


Review

Shade and microbes enhance drought stress tolerance in plants by inducing phytohormones at molecular levels: a review

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Handling Editor: Kai Xue

Received: 10 May 2021, **First Decision:** 27 June 2021, **Accepted:** 13 February 2022, **Online Publication:** 19 March 2022

Abstract

Plants are frequently exposed to adverse environments during their life span. Among them drought stress is one of the major threats to agricultural productivity. In order to survive in such unstable environment, plants have developed mechanisms through which they recognize the severity of the stress based on the incoming environmental stimuli. To combat the detrimental effects of drought, the plants have evolved various strategies to modulate their physio-hormonal attributes. These strategies that can be modulated by shade and microbes contribute to enhancing tolerance to drought and reducing yield loss. Plant hormones, such as abscisic acid, auxin and ethylene have a major role in the shade- and microbe-associated improvement of drought tolerance through their effects on various metabolic pathways. In this process, the CLAVATA3/EMBRYOSURROUNDING REGION-RELATED 25 peptide has a major role due to its effect on ABA synthesis as shown in our regulatory model.

Keywords abscisic acid, CLAVATA3/EMBRYOSURROUNDING REGION-RELATED 25, auxin, plant growth promoting rhizobacteria, ethylene

荫蔽和微生物可在分子水平上调控植物激素以增强植物的耐旱性

摘要: 植物在其生命周期内会受到一系列不利环境的影响。其中，干旱胁迫是限制农业生产力的重要因素之一。为了在逆境的环境中得以生存，植物进化出了识别环境胁迫严重性的机制。植物可通过多种方式调节激素活性，以减轻干旱带来的不良影响。受荫蔽和微生物调控，植株的抗旱性提高，产量损失减少。脱落酸、生长素和乙烯等植物激素，可调控多种代谢途径，在荫蔽和微生物介导的植株抗旱

性增强过程中起着重要作用。如我们的调控模型所示, CLAVATA3/EMBRYOSURROUNDING REGION-RELATED 25多肽因其可影响ABA合成而在此过程中具有重要作用。

关键词: 脱落酸, CLAVATA3/EMBRYOSURROUNDING REGION-RELATED 25, 生长素, 植物生长促进根瘤菌, 乙烯

INTRODUCTION

Among several abiotic factors, drought stress is the most harmful one. It is the major threat to global agricultural production. Drought is responsible for at least 40% of yield losses worldwide (Boyer 1982; Conesa *et al.* 2018). Drought and other climate change drivers are expected to intensify in frequency and magnitude, posing serious threats to sustainable agriculture under the changing climate. These climate change drivers often interact with each other under field conditions, which results in markedly different impacts compared with individual stress exposure (Ahmad *et al.* 2012; Ahmad and Prasad 2012). More specifically, drought adversely affects the plant growth, productivity and nutritional potential of plants (Abdelaal *et al.* 2020; Katare *et al.* 2012; Kreszies *et al.* 2019). To survive under unfavorable environmental conditions, plants activate various protective mechanisms.

Protection against drought stress has been associated with many strategies which enable plants to enhance their growth and development. The selection and breeding strategies, molecular and genomic practices, use of plant growth regulators and osmoprotectants, seed priming, microorganisms and moderate shading are the major techniques, which can be used to mitigate the drastic effects caused by water deficit conditions. Among them, the exploitation of shade and microbial communities could be potential strategies to mitigate the drastic effects of water deficit conditions. Both can induce the production of various hormones, especially that of ABA. It has an important role in the regulation of drought response at physiological and molecular levels (Fig. 1). It has been previously shown that shade has significantly improved the drought tolerance in plants by modulating the physiological and biochemical mechanisms through the upregulation of the ABA-induced genes (*AAO3*, *NCED3*, *ABI4* and *ABI5*) (Asgar *et al.* 2020; Cagnola *et al.* 2012; Huang *et al.* 2008; Setter *et al.* 2001). There are many studies which had focused on the biosynthesis of ABA in different plant organs. Among them, one

of the recent studies had elaborated that, leaves are the main source of ABA biosynthesis but not the roots or floral tissues (Zhang *et al.* 2018). A recent study has introduced a new factor, the CLAVATA3/EMBRYOSURROUNDING REGION-RELATED 25 (CLE25) peptide, which is involved in the drought stress regulation through its effect on the ABA biosynthesis (Takahashi *et al.* 2018). This molecule is synthesized in the roots from where it is translocated to the leaves. There it influences the accumulation of ABA together with some meristem receptors (β -amylase-1 and 3 receptor-like kinases) in a coordinated way, and consequently increases plant drought stress resistance (Takahashi *et al.* 2018). Therefore, studies on this peptide molecule, its relationship with ABA and other phytohormones and their role in mitigating drought stress through shade might be an appealing topic for biologists.

