to induce heat stress tolerance.

2.2. Improving crop production by enhancing environmental stress tolerance

In nature, crop yield is usually reduced by stress related to both biotic and abiotic causes; surprisingly, abiotic stress is the major inhibiting constraint, by up to 70% of potential production, in contrast to 10% for biotic stress (**Figure 3**, reconstituted from [13]). This indicates that crop production is on average only producing 20% of potential yield. Thus, if crop plants were liberated from abiotic stresses, by even only 10% of potential yield, then net crop production would increase by an average of 50%. Achieving this would be dependent on fertilizer-independent crop improvements, based on agriculturally beneficial biostimulants.

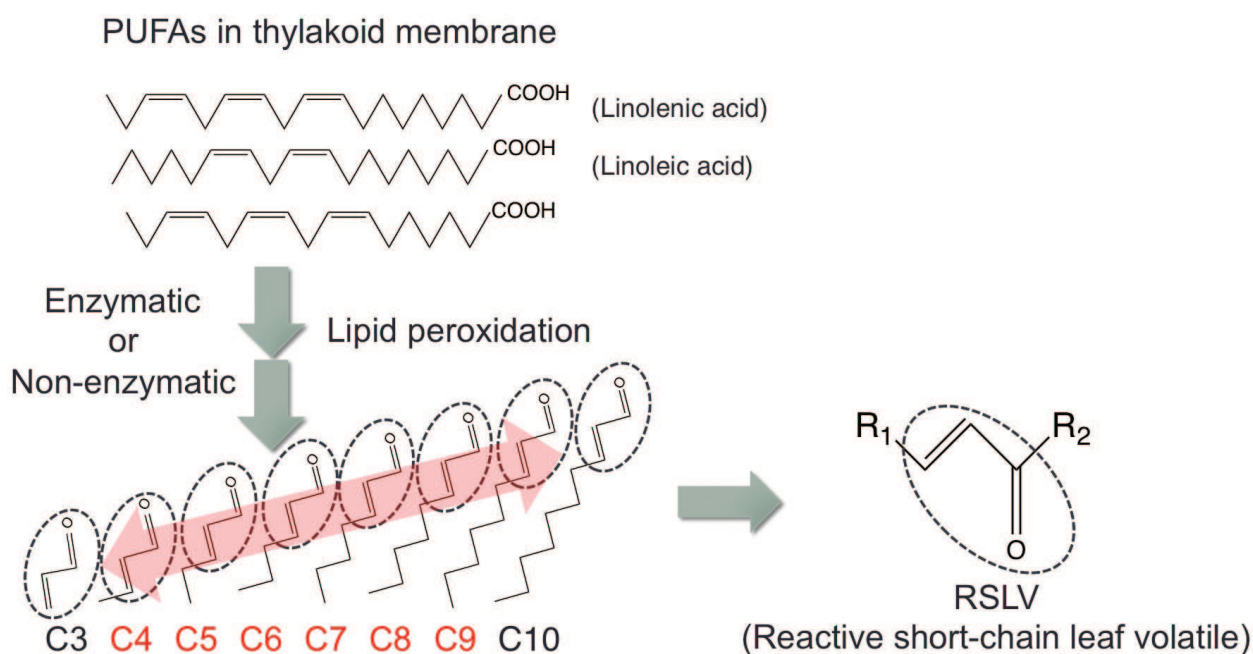


Figure 2. Reactive short-chain leaf volatiles (RSLVs) are signaling chemicals involved in the response to heat and oxidative stresses [12]. They are produced from oxidized polyunsaturated fatty acids, such as linolenic and linoleic acid, in thylakoid membranes through both enzymatic and nonenzymatic mechanisms. The essential chemical structure revealing signal activity is a straight chain carbonyl between C4 and C9, which has an α,β -unsaturated carbonyl bond (indicated by dotted circles). Of these, 2-hexenal is an RSLV produced enzymatically that is also known as a green leaf volatile.

