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Hormone balance and abiotic stress tolerance in crop plants

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Plant hormones play central roles in the ability of plants to adapt to changing environments, by mediating growth, development, nutrient allocation, and source/sink transitions. Although ABA is the most studied stress-responsive hormone, the role of cytokinins, brassinosteroids, and auxins during environmental stress is emerging. Recent evidence indicated that plant hormones are involved in multiple processes. Cross-talk between the different plant hormones results in synergistic or antagonistic interactions that play crucial roles in response of plants to abiotic stress. The characterization of the molecular mechanisms regulating hormone synthesis, signaling, and action are facilitating the modification of hormone biosynthetic pathways for the generation of transgenic crop plants with enhanced abiotic stress tolerance.

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Introduction

As sessile organisms, plants must regulate their growth and development in order to respond to numerous external stimuli and an ever-changing environment [1^{••}]. These adaptations include the responses to temperature fluctuations, water and nutrients imbalance, and pathogens, etc. These responses are mediated by plant growth regulators (phytohormones), compounds derived from plant biosynthetic pathways that can act either at the site of synthesis or following their transport, elsewhere in the plant. Collectively, plant hormones regulate every aspect of plant growth and development and the responses of plants to biotic and abiotic stresses. Plant growth regulators include the five classical phytohormones: abscisic acid (ABA), ethylene, cytokinin (CK), auxin (IAA), gibberellin (GA), jasmonate (JA), as well as brassinosteroids (BR), salicylic acid (SA), nitric oxide (NO), and strigolactone (SL), and it is likely that additional growth regulators are yet to be discovered. In recent years, significant research progress

contributed to the understanding of processes associated with the biosynthesis of plant hormones, their metabolism, as well as their role in signaling. Studies using plants bearing mutations in hormone-biosynthetic pathways have been instrumental in advancing our understanding of the processes associated with the plant responses to changing environments. However, hormones do not act in isolation but are interrelated by synergistic or antagonistic cross-talk so that they modulate each other's biosynthesis or responses. Reviews on hormone action and signaling of ABA [2–4], CK [5–7], ethylene [8], BR [9,10] and JA [11], and on hormone cross-talk [12^{••},13] have been published recently. Here, we highlight the latest advances in our understanding of the role of hormones and hormone cross-talk in plant responses to abiotic stresses. We then discuss the recent progress in the engineering of hormone-associated genes aimed at improving crop stress tolerance.

Hormones and the response to abiotic stress

Phytohormones are essential for the ability of plants to adapt to abiotic stresses by mediating a wide range of adaptive responses [13–15,16[•]]. They often rapidly alter gene expression by inducing or preventing the degradation of transcriptional regulators via the ubiquitin–proteasome system [17]. One of the most studied topics in the response of plants to abiotic stress, especially water stress, is ABA signaling and ABA-responsive genes. ABA synthesis is one of the fastest responses of plants to abiotic stress, triggering ABA-inducible gene expression [18] and causing stomatal closure, thereby reducing water loss via transpiration [19] and eventually restricting cellular growth. Numerous genes associated with ABA *de novo* biosynthesis and genes encoding ABA receptors and downstream signal relays have been characterized in *Arabidopsis thaliana* (reviewed by [2]). At least ten viviparous mutants have been identified in maize (*Zea mays*), most of which (*vp2*, *vp5*, *vp7*, *vp9*, *w3*, *y3*, and *y9*) were blocked in the biosynthesis of the carotenoid precursors for *de novo* ABA synthesis. In rice (*Oryza sativa*), four *phs* mutants, defective in phytoene desaturase (*OsPDS*), ζ -carotene desaturase (*OsZDS*), carotenoid isomerase (*OsCRTISO*), and lycopene β -cyclase (β -*OsLCY*) were found to impact on the biosynthesis of carotenoid precursors of ABA [20]. ABA also plays an important role during plant adaptations to cold temperatures. Cold stress induces the synthesis of ABA and the exogenous application of ABA improves the cold tolerance of plants [21]. Other hormones, in particular CK, SA, ethylene, and JA, also play substantial direct or indirect roles in the response of plants to abiotic stress. CK is an antagonist to ABA, and the exposure of plants to water limiting conditions results in decreased levels of CK. Examination of public microarray expression data for

A. thaliana revealed numerous genes encoding proteins associated with CK signaling pathways that were differentially affected by various abiotic stresses [14]. BR was reported (mainly based on the exogenous application of BR) to induce the expression of stress-related genes, leading to the maintenance of photosynthesis activity, the activation of antioxidant enzymes, the accumulation of osmoprotectants, and the induction of other hormone responses [10]. The overlap between hormone-regulated gene suites during the adaptive responses of plants to environmental stresses suggests the existence of a complex network with extensive cross-talk between the different hormone signaling pathways.

