Chapter

The Modification of Abscisic Acid and Cytokinin Signaling with Genome Editing to Increase Plant Drought Tolerance

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Abstract

Due to climate aridization, the need to increase the resilience of plant productivity lo water stress becomes urgent. Abscisic acid and cytokinins have opposing biological roles during water deficit and post-drought recovery, but both these regulators can be utilized to maintain plant productivity under water stress. Downregulation of abscisic acid biosynthesis and signaling can aid in the maintenance of photosynthesis, growth, and productivity in plants, although increasing the susceptibility to severe stress. Cytokinin upregulation can maintain photosynthesis and productivity during water stress and aid recovery processes, whereas downregulation can lead to increased root growth, thus improving plant water balance, nutrient absorption, and hence productivity in water-limited conditions. The use of modern genome editing methods makes it possible to specifically modify genes involved in the implementation of complex traits in plants, such as resistance to stress factors. This review will examine the main areas of work on genome editing of gene families involved in plant responses to water deficiency using CRISPR/Cas technologies. Our current work on editing the ABF gene family, encoding transcription factors for ABA (AREB1/ABF2, AREB2/ABF4, and ABF3), as well as the CKX gene family (CKX1 and CKX4), encoding cytokinin oxidase/dehydrogenases, will be presented.

Keywords: stress factors, plant drought resistance, molecular methods, plant genome modification, genome editing, abscisic acid, cytokinin signaling

1. Introduction

Drought is the most important abiotic factor challenging plant survival, performance, and productivity on the planet. The rapidly increasing risk of coupled negative effects of water deficit and heat stress implies that we need to adapt the physiology of major crop plants to a hotter and drier future. Generally, the adaptation of annual crop plant to water stress can be confined to two capabilities:

- to survive water stress without major irreversible effects on plant performance
- to quickly and fully recover after restoration of water supply, thus decreasing the cumulative drought impact on plant performance

It is clear that these capabilities depend on different or even conflicting plant traits. Survival during water deficiency depends mainly on the ability to prevent the irreversible desiccation of plant tissues and maintain the hydraulic integrity of a plant [1]. Plants need to minimize water losses, whereas photosynthesis and growth can be drastically diminished during this period without the major threat for plant survival during drought. In contrast, recovery occurs when water is plenty again and depends on the ability to recover photosynthesis, growth, and resource allocation to productive organs. Therefore, it is likely that opposing regulatory mechanisms would be required to make plants more tolerant to water stress per se and to make them abler to recover form water stress [2]. A clear example of such opposing pair of regulatory mechanisms are abscisic acid (ABA) and cytokinins (CKs). This chapter is devoted to the effects of these regulatory molecules on plant performance during water stress and recovery, on their regulatory modes, and on the usage of genome editing technologies to change plant ABA and CK balance to increase drought tolerance and post-drought recovery.

2. Abscisic acid

The response to water deficit is the major biological function of abscisic acid, and ABA can be considered as a versatile hormone that regulates plant water status in an integrated fashion. Abscisic acid increases water acquisition by affecting root growth and plant osmotic balance, affects water transport from the root surface to leaf tissues through regulation of aquaporin genes, and regulates water spending through the regulation of stomatal conductance, possibly influencing cuticular conductance. In case of stress severe enough to exert a substantial degree of dehydration in plant cells, ABA regulates the biosynthesis of stress-protective compounds such as dehydrins [3], but such intensive stress is likely of minor importance for agricultural plants [4]. The major biological functions of ABA during water stress are considered below.

2.1 Abscisic acid and plant water spending

The most-studied biological effect of ABA is stomatal closure, which enables plants to greatly diminish water losses, thus making a major contribution to the maintenance of plant water status during drought [5]. The paramount importance of ABA for stomatal regulation is clearly illustrated by ABA-deficient mutants, which are extremely sensitive to increasing vapor pressure deficit even under well-watered conditions [6]. In more ancient plant lineages, ABA biosynthesis is rather slow, likely due to a reliance on non-specific enzymes during ABA biosynthesis, and therefore, ABA accumulation can occur only if water stress is rather prolonged [7–9]. For example, in [10], ABA increment in drying leaf tissues in several coniferous species occurred only after 2-h of dehydration. In contrast, angiosperms can induce ABA biosynthesis rapidly within few tens of minutes through the activation of NCED gene expression in ABA-producing tissues [7]. ABA catabolism genes are often upregulated simultaneously with ABA biosynthesis genes, but the expression of the former is lower than