ABA regulates plant growth during abiotic stresses as a signaling mediator (Finkelstein 2013). It efficiently modulates ROS homeostasis and stomata activity during water deficit conditions (Hubbard *et al.* 2010). In this signaling pathway, micro-RNAs are also involved as shown for the *miR169* family. In addition, ABA also activated target genes of the *NUCLEAR FACTOR-Y SUBUNIT A (ZmNF-YA)* family in maize. Hence, ABA modulation in plants is an important drought regulator (Luan *et al.* 2015). Likewise ABA, auxin also plays crucial role in the regulation of plant growth during drought by the activation of the antioxidant system. It was reported that, its interactive action with ABA-responsive genes resulted in the maintenance of the root growth in maize, *Arabidopsis* (*Arabidopsis thaliana*), and rice, thereby improving the drought tolerance (Thirunavukkarasu *et al.* 2014; Xu *et al.* 2013). Along with growth regulation, the (cytokinin) CK-mediated responses are also important for protection of plants against stress (Zwack and Rashotte 2015). Besides stimulating cell division and elongation, GA modulation and its interaction with various other phytohormone signaling activated the stress-responsive genes (Colebrook *et al.* 2014; Jogawat 2019). The ETH

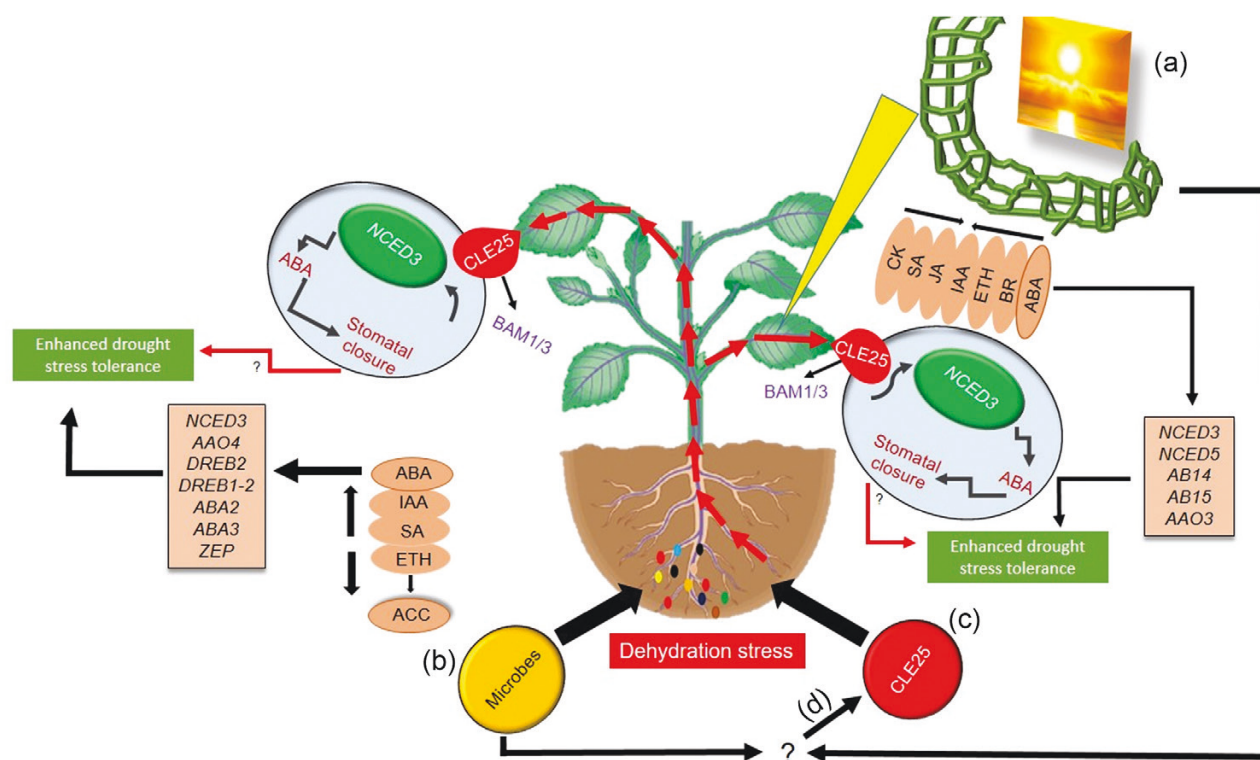


Figure 1: This model demonstrates how shade and microbes enhance the drought tolerance in plants by altering the endogenous hormonal levels. (a) The influence of shade on the endogenous levels of phytohormones and especially on ABA, which results in the upregulation of numerous genes, and consequently leads to drought stress resistance enhancement. (b) The effect of microbes on the concentrations of phytohormones, especially the ABA, which leads to the upregulation of several genes and hence enhances the drought tolerance. (c) How CLE25 is produced in the roots and how it moves to the shoots where it interacts with BAM1/3 receptor-like kinase and is responsible for the biosynthesis of ABA, which induces the stomatal closure. As a result, drought tolerance enhances. However, this mechanism has not been established yet in response to microbes and shade. (d) The phenomenon which reveals how shade and microbes regulate the production of CLE25 in the roots. The CLE25 moves further to the shoot where the rest of the function is completed is unknown yet. The explanation of hormones