Hormone cross-talk

Evidence supporting hormone cross-talk comes mainly from analysis of *A. thaliana* mutant phenotypes [13]. The synergistic or antagonistic hormone action and the coordinated regulation of hormone biosynthetic pathways play crucial roles in the adaptation of plants to abiotic stress. Recently, the role of auxins in drought tolerance was postulated; *TLDI/OsGH3.13*, encoding indole-3-acetic acid (IAA)-amido synthetase, was shown to enhance the expression of *LEA* (late embryogenesis abundant) genes, which correlated with the increased drought tolerance of rice seedlings [22]. The expression of many other genes associated with auxin synthesis, perception, and action has been shown to be regulated by ethylene [8]. Among them, are the auxin-responsive factors *ARF2* and *ARF19* [23,24], the auxin transporters *PIN1*, *PIN2*, *PIN4*, *AUX1* [25], and genes encoding auxin biosynthetic enzymes (*ASA1/WEI2/TIR7*, *ASB1/WEI7*, *TAA1/SAV3/WEI8*) [26,27]. Conversely, auxin was found to affect ethylene biosynthesis. Several members of the *1-amino-cyclopropane-1-carboxylate synthase (ACS)* gene family, encoding rate-limiting enzymes in ethylene biosynthesis, were shown to be regulated by auxin treatment [28]. Recently, CK was also shown to be a positive regulator of auxin biosynthesis, and it was postulated that a homeostatic feedback regulatory loop involving both CK and IAA signaling acts to maintain appropriate CK and IAA concentrations in developing root and shoot tissues [29]. GA and BR regulate many common physiological processes. *OsGSR1*, a member of the *GAST* (GA-stimulated transcript) gene family, was found to play key roles in both BR and GA signaling pathways, and to mediate the interaction between them [16]. RNAi transgenic rice plants with reduced *OsGSR1* expression displayed phenotypes similar to plants deficient in BR, including short primary roots, erect leaves and reduced fertility. GA is also associated with SA. The exogenous application of GA (GA_3) induced increased expression levels of *ICS1* (*isochorismate synthase1*) and *NPR1* (*nonexpressor of pathogenesis related genes 1*), genes involved in SA biosynthesis and SA action, respectively [30]. Transgenic *A. thaliana* plants constitutively overexpressing a GA-responsive gene from *Fagus sylvatica* encoding *FsGASA4*,

a member of the GA_3 gene family, showed improved tolerance under abiotic stress and the stress tolerance was correlated with increased endogenous levels of SA [30].

ABA regulates stomatal opening during stress, however, recent studies suggest that other hormones such as CK, ethylene, BR, JA, SA, and NO also affect stomatal function (reviewed by [31]). While ABA, BR, SA, JA, and NO induce stomatal closure, CK and IAA promote stomatal opening. NO operates as a key intermediate in the ABA-mediated signaling network that regulates stomatal closure [32]. ABA is also a regulator of strigolactones biosynthesis, as shown using tomato ABA-deficient mutants of different steps in the ABA biosynthetic pathway and specific inhibitors for different carotenoid cleaving enzymes [33]. Recently our own work has shown that expression of *IPT* (*isopentenyl transferase*, a gene encoding a key step in the biosynthesis of CK) under the control of a drought-inducible and senescence-inducible promoter (*P_{SARK}*) in tobacco (*Nicotiana tabacum*) and rice results in a significant alteration of gene expression associated with hormone biosynthesis, response, and regulation [34,35]. Transgenic tomato (*Solanum lycopersicum*) rootstocks expressing *IPT* had enhanced root CK synthesis that was shown to modify shoot hormonal balance under salinity stress [36]. The *P_{SARK}::IPT* tobacco and rice plants showed an upregulation of BR-biosynthesis and BR-regulation and signaling genes, suggesting an interaction between CK and BR [34,35]. BR-mediated signaling was regulated by ABA through *BIN2* or its upstream components via the *PP2C* (*protein phosphatase 2C*) family of genes [37]. ABA was also shown to inhibit BR-induced responses during the exposure of plants to abiotic stress [38]. Whether the positive interaction between CK and BR is a consequence of direct cross-talk between CK and BR or indirectly mediated by ABA remains unclear at this stage [34].