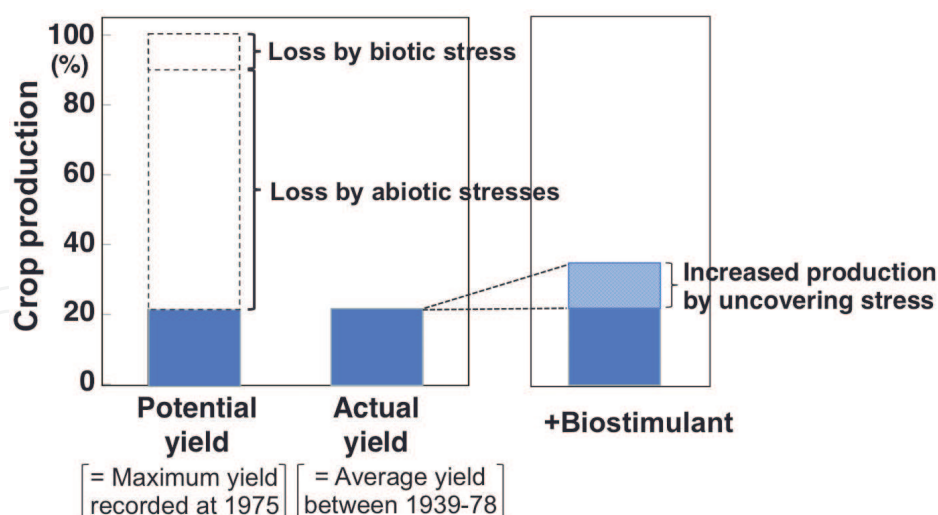


Figure 3. Concept of increase in crop production by using biostimulants. A comparison between the maximum yield recorded in 1975 (potential productivity) and average yield over a period of 50 years (1939–1978, actual productivity) shows that a large proportion of crop production is lost due to abiotic (up to 70%) and biotic (10%) stress (adapted from Table 1 in [13]). As shown in the right panel, if biostimulant treatment were to remove only 10% of the damage due to abiotic stress, net production would increase 1.5-fold when compared to no treatment being applied.

2.3. Use of biostimulants in enhancing tolerance to environmental stress

Crop yield has traditionally been improved by the application of fertilizers, pesticides, and irrigation to agricultural fields. Biostimulants are also products that have positive effects on yield by increasing stress tolerance and repairing damage already caused by unfavorable conditions [14, 15]. They can be either natural or synthetic in origin and usually consist of various organic and inorganic components. Naturally derived biostimulants include preparations based on free amino acids, seaweed and fruit extracts, effective microorganisms, humic substances, and chitosan [14, 15]. Synthetic biostimulants include plant growth regulators, phenolic compounds, inorganic salts, essential elements, and other substances with plant-stimulating properties. Hereafter, in this chapter, some major biostimulants, in particular chemical biostimulants with the potential to mitigate the effects of environmental stresses, are introduced.

2.4. Use of Pyrabactin as an ABA derivative for controlling water use

Under drought-stress conditions, plants often produce elevated levels of ABA to reduce transpiration by closing the guard cell aperture, resulting in a reduction in water loss. In order to control water use by plants, ABA derivatives have therefore been developed to activate the ABA receptors. Pyrabactin is representative of these synthetic ABA derivatives that mimic ABA; it activates the ABA receptors needed for improving drought tolerance [16]. Unlike natural ABA, Pyrabactin is not sensitive to light, is easy to synthesize, and relatively inexpensive, and its manufacture for agricultural use is therefore practical.

2.5. Acetic acid

The external application of acetate enhances drought tolerance in various plant species, such as *Arabidopsis*, maize, rapeseed, rice, and wheat [17]. This effect is related to a novel drought tolerance mechanism in plants involving the acetate-jasmonate signaling pathway, which is regulated epigenetically and conserved in plants. In *Arabidopsis*, exogenous acetic acid promotes JA synthesis and enriches histone H4 acetylation using ON/OFF switching, which is dependent on histone deacetylase HDA6, influencing the priming of the JA signaling pathway for plant drought tolerance. Thus, the external application of acetate to crops is potentially a useful, simple, and low-cost method of enhancing drought tolerance in various plant species.