used in the figure: ABA (abscisic acid), IAA (indole 3-acetic acid), BR (brassinosteroids), CK (cytokinins), ETH (ethylene), GA (gibberellins), JA (jasmonic acid) and SA (salicylic acid). Explanation of other components: ACC (1-aminocyclopropane-1- carboxylate), AAO3 (Arabidopsis aldehyde oxidase 3), *ABI4* (ABA-INSENSITIVE 4), *ABI5* (ABA-INSENSITIVE 5), *ABA2* (ABA-aldehyde 2), *ABA3* (ABA-aldehyde 3), *AAO4* (Arabidopsis aldehyde oxidase 4), CLE25 (CLAVATA3/EMBRYOSURROUNDING REGION-RELATED 25), *DREB2* (dehydration-responsive element-binding protein 2) and *DREB1-2* (dehydration-responsive element-binding protein1-2), *NCED3* (NINE-CIS-EPOXYCAROTENOID DIOXYGENASE3), *NCED5* (NINE-CIS-EPOXYCAROTENOID DIOXYGENASE5) and *ZEP* (zeaxanthin epoxidase).

signaling significantly contributes to the induction of drought tolerance by modulating various ET-RESPONSIVE FACTORS (ERFs) and affecting other phytohormone pathways (Jogawat *et al.* 2021). The interactive effect of BRs with other phytohormones regulates various growth and stress regulatory pathways. It was noticed that the BR deficiency resulted in the increment of sensitivity to drought (Tůmová *et al.* 2018). The association of SA signaling pathway with ABA, ET and JA pathways is also very crucial in the control of plant growth and stress responses (Jogawat 2019). SA

is involved in the detoxification of ROS produced during stress. It also affects the isoprenoid levels such as chlorophylls, monoterpene, tetraterpenes, α -tocopherol and carotenoids, which are important antioxidants regulating plant growth in stressful environments (Munné-Bosch *et al.* 2007).

There is a series of studies, which describe that the microbes and shade induce the production of several plant hormones during stressful environments. However, these articles did not confirm that this change in the hormone levels might improve drought tolerance. Therefore, identification of common and

specific components of the shade- and microbe-induced protective mechanisms can contribute to the better understanding of the molecular background of the reduction of drought-induced damages. The role of the various plant hormones, especially ABA and the CLE25 peptide in this process is of great importance and will be presented in this review.

THE SHADE: A DROUGHT MITIGATING APPROACH

Shade enhances the drought tolerance of plants through its effect on phytohormones