the latter, resulting in net ABA accumulation under inductive conditions [11]. ABA accumulation in dehydrating cells occurs due to the decreasing of cell volume, rather than of turgor or water potential [12]. After water stress relief, the activity of ABA biosynthesis genes stayed elevated in guard cells, thus achieving drought memory effects favorable in case of subsequent droughts [13]. ABA controls memory processes through signaling pathway SnRK2/ABF/ABRE [14]. ABA biosynthesis in roots also occurs under water stress, but leaf is likely a major site of ABA biosynthesis [15, 16]. There are conflicting evidences whether leaf ABA biosynthesis occurs mainly in vascular buds and guard cells or in mesophyll tissues, with solid evidences in favor of mesophyll as the main site of ABA biosynthesis [7, 10, 15]. Additionally, in angiosperms, rapid ABA-induced stomatal closure occurs within seconds to minutes, thus indicating the presence of ABA-dependent non-transcriptional mechanisms in stomatal closure [17]. Abscisic acid is among the key mechanisms underlying the difference between isohydric (R-type) and anisohydric (P-type) strategies under drought stress, as isohydric plants achieve high leaf ABA levels during stress, whereas anisohydric plants respond to stress with an initial peak and subsequent decline of ABA content [5, 18, 19]. In ferns or lycophytes, the stomatal closure is independent of ABA [5], thus indicating that stomatal regulation by ABA is a relatively late evolutionary achievement.

Besides regulation of stomatal conductance, ABA probably plays a role in the regulation of residual cuticular conductance. This type of conductance, although quantitatively minor in well-watered plants, becomes the major determinant of plant survival during prolonged water deficiency, when water absorption by the plant root system reaches zero, and the ability to preserve the water already present in tissues becomes crucial [1]. Cuticle is often viewed as a rather stable structure hard to be modified, but in fact, recent assimilates can be incorporated rapidly in the cuticle [20], indicating that cuticle can be probably abler to modifications than it is thought currently. Plant minimum leaf conductance can decrease under drought stress from −4 to −70%, with a decrease of 30–40% being typical [21]. It is known that ABA can change the chemical composition of cuticle, but whether it aids in decreasing minimal conductance is unknown and requires further clarification [21].

2.2 Abscisic acid and regulation of water acquisition and transport

ABA effect on root growth is biphasic, with mild ABA increase stimulating root growth through ethylene-dependent mechanisms, whereas higher ABA concentrations inhibit growth through auxin signaling pathway [22, 23]. The ABA-dependent increase in main root elongation concomitantly with the inhibition of lateral root formation aids plants in reaching deep water-containing soil horizons with minimal carbon expenditure on root growth, and the biological effects of ABA and drought on root growth are similar [24]. However, in [25], ABA increased lateral root number and length at mild water deficit, likely also suppressing primary root growth to a certain extent. Not only the biological effects of ABA but also the source of ABA in root remain somewhat controversial. Mild drought leads to local ABA accumulation in roots [26]. Earlier, root tip was thought to be the main source of ABA biosynthesis during water stress, but now, it is clear that leaf-derived ABA plays a major role in shaping root growth [27], whereas ABA biosynthesis in roots can be limited by carotenoid substrate limitation under water stress [10]. ABA effects on root growth take place via interacting network with cytokinins, ethylene, and auxin [23]. Synthesis of ABA in roots of transgenic poplar increased root growth and drought tolerance [28].

ABA is involved in the stimulation of reversible suberization of root endodermis, which is required to regulate the apoplastic movement of water [17]. The decrease in root hydraulic conductance, in turn, leads to stomatal closure and water economy, aiding in adaptation to water deficiency [29]. The decrease in ABA accumulation in root tissues during water stress can be also observed, probably due to increased ABA translocation to the above-ground plant part [30]. In addition to root growth, ABA can also positively affect osmotic adjustment, thus increasing plant water absorbing capacity [31].

Abscisic acid participates in regulation of the plant aquaporin system [32]. ABA positively affects root hydraulics [25] and is involved in jasmonate-mediated increase of root hydraulic conductivity [33]. However, not only promoting but also inhibiting effects of ABA on root hydraulic conductivity are observed [26]. Also, ABA can play a role in increasing the water-transporting ability of mycorrhizal fungi [26]. ABA is among the key regulators of expression of aquaporin genes [26]. In Zea mays, ABA increases both gene expression and protein content of different PIP aquaporins, although the results can vary between studies [34]. Also, ABA participates in the regulation of aquaporin activity through phosphorylation [32]. Although ABA is generally viewed as hormone inhibiting the above-ground growth, the positive ABA influence on plant hydraulic conductance through the regulation of aquaporin system can translate into positive effect on leaf extension growth, thus making the total ABA effect on growth less straightforward [34]. ABA-induced decrease of leaf hydraulic conductivity can participate in the regulation of stomatal closure [35].