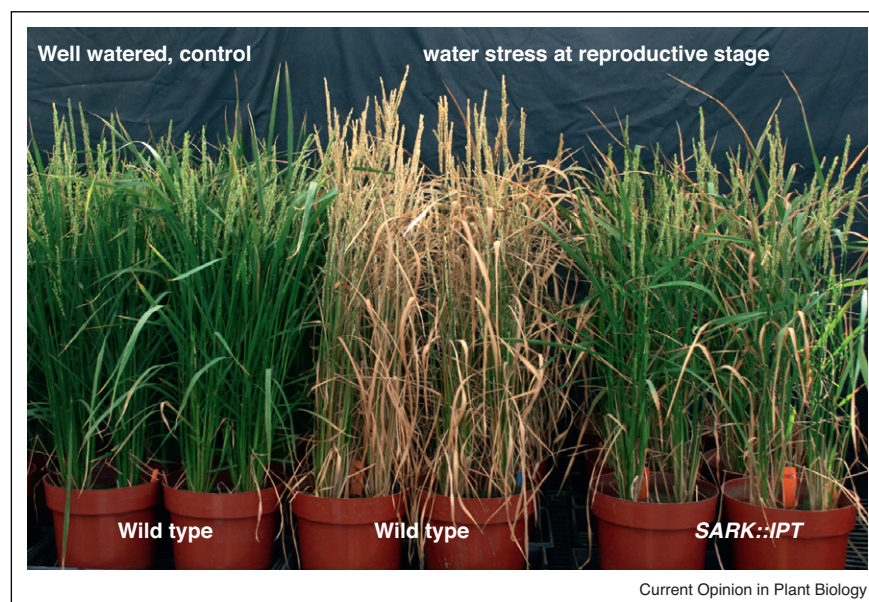
Biotechnological applications

A large number of genes associated with *de novo* ABA biosynthesis and genes encoding ABA receptors and downstream signal relays have been characterized in *Arabidopsis* (reviewed by [2]). The catalytic steps of ABA biosynthesis involving the conversion of β -carotene to ABA is mediated by the action of enzymes encoded by *ABA1/LOS6*, *ABA4*, *NCED*, *ABA2*, and *ABA3/LOS5* [39]. Some of these genes have been manipulated in crops. *ABA3/LOS5* encodes a Mo-cofactor sulfurase (MCSU) that catalyzes the final conversion of abscisic aldehyde to ABA. The expression of *ABA3/LOS5* was enhanced when *A. thaliana* plants were exposed to drought or salt [40]. Overexpression of *ABA3/LOS5* under the control of constitutive or drought-inducible promoters resulted in a significant increase in transgenic rice yield under drought conditions in the field [41]. *NCED* encodes 9-cis-epoxy carotenoid dioxygenase, an enzyme that catalyzes the conversion of neoxanthin to xanthoxin, a rate-limiting reaction in the synthesis of ABA. In *A. thaliana*, *AtNCED3*

plays a crucial role in drought-stress-inducible ABA biosynthesis, and T-DNA insertional *nced3* mutants have defects in ABA accumulation under drought stress and impaired drought tolerance. Tobacco plants constitutively overexpressing *SgNCED1* (from *Stylosanthes guianensis*) displayed a 51–77% increase in leaf ABA accumulation, which resulted in enhanced tolerance of the transgenic plants to drought and salinity [42]. The transgenic *NCED1* plants were similar in size to the wild-type plants, and under drought they were able to maintain relative growth rates similar to that of the wild-type plants under normal conditions. The constitutive overexpression of *LeNCED1* in tomato also resulted in increased ABA accumulation in the transgenic plants [43]. Under well-watered conditions, the transgenic plants showed a reduction in assimilation rates, leaf chlorosis. Under water-deficit conditions, these effects did not reduce biomass production, presumably because of counteracting positive effects of ABA on leaf expansion through improved water status [43]. The modification of genes involved in the regulation of the plant responses to ABA is an alternative approach to enhance of plant stress resistance. *ERA1* encodes the β -subunit of farnesyltransferase, an enzyme associated with ABA-dependent signal transduction [44]. Transgenic canola (*Brassica napus* L.) carrying an *era1* antisense construct driven by the drought-inducible *rd29A* promoter from *A. thaliana* displayed enhanced yield under a mild drought stress [45]. CK is an antagonist to ABA, and the exposure of plants to drought results in decreased levels of CK. Elevated CK levels promoted survival under water-stress conditions, inhibited leaf senescence and induced increased proline