High vegetation density influences the plant growth and development. Among them, the most widely documented effect is shade. It can be either detrimental or very useful depending on its severity in a given situation (Asghar *et al.* 2020; Franklin *et al.* 2020; Jiang *et al.* 2020; Romero-Montepaone *et al.* 2020; Wan *et al.* 2020; Ward 2020). Shade can significantly improve the growth and development of plants by adjusting the appropriate physiological, hormonal, metabolic and molecular profiles under water deficit conditions. The most prominent plant hormone that regulates the responses to shade conditions is auxin (Fig. 1a, Yang *et al.* 2017). In shade, the low red:far-red (R:FR) ratio stimulates the expression of *TRYPTOPHAN AMINOTRANSFERASE OF ARABIDOPSIS1 (TAA1)*, which is involved in encoding the protein catalyzing the synthesis of the main precursor of auxin biosynthesis in the tryptophan-dependent pathway (Stepanova *et al.* 2008; Tao *et al.* 2008). Similar to auxin contents, the endogenous levels of GA have also been proliferated by shading condition through the upregulation of *GA20ox1*, *GA20ox2*, and *GA3ox* enzymes (Fig. 1a, Yu *et al.* 2015; Zhou *et al.* 2004). The CK, jasmonic acid (JA) and SA levels were downregulated (Boonman *et al.* 2007; de Wit *et al.* 2013; Moreno *et al.* 2009), whereas positive impact of shade was found on ethylene (ETH) and brassinosteroids (BR) (Fig. 1a, Kozuka *et al.* 2010; Pierik *et al.* 2004). Another very interesting finding is that karrikins (structurally similar to strigolactones, mimicking their signaling effect) increased the susceptibility of the seedlings to low light conditions (Waters *et al.* 2013). Under shade, the identified role of strigolactone is the mediation of lateral shoot growth and development (Aguilar-Martínez *et al.* 2007; González-Grandío *et al.* 2013). All these studies have shown that shade influences the biosynthesis of several plant hormones. It would be interesting to know whether the shade-induced

hormonal adjustments could lead to the improved drought tolerance.

ABA: the main modulator in shade-induced drought tolerance

Since ABA has an important role in response to drought, shade-induced increase in its level can improve the tolerance to water deficit. ABA is involved in two main regulatory mechanisms: modulating core transcription factors and controlling its own transport and metabolism. These processes are still not fully identified. To sustain the appropriate hormonal concentration in plant tissue, the coordinated adjustment of ABA metabolism, transport and level of core signaling elements are very important for proper growth and development (Bielach *et al.* 2017; Finkelstein 2013). Shade induces the transcription of ABA biosynthetic genes, i.e. *NCED3* and *NCED5*, mainly in hypocotyls (Fig. 1a, Kohnen *et al.* 2016). Likewise, the expression of various auxin-related genes (e.g. *PIN* and *AUX/IAA* genes) is also increased in response to low R:FR, being characteristic for shade (Pierik *et al.* 2009; Salisbury *et al.* 2007). In a study on tomato, it is proposed that the low R:FR treatment potentially amplified the ABA levels (Cagnola *et al.* 2012), and this increase could have positive impacts on the growth of the plants under water limiting conditions. An investigation on *Amaranthus palmeri* seedlings showed that shade significantly enhanced the ABA levels, indicating its involvement in the control of the plants' response to shade (Jha *et al.* 2010). In a study on *Helianthus annuus* showed that the endogenous IAA, GA and ABA contents were increased significantly as the R/FR ratio decreased (Kurepin *et al.* 2007). Thus, it can be concluded that shade can contribute to the improvement of drought tolerance through a regulatory network of various plant hormones.

Moreover, it is widely known that phytochromes are potentially involved in shade-regulating responses. Specifically, phytochrome B (phyB) is responsible for the adjustments of the morphological, physiological and molecular responses of plants according to changes in the R:FR ratio. Phytochromes significantly enhanced the ABA levels, leading to increased drought tolerance in plants (González *et al.* 2012). PhyB-driven acclimation of plants to open environments (high R:FR ratio) increased carbon gain at the expense of greater water loss. In phyB mutants, the lower sensitivity to ABA led to lower drought tolerance (González *et al.* 2012).

Furthermore, it was documented that high light stress had reduced the level of ABA, whereas it had been enhanced under low light conditions (Nambara *et al.* 2005). More specifically, the shade had upregulated the ABA metabolism-related genes remarkably (*AAO3*, *NCED3*, *ABI4* and *ABI5*) and hence the ABA levels (Fig. 1a, Asghar *et al.* 2020). The authors further proved that the ABA induction had positively regulated the antioxidant enzymatic activities and photosynthetic parameters, which had consequently led to the increase in drought tolerance. These studies demonstrated well that shade significantly enhanced the ABA hormone levels (Fig. 2). However, the interaction between ABA and other phytohormones is still unidentified, although it is known that the auxin is involved in the shade-regulated responses. Therefore, future studies are necessary to explain the coordinated participation of phytohormones in the modulation of shade-induced drought tolerance. In addition, it would be very striking to demonstrate how the ABA synthesis-associated (*NCED3*-upregulation) CLE25 peptide might be involved in these mechanisms

through its effect on genes induced by ABA or other hormones (Fig. 1c).