The role of ABA in the regulation of axial water transport through xylem is less well studied, compared to cell-to-cell transport through aquaporins. ABA is well-known to regulate the blockage of plasmodesmata in dormant cambium, making it unresponsive to activating environmental signals, and is involved in the termination of wood differentiation [36]. Exogenous ABA treatment often leads to reduced stem growth through inhibition of cambial activity, whereas occasional reports of secondary growth stimulation by ABA treatment likely stem from specific experimental approach rather than from ABA effects per se [37]. In cambial and xylem tissues of *Eucommia ulmoides* trees, the seasonal dynamics of ABA and IAA was the opposite, and ABA negatively influenced cambium reactivation by IAA [38]. ABA treatment decreases the hydraulic diameter of vessels, which negatively affects xylem hydraulic conductance [39]. Therefore, ABA likely plays a negative role in the formation of water-transporting tissues during plant secondary growth.

2.3 Trade-offs of ABA effects on plant performance

The above-described integrative positive effects of ABA on plant drought tolerance are linked with several important trade-offs. Although ABA biosynthesis is down-regulated quite rapidly during post-stress period, the major increment of ABA in leaves can sustain for prolonged period after drought release [5]. This limits a plant's ability to rapidly restore gas exchange and photosynthesis and underlies, at least partially, the hysteresis between stomatal conductance and other leaf hydraulic characteristics post-drought [40], although these limitations can be also unrelated to ABA accumulation. However, it should be noted that sustained ABA accumulation may aid the recovery processes by facilitation of embolism repair by decreasing stomatal conductance and water loss, which favors embolism refilling processes [41, 42]. Also, memory effects due to ABA increase during the first stress encounter can increase the tolerance to subsequent stresses and yield [43]. The negative

influence of ABA on leaf growth can be mainly due to the inhibition of assimilation resulting in source limitation of growth [10]. However, direct negative ABA effects on growth processes through ABF transcription factors is also well-known [44]. The ABA-induced increase of biosynthesis of osmolytes and protective compounds would distract these resources from growth and reproduction. Also, allocation of belowground growth to the deeper root system would probably lead to deterioration of mineral nutrition, since the deeper soil layers are deprived with mineral nutrients compared to upper layers [45].

Given these trade-offs, it is not surprising that constitutively increased ABA biosynthesis and ABA signaling results in depressed growth and productivity in non-stressed conditions, whereas the suppression of ABA signaling increases growth in the absence of abiotic stressors [46]. Crop plants are usually grown in more favorable conditions compared to native plants, and severe water stress is less prevalent for agricultural ecosystems compared to native ones [4]. Also, the maintenance of productivity during mild water stress is obviously more important from the economical point of view compared to the ability to survive severe water deficiency, since in the latter case, the productivity would be anyway lost. Therefore, for annual crops, the downregulation rather than upregulation of ABA biosynthesis and/or signaling can be a more promising strategy to maintain productivity during mild stress, although making plants more susceptible to severe stresses, which are devastating for plant productivity no matter whether plants survive the stress period or are desiccated. However, it is known that the logarithmic character of dependence of carbon fixation on stomatal conductance means that plants can decrease stomatal conductance to a certain extent without trade-off with CO2 uptake and assimilation activity [5]. It can be therefore proposed that mild increase in ABA biosynthesis/signaling with the associated moderate decrease of stomatal conductance can result in substantially improved water use efficiency without compromising plant productivity, making such plants more effective from the economical point of view.

3. Cytokinins and their effects on plant performance during drought and recovery

Generally, biosynthesis and signaling of cytokinins are negatively affected by drought, consistent with the view on CKs as negative regulators of drought tolerance [11]. However, the regulation of CK metabolism under water-stress conditions can be rather specific, with different IPT genes demonstrating differently directed regulation under drought, whereas for CK OXIDASES/DEHYDROGENASES (CKX), more uniform upregulation is observed [47]. The directional changes in CK biosynthesis and signaling can have rather contrasting effects on plant ability to tolerate drought and to recover from its impact. Both CK signaling mutants and transgenic plants with enhanced CK signaling often demonstrate increased drought tolerance (Hai 2020). CKs generally exacerbate water loss by plants, thus making them more prominent to severe drought, whereas decreased CK levels contribute to more parsimonious water spending and better maintenance of plant water status during stress [48]. Also, CKs are positive regulators of shoot meristem activity and hence shoot growth [11, 49], and the increased above-ground growth can be maladaptive under severe water deficiency. The decreased CK accumulation can be associated with higher tolerance of photosynthetic processes during drought [48]. CKs and ABA reciprocally downregulate the biosynthesis and signaling of each other, thus exerting contrasting