2.6. Nonprotein amino acids and derivatives

The nonprotein amino acid β -aminobutyric acid (BABA), a potent inducer of resistance to infection by various pathogens [18], exerts its functions via priming of the SA-dependent defense mechanisms in *Arabidopsis* [19]. In other cases, BABA acts through potentiation of the ABA-dependent signaling pathways [20]. As both pathways can contribute to water stress tolerance, BABA is also able to protect *Arabidopsis* against abiotic stress, such as drought and high salinity [21], although BABA is a rare amino acid in plants [18]. This result suggests that BABA can be used as a biostimulant to protect plants from drought and salinity stress when it is based on ABA-dependent but not on SA-dependent defense mechanisms.

Glycine betaine is a major organic osmolyte that accumulates in various plant species in response to stresses such as drought and salinity [22]. It is an endogenous osmolyte produced by two enzymes: choline monooxygenase converts choline to betaine aldehyde, which is then catalyzed by betaine aldehyde dehydrogenase to form glycine betaine. As an osmolyte, glycine betaine is considered to have positive effects on the enzyme and membrane integrity in plants growing under stressful conditions; its role as a biostimulant has been subjected to field tests, and it is already being produced commercially. However, although many plant species show a significant increase in growth and final crop yield under conditions of environmental stress when treated with glycine betaine, others do not. Thus, the most useful and economic application of these compounds requires further investigations in order to determine the most effective concentrations and number of applications, as well as the most responsive growth stage(s) of the plant.

2.7. Controlling cold tolerance by modifying membrane fluidity

There is a close correlation between the chilling sensitivity of plants and the level of unsaturated fatty acids in the phosphatidylglycerol (PG), a phospholipid found in the thylakoid membranes of the chloroplasts [23]. When glycerol-3-phosphate acyltransferase, a key enzyme in determining the extent of unsaturated fatty acids in PG, is overexpressed, then increases in the relative levels of saturated and monounsaturated fatty acids in PG have been shown to increase the sensitivity of tobacco plants to low temperatures during the growth of young seedlings and maturation of reproductive organs [24]. As increases in the unsaturation of fatty acids result in decreases in biomembrane rigidification, then chemicals that enhance

membrane rigidification can be used to promote tolerance against cold-induced stress. Furuya et al. [25] suggest that a treatment of dimethyl sulfoxide, which is a membrane rigidifier, enhanced the cold acclimation of *Arabidopsis* by activating the MEKK1-MKK2-MPK4 cascade. These results indicate that chemicals modifying lipid fluidity are a possible means of cold adaptation in plants.

2.8. Nitrophenolates

Nitrophenolates are biostimulants and are already being manufactured commercially in Japan under the name Atonik, a synthetic product composed of three phenolic compounds: sodium *p*-nitrophenolate (0.3%), sodium *o*-nitrophenolate (0.2%), and sodium 5-nitroguaiacolate (0.1%), together with water. Atonik has been used successfully for many years in the cultivation of most globally important crops. Its mode of action is still not understood but might be involved in hormone regulation, nutrient uptake, and nitrogen metabolism [26]. Atonik therefore stimulates plant growth and development and contributes to enhancing biomass accumulation, increasing water uptake, protecting against drought, and mitigating stress due to noble metals.

2.9. Use of RSLVs as biostimulants

As described in Section 2.1, RSLVs potentially act as signaling chemicals involved in heat and oxidative responses. A representative RSLV, 2-hexenal, is a green leaf volatile that induces gene expression in response to heat and oxidative stresses [12] and thus enhances thermotolerance in plants. Terada et al. suggest that this effect is partly explained by transpiration being sustained at higher temperatures [27]. The field use of 2-hexenal is being progressed commercially in Japan. 2-hexenal is a volatile; its vaporization from a tablet form by sublimation has enabled the effective concentrations for use in closed greenhouses to be determined. A preliminary examination showed that its application in greenhouses improved the production of crops such as tomato, strawberry, and cucumber in the summer (unpublished data), suggesting that its use as a biostimulant is effective in overcoming heat stress.