THE MICROBES: A DROUGHT ALLEVIATION STRATEGY

PGPR improve drought tolerance by inducible phytohormones

It is widely acknowledged that soil microbes are also able to cope with abiotic stresses, especially with drought stress, and consequently they also enhance the drought tolerance in plant species by changing the soil physical environment through bioturbation, decomposition of soil organic matter (e.g. wood and leaf litter) and facilitation of nutrient cycling (Ashton *et al.* 2019; Kim *et al.* 2012). Drought stress tolerant microbes have the ability to enhance plant growth and development under water-deficient conditions. Microbes have evolved, adapted and/or developed a tolerance mechanism to survive under low water potential (Fitzpatrick *et al.* 2019; Naylor and Coleman-Derr 2018). They may form thick walls

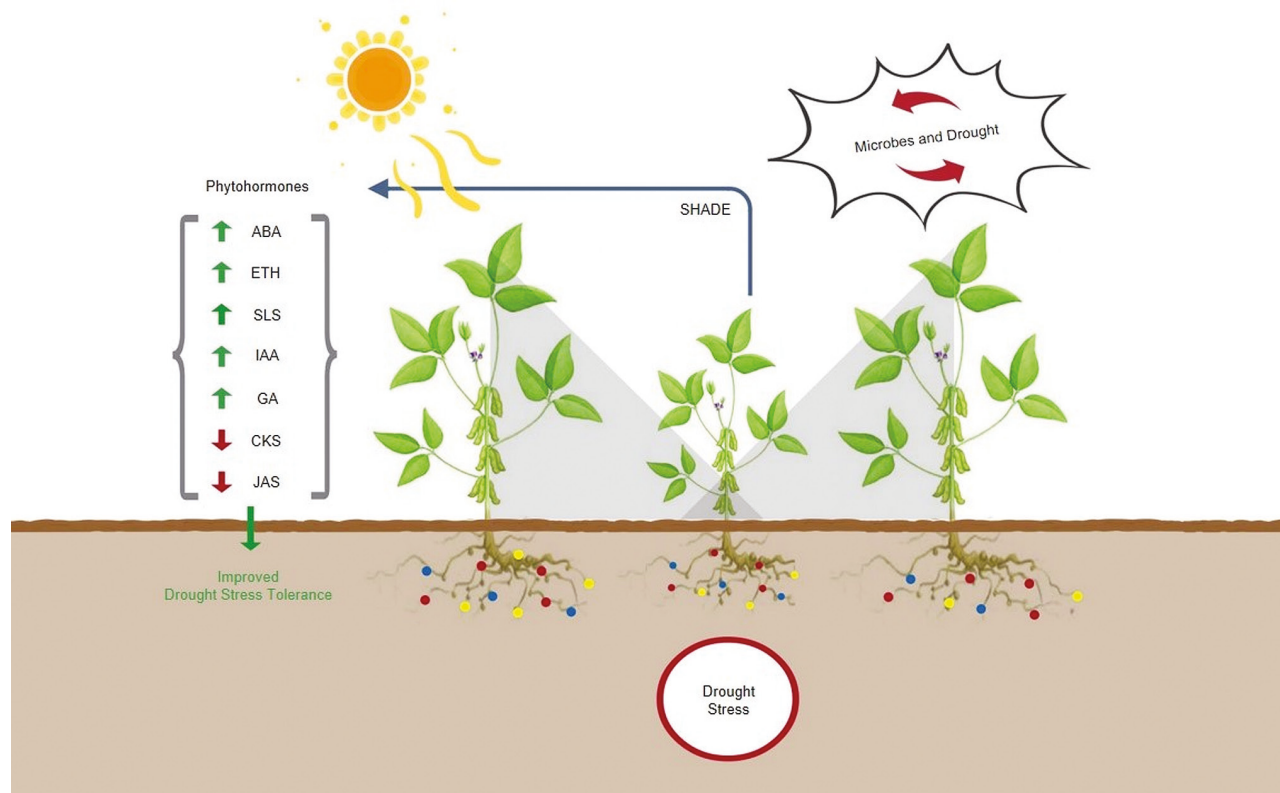


Figure 2: A model depicting how shade enhances the drought tolerance by inducing several plant hormones. Green arrows represent the upregulation of phytohormones and red arrows show the downregulation of phytohormones. The ABA, Cks, ETH, GA, IAA, JAS and SLS refer to abscisic acid, cytokinins, ethylene, gibberellins, indole acetic acid, jasmonic acid and strigolactone, respectively.