effects in plants under non-stressed conditions and under drought stress [50]. ABA decreases CK contents, which increase plant sensitivity to ABA, thus making plants abler to respond to water deficiency [50]. Cytokinins repress SnRKs as major components of ABA signaling, thus inhibiting ABA effects on plant under non-stressed conditions [50].

On the other hand, when water stress is not severe and plants are not at risk of desiccation, CKs can have numerous positive effects on plant drought and post-drought performance. Both exogenous CK treatment and modulation of endogenous CK levels were reported to positively affect plant drought tolerance [48]. Increased CK biosynthesis delayed drought-induced leaf senescence in tobacco and maintained photosynthesis, thus decreasing yield loss [4]. Under water deficiency, CKs promote stomatal conductance and chlorophyll biosynthesis [47], which can be detrimental under severe stress but is advantageous for productivity under relatively mild water stress conditions. CK-mediated inhibition of stomatal closure is the conserved response in diverse plant species [5]. CKs promote plant antioxidant defense by increasing the activity of antioxidant enzymes and decreasing the activity of ROS-generating systems such as xanthine oxidase [47]. CKs may positively affect plant osmotic adjustment under water deficiency [51]; in contrary, in [4], much lower proline accumulation in tobacco plants with increased CK biosynthesis was observed, likely due to their higher drought tolerance and lower degree of stress compared to wild-type plants. Also, CKs positively influence cambial activity and radial growth, thus increasing stem hydraulic conductance [37]. Generally, many of the positive CK effects during mild stress can be due to a delay in activation of drought response, thus decreasing stress impact [48]; CKs are known to suppress SnRK2 functioning and thus stress response [50]. It can be very promising for agricultural plants, since plant productivity and not plant survival is of the most interest for agriculture, thus making CK-induced desensitization of plants to environmental stress a promising strategy to maintain crop productivity under relatively mild stresses, typical for agricultural conditions [4].

CKs have numerous positive effects on plant post-drought recovery processes, making plants with upregulated CK content superior in recovery compared to wild-type plants. CKs in plants decreases under drought [11] while increasing prominently during the recovery period, together with compensatory growth acceleration compared to non-stressed plants [48]. Higher CK content during the post-drought recovery period can elevate auxin content in leaves [48] and also in cambium [52], which is necessary for active post-drought growth. CKs positively affect stomatal opening in post-drought period [53], helping to restore photosynthesis and to minimize cumulative negative drought impact on assimilation. Therefore, despite negative effects of CKs on tolerance to severe stress, their upregulation can be a promising way to increase the performance and productivity of crop plants.

However, downregulation of CKs can also have positive effects on crop performance under water shortage. Cytokinins are negative regulators of root meristem activity [11, 54], suppressing both primary root elongation and root branching [50, 55]. Negative CK effect on primary root growth is exerted through increase in ethylene biosynthesis [54]. As a result, CKs decrease both drought tolerance and absorption of mineral nutrients [56], whereas reduction of endogenous CKs can have prominent positive effects on root growth, increasing the number and length of lateral roots and root biomass accumulation [47]. For example, root-specific expression of CKX gene in Zea mays improved both root growth and mineral nutrition of plants, which was surprisingly achieved without trade-offs with above-ground growth [57].

The fact that such prominent changes in whole-plant architecture were made possible by expressing a single gene is quite promising for plant improvement. Therefore, both decrease and increase in CK biosynthesis and signaling can be viewed as a potential way to increase the resilience of crop productivity to water shortage.

4. Methods for modifying plant genomes

Plant genetic traits are inherited from parents from generation to generation and are encoded by genetic information contained in DNA. At the same time, genetic information is subject to constant changes due to the presence of spontaneous or induced mutations, errors arising during transcription, the activity of transposable elements, the processes of meiotic crossing over, and cross-fertilization. Some pathogenic and symbiotic bacteria, such as Agrobacterium spp. [58], can transfer part of their DNA into the genome of the host cell, thereby changing the functioning of the host cell to suit their needs. Thus, genome modification occurs constantly in a plant cell.