3. Perspective: toward an integrated chemical control against environmental stress

Historically, the use of pesticides, irrigation, and fertilizers, especially chemical fertilizers, has proven highly successful in increasing crop yields and thus in meeting the demands of increasing population levels. However, recent climate change is having an adverse impact on crop production, and therefore, more efficient methods of crop production need to be established. The use of genetically modified organisms (GMOs) is undoubtedly a solution to combat losses in plant production caused by global environmental changes. However, GMO is limited to major crops, and its use is also either strictly restricted or not even permitted legally in several countries. Therefore, chemical control of abiotic stress tolerance is required as an alternative solution for ensuring unrestricted agricultural production.

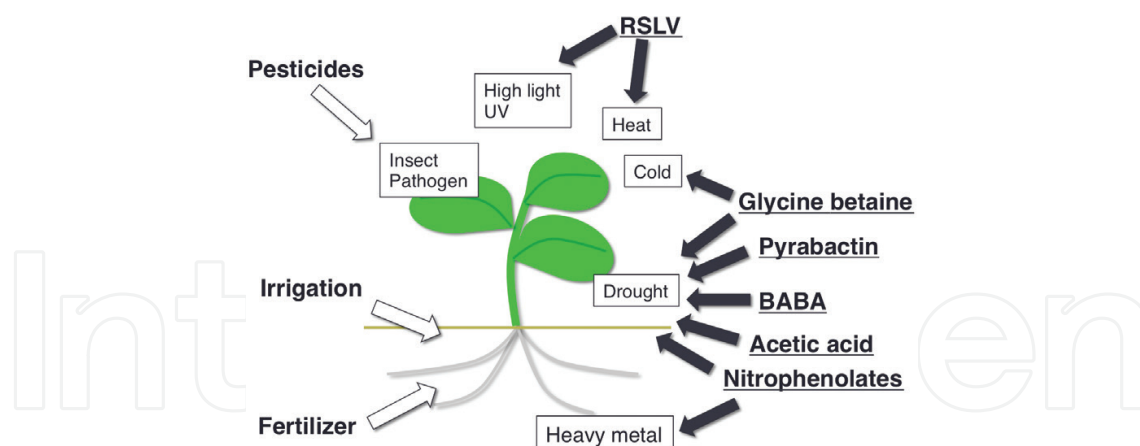


Figure 4. Integrated chemical control of abiotic stress tolerance using biostimulants. Traditional countermeasures, such as irrigation and fertilizers, contribute to improving the basal level of crop production. On the other hand, unusual losses in crop yield caused by biotic and abiotic stress can be mitigated by pesticides and biostimulants, respectively. In this figure, unusual adverse events and biostimulants are boxed and underlined, respectively. The stresses targeted by traditional countermeasures and biostimulants are indicated by white and black arrows, respectively.

As introduced in this chapter, the use of biostimulants has potential as a powerful countermeasure for improving crop production under conditions of environmental stress. Traditional methods for enhancing yield, such as fertilizer and irrigation support, enable a basal level of production to be maintained (**Figure 4**). However, when crops are subject to unusual abiotic and/or biotic stresses, the transient use of adequate biostimulant(s) helps to overcome these stresses, sustaining production to at least the basal level, and sometimes bringing about an increase in crop production, as explained in **Figure 3**. Some biostimulants are already commercially available, and their use will become increasingly popular. However, there is still a lack of technical information for each biostimulant, such as the application period, concentration, and target plant species; these points must be established if biostimulant application is to become a reliable technique. Moreover, the combined use of biostimulants and traditional pesticides must be examined in order to realize the integrated chemical control of abiotic/biotic stress tolerance. In addition to the chemical biostimulants that are the focus of this chapter, other types of biostimulants that are derived from natural materials, such as microorganisms and algae, are also useful in reducing damage caused by abiotic stress [14, 15]. While the mode of action in chemical biostimulants can be explained scientifically, this is not the case for natural biostimulants. Therefore, more details on scientific analyses of the mechanisms used by biostimulants are necessary to support their availability for widespread use in the field.

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Conflicts of interest

The authors declare no conflict of interest.

Abbreviations

ABA	abscisic acid
BABA	β -aminobutyric acid
GMO	genetically manipulated organism
JA	jasmonic acid
PG	phosphatidyl glycerol
PUFA	polyunsaturated fatty acid
ROS	reactive oxygen species
RSLV	reactive short-chain leaf volatile
SA	salicylic acid

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