or enter dormant stage, can accumulate osmolytes and produce exopolysaccharides (EPS). These plant-associated microbes have various mechanisms to cope with the negative impacts of drought on plant as well as on soil. Irrespective of water content, they provide nutrient and better environment condition for the continuous growth of plants. The beneficial microbes colonized around rhizosphere and promote plant growth and development through various direct and indirect mechanisms. The potential mechanism includes (1) the production of phytohormones, such as IAA, CK and ABA; (2) bacterial exopolysaccharides; (3) 1-aminocyclopropane-1-carboxylate (ACC) deaminase and (4) induced systemic tolerance. In addition, plant growth-promoting rhizobacteria (PGPR) have the ability to synthesize plant hormones that stimulate plant growth and division under stress condition. IAA, the most active auxin, regulates the vascular tissue differentiation, adventitious and lateral root differentiation, cell division and shoot growth during drought stress (Kumar and Verma 2018). PGPR play an important role in the mitigation of biotic and abiotic stresses in plants. Some of the studied soil microorganisms are identified to play role in combating the harmful effects of different environmental hazards (Berg *et al.* 2013). For instance, PGPB improved the plant growth either by increasing the acquisition of nutritional resources, such as nitrogen, iron, phosphorus; reducing the damage of plants by pathogenic organisms or by directly modulating the plant growth by either providing plant hormones, such as ABA, CK, IAA, GA or decreasing ETH levels through the activity of 1-aminocyclopropane-1-carboxylate (ACC; Fig. 1b, Armada *et al.* 2016; Ilyas *et al.* 2020; Kartik *et al.* 2020; Ma *et al.* 2016; Moreno-Galván *et al.* 2020; Tiepo *et al.* 2018; Tiwari *et al.* 2020). The various hormones are involved in the modulation of the cellular activities in higher plants and play remarkable role in the development of several signal transduction pathways in response to abiotic stresses (Pieterse *et al.* 2009). One of the stress-escaping strategies of microbes is the increment in the production of several saccharides including trehalose, which prevents or decreases the breakdown of various compounds and molecules in plants under unfavorable environments and allows the plants to tolerate stresses. It is also confirmed that the inoculation of bacterium reduced the level of malondialdehyde, while it intensified the activities of antioxidants (Khan *et al.* 2016).

The microbe-mediated induced systemic tolerance against drought stress is linked with altered ETH

levels in plants. The *A. piechaudii* ARV8 (root colonizing bacterium) decreased the ETH production in roots because it deaminates the precursor of ETH, i.e. ACC in roots (Glick *et al.* 2007). The reduction in ETH levels could be associated with stomatal closure because the antagonism between ETH and ABA is condensed (Mayak *et al.* 2004). Several reports describe that exogenous IAA alleviates certain abiotic stresses. Thus, bacterial IAA was reported to stimulate increased root and shoot length of wheat seedlings exposed to high levels of salt (Egamberdieva 2009). In another study, it was described that PGPR had significantly raised the levels of GA, SA and IAA in *Brassica oleracea* (Fig. 1b, Turan *et al.* 2014). It was revealed that the *Bacillus* strains had shown a stress-induced increase in the levels of IAA, CK and GA (Ghosh *et al.* 2019). A study on *Pseudomonas poae* s61 and *Astragalus mongholicus* described that it had been mainly involved in massive production of IAA under stressful environments (Sun *et al.* 2019). In drought stress environments, rhizobacteria was involved in the upregulation of GA, IAA, BR, SA, JA and CKs hormones (Carlson *et al.* 2020). Other recent findings have demonstrated that the *Azospirillum lipoferum*, *Bacillus* spp., *Pseudomonas putida* H-2-3, *Bacillus amyloliquefaciens* 5113, *Azospirillum brasilense* NO40, *Rhizobium leguminosarum* (LR-30) and *Azospirillum Brasilense* Sp 245 are potentially involved in the mitigation of drought stress in numerous plant species (Cohen *et al.* 2015; Kasim *et al.* 2013; Vurukonda *et al.* 2016). Although several investigations have revealed the effect of microbes on hormonal levels, there are only a very few studies which explain the role of microbes in mitigating drought stress by altering the phytohormone concentrations at molecular levels. Therefore, further researches are required to gain deeper insights into the role of numerous phytohormones in the regulation of the microbe-induced drought tolerance (Fig. 3).