levels [46]. Manipulation of endogenous CK levels was effective in delaying senescence. The *IPT* gene has been overexpressed in several plant species under different promoters and the transgenic plants were tested for tolerance to various environmental stresses (reviewed by [47]). The constitutive overexpression of *IPT* increased endogenous CK concentrations up to 150-fold and resulted in decreased root growth and in water stress [48]. The use of inducible promoters for the conditional expression of hormone biosynthetic genes makes it possible to control hormone levels without the negative effects on growth and development produced by very large changes in hormone concentrations. The senescence-induced promoter *P_{SAG12}* [49] has been used to drive the *IPT* expression, resulting in a significant delay in plant senescence. However, a significant delay in flowering and reduced yield were also observed (reviewed by [47]), probably due to altered source/sink relationships brought about by the lack of chlorophyll and protein degradation in source leaves [50]. The use of maturation-induced and stress-induced promoters (*SARK*, *senescence associated receptor kinase* [50]) to drive *IPT* expression in both dicots and monocots provided an alternative approach for the induction of *IPT* and the concomitant biosynthesis of CK, without the negative effects of constitutively high CK content on plant phenology (i.e. flowering time, plant architecture, etc.) [34^{**},35^{**},50,51^{*}]. *IPT* was expressed in the whole plant, its maximal expression was attained during the drought episode and the transgenic plants displayed enhanced drought tolerance and superior yields (Figure 1) [34^{**}]. Tomato plants grafted onto rootstocks constitutively

Figure 1



Effects of water-stress on growth of rice (*Oryza sativa*) plants. Wild-type (WT) and transgenic plants expressing *P_{SARK}::IPT* grown under well-watered conditions and plants subjected to water-stress at pre-anthesis follow re-watering as described [34^{**}].

expressing *IPT* resulted in a decrease of root biomass under control conditions. However, under salinity-stress conditions the transgenic plants yielded 30% more than the wild type plants [36^{••}]. An alternative approach to introducing the *IPT* gene is to fuse *IPT* to the 3' end of other genes under the control of a single constitutive promoter. The distance of the *IPT* gene from the constitutive promoter resulted in a moderate *IPT* expression and only a 2–3 fold increase of CK levels. This resulted in improved stress tolerance in transgenic plants [52], supporting the notion that moderate increases in CK can be an effective strategy for improving stress tolerance. Exogenous application of BRs was reported in diverse plant species to induce drought tolerance [38[•]]. Nevertheless, using BR-deficient mutant it was shown that endogenous BRs or perception of the same are not required for plants to respond to water stress [53]. The overexpression of *AtDWF4*, a gene involved in BR biosynthesis, under the control of a seed-specific *oleosin* promoter resulted in improved germination of seeds that were previously treated with ABA, which suggests an antagonistic effect of BR on ABA-regulated processes. Furthermore transgenic seedlings were more tolerant to cold stress than wild-type seedlings [54]. A knockout T-DNA insertion mutant of *Osgsk1* (a rice *GSK3/SHAGGY-like protein kinase* gene, ortholog of *AtBIN2/AtSK21*, a negative regulator of BR-signaling), showed greater tolerance to abiotic stresses, while *Osgsk1* overexpression in *Arabidopsis* resulted in stunted growth [55]. Antagonism between BR and ABA was recently demonstrated in transgenic *P_{SARK}::IPT* rice plants, where the increase in CK induced BR-associated genes and repressed ABA-related processes [34^{••}]. These results further highlight the importance of hormone cross-talk during the response of plants to abiotic stress.

Conclusions

The molecular mechanisms regulating hormone synthesis, signaling, and action have been elucidated during the past few years, and the roles of plant hormones for responses to changing environments have been demonstrated. These findings will facilitate the modification of hormone biosynthetic pathways for the generation of transgenic plants with enhanced abiotic stress tolerance. Controlling the hormone dose/response ratio remains a challenge, since the hormone levels attained should be moderate in order to maintain a balance between the positive effects of plant hormones on stress tolerance and the negative effects on growth and development. The use of conditional promoters driving gene expression at specific developmental stages, in specific tissues/organs and/or in response to specific environmental cues circumvents this problem and will facilitate the generation of transgenic crops able to grow under various abiotic stresses with minimal yield losses.

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