ABA: the prominent modulator in microbe-mediated drought tolerance

It is extensively documented that ABA is a main mediator in microbe-induced drought tolerance in plants. The microorganisms can synthesize ABA or its analogues (xanthoxin-like compounds) and alter the expression of several genes (e.g. *NCED3*, *ABA2* and *ABA3*) which are responsible for ABA biosynthesis *in planta* (Fig. 1b, Gowtham *et al.* 2021). A study on microbe-ABA interaction demonstrated that *Azospirillum brasilense* had significantly amplified the ABA content (Cohen *et al.* 2015).

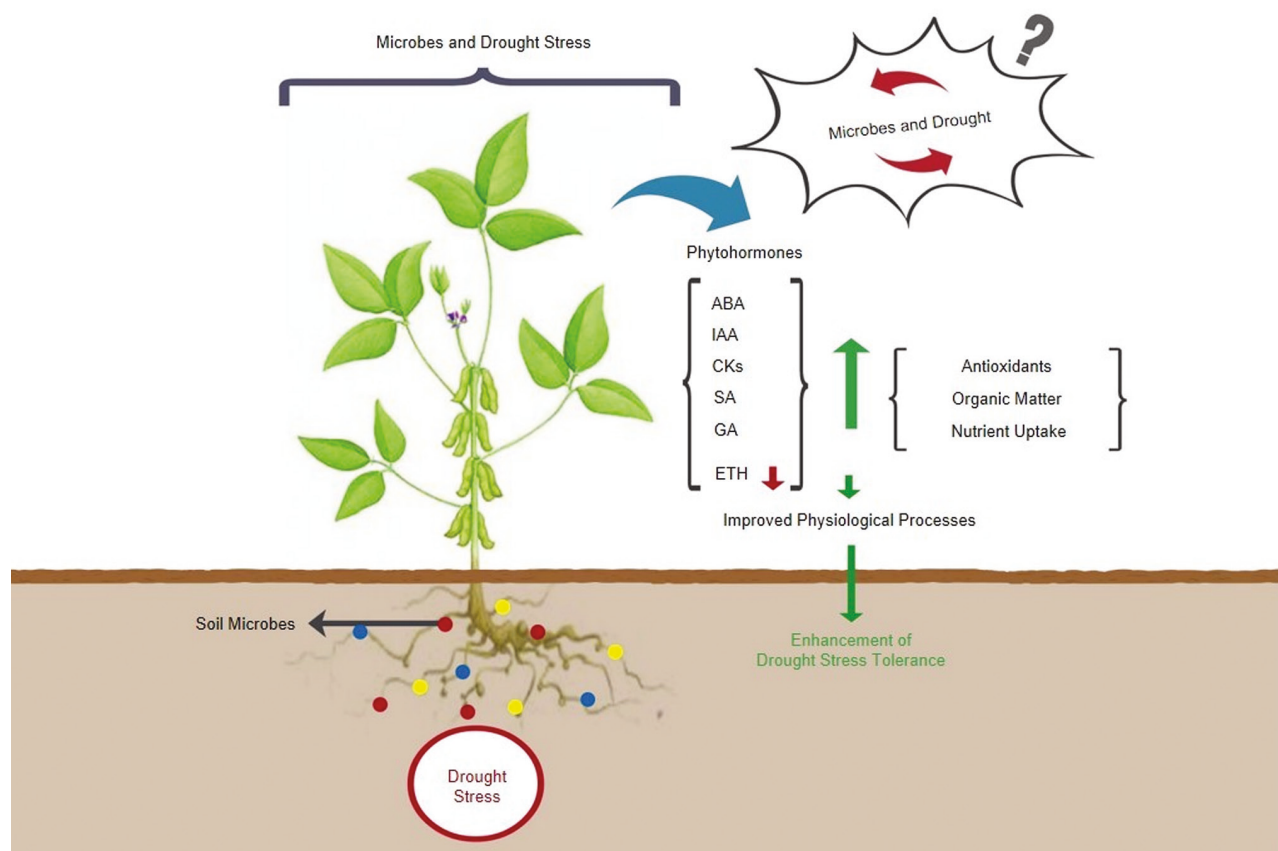


Figure 3: An overview of how soil microbes induce phytohormones accumulation, which results in the improvement of several physiological processes leading to greater drought stress tolerance. Green arrows represent the upregulation of phytohormones and other physiological processes. The ABA, Cks, ETH, GA, IAA, and SA represent abscisic acid, cytokinins, ethylene, gibberellins, indole acetic acid and salicylic acid, respectively.

In addition, it was shown in *Oryza sativa* that the endophytic bacteria had enhanced the SA and ABA concentrations, which had potentially improved the stress tolerance (Shahzad *et al.* 2017). *Pseudomonas putida* strain FBKV2 inoculation increased the amount of the SnRK2 family proteins, which facilitated the transcription of ABA-responsive genes (SkZ *et al.* 2018). Furthermore, it was reported that the poly- γ -glutamic acid produced by microbes had considerably upregulated the ABA-related genes, i.e. *BnNCED3*, *BnZEP* and *BnAAO4* and consequently the ABA levels (Xu *et al.* 2013). In addition, it was demonstrated that mycorrhization altered the leaf ABA levels under drought stress conditions in olive plants (Ouledali *et al.* 2019). These studies demonstrate that ABA has an important role in microbe-mediated drought tolerance.

An investigation on *Pseudomonas simiae* strain AU-inoculated plants have confirmed the involvement of various transcription factors (*DREB/EREB*), osmoprotectants (*P5CS* and *GOLS*) and water

transporters (*PIP* and *TIP*) in the induction of drought tolerance (Vaishnav *et al.* 2019). The expression of *DREB2* and *DREB1-2* genes was considerably enhanced due to the inoculation under water deficit stress, which indicates their involvement in *Bacillus*-mediated priming of drought tolerance (Fig. 1b, Bandedepa *et al.* 2019). It was proved that integrated application of ACC-deaminase containing rhizobacteria could be helpful in the adaptation to water deficit (Yaseen *et al.* 2019). In contrast to the results of other studies, *B. amyloliquefaciens* RWL-1 strain had amplified the endogenous GA and SA contents, while it had reduced the ABA and JA levels (Shahzad *et al.* 2016). The findings of this investigation have drawn the attention of the researchers to the necessity of discovering the exact effects of PGPB in the production of phytohormones, especially JA and ABA, since these were enhanced by another bacterium strain, too (Carlson *et al.* 2020, Shahzad *et al.* 2017). In a very recent study, it has been documented that microbial inoculation in rice

regulates anti-oxidative reactions and defense-related genes to mitigate drought stress. It has been found that the microbial inoculation enhances the activity of the antioxidant enzymes, such as superoxide dismutase, ascorbate peroxidase and catalase, and simultaneously the ABA levels in rice (Singh *et al.* 2020). However, these mechanisms of drought stress adaptation in plants at molecular levels have not been recognized yet. Above and beyond all the mentioned findings, it is still not fully understood how microbes interact with CLE25, which is ultimately involved in ABA biosynthesis through *NCED3* upregulation and consequently in the integration of drought tolerance in plants. To answer this question, some potential investigations are required to explain the mechanism of CLE25-microbe interaction-based improvement of drought tolerance in plants. Furthermore, the current knowledge about this process also lacks the information about the associations of different plant hormones at molecular level in this module regulation. Therefore, the understanding of this question is of great importance since it will uncouple the complexity of drought stress adaptation processes, which ultimately leads to the establishment of better ecological environment for plant growth.

CONCLUSIONS AND FUTURE PERSPECTIVES

In conclusion, it was observed that shade had significantly affected the endogenous levels of several phytohormones, which could enhance the drought tolerance in plants. In this process the upregulation of the ABA synthesis-related genes, and consequently the increase in ABA levels are very important. An interesting future research direction could be the discovery of the possible regulatory interaction between ABA and CLE25 in mitigating drought stress through shade. Moreover, the involvement of auxin is also unidentified in this shade-induced drought resistance mechanism. The microbes also affected the phytohormones thereby enhancing the drought tolerance. More specifically, it was found that soil microbial communities had significantly reduced the levels of ETH; consequently, the drought stress resistance had increased (Fig. 1b). The soil microbial communities (especially the bacteria) had amplified the ABA levels; however, the interaction of microbes with CLE25 has been unsettled yet. The clarification of this process will uncover the several pathways of microbe-induced drought tolerance

mechanism. In addition, the point that the shade and microbes could be interlinked with each other (CLE25, which is produced in roots and responsible for ABA biosynthesis in leaves) in the enhancement of drought tolerance could be an important matter of debate for future studies (Fig. 1d). Therefore, understanding the role of shade and microbes in mitigating the drastic effects of drought stress is of great significance since both of them are based on ABA-modulation.

Funding

This work was supported by the National Research, Development and Innovation Office (grant no. K131638), National Natural Science Foundation of China (grant nos. 31871552, 31671445), Sichuan Science and Technology Program (grant no. 2018HH0108).

Conflicts of interest statement. The authors declare that they have no conflict of interest.

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