Plant breeding is the process of obtaining new varieties of plants that contain in their genome a set of genes that make it possible to grow plants that are suitable for agricultural production, processing, and consumption and at the same time have properties beneficial to humans and animals. Thus, plant breeding involves systematic selection among the entire population of plants of samples bearing target properties. It is estimated that humans have been successfully breeding plants for over ten thousand years [59] when seeds of plants with favorable features were saved for the next plantation, a practice known as domestication. The most significant advances in plant breeding techniques have been achieved as knowledge and understanding of plants and their genetic structures have accumulated. In the second half of the twentieth century, with an increase in the quantity and quality of food consumption, a revolution in plant breeding occurred, the key achievements of which were achieved in the creation of hybrids and transgenesis. The most important stage in plant breeding was the Green Revolution, which made it possible to dramatically increase the productivity of agricultural crops through the development of high-yielding varieties of cereals, particularly dwarf wheat and rice. Norman Borlaug, Nobel Prize laureate and father of the Green Revolution, emphasized that the key to the success of these semi-dwarf varieties was their wide adaptability, short plant height, high sensitivity to fertilizers, and resistance to disease, which ultimately made it possible to obtain more yield at a lower cost [59]. Later, these requests were addressed to the emerging technology of transgenesis, which led to its rapid development. Transgenic crops are now widespread globally and are increasingly accepted as food and feed. Transgenesis changes the genetic information of a plant cell, resulting in a so-called genetically modified organism (GMO) that carries in its genome a fragment of foreign DNA that gives the plant new useful traits that cannot be obtained by conventional breeding methods. However, GMO organisms were perceived ambiguously by society, which led to the fact that obtaining state registration for a GMO variety in some countries is significantly difficult or completely impossible.

4.1 Development of the genome editing tools

With the development of genetic engineering methods and the accumulation of data on plant genomes, gene editing technologies began to develop—making

it possible to make site-specific changes in the target site of the genome. The first methods that appeared were zinc-finger nuclease (ZFN) and later transcription activator-like effector nucleases (TALEN). Both TALEN and ZFN are composed of repeated tandem sequences of DNA-binding domains and an attached Fok1 nuclease protein, such that the recombinant protein can be targeted to recognize a target DNA sequence and therefore create double-strand breaks (DSBs) at the target site. For each target site, a new TALEN or ZFN protein must be prepared to recognize the target DNA sequence, which required labor-intensive genetic engineering and significantly limited the widespread use of these gene editing technologies [60, 61]. However, there are examples of successful use of ZFN to manipulate genes in tobacco, Arabidopsis, and maize [62–64]. TALENs, which are easier to target to a specific DNA region because each TALEN domain recognizes one target nucleotide, as opposed to ZFN, where each domain recognizes a triplet of nucleotides, have been successfully used in horticultural crops such as soybeans, wheat, rice, tomatoes, and potatoes [65, 66]. However, the major drawback related to ZFNs and TALENs are their off-targeting effects, prolonged screening process, toxicity to the host cell, and complex genetic engineering procedures, limiting their applicability. The most modern method of genome editing is CRISPR technology; the first article on the successful application of this technology on plant cells was published in 2013, and the first edited plants were *Arabidopsis thaliana* and *Nicotiana benthamiana* [67].

Typically, CRISPR/Cas9 is a complex consisting of two components: the Cas9 endonuclease protein and a single guide RNA (sgRNA) with 20-nucleotide homology to the target DNA region [68–70]. The Cas9 endonuclease binds to the protospacer adjacent motif (PAM) DNA sequence (for Cas9 the PAM site is NGG), the sgRNA complementarily binds to the DNA sequence adjacent to the PAM site, and if the binding is successful, Cas9 carries out a DSB in the target site [68, 71]. DSBs caused by the Cas9 endonuclease lead to the activation of DNA repair systems, which can take two pathways, the error-prone non-homologous end-joining (NHEJ) or homology-directed repair (HDR). Errors of DNA repair system result in deletions, insertions, or substitutions of DNA at DSB sites, which in turn disrupt gene function or cause a reading frameshift, known as a gene mutation or knockout [68–70]. As a result of DSB repair via the NHEJ pathway, insertions/deletions (indels) of several bases are usually observed during plant genome editing based on CRISPR/Cas9. The use of the mechanism of HDR, in turn, makes it possible, using editing systems, to replace individual nucleotides in the DNA sequence and even obtain a site-specific insertion of a gene or group of genes.

At the moment, editing technologies have become so widely developed that they make it possible to influence any stage of the implementation of genetic information in a cell—at the level of transcription, translation, post-translation, epigenetic and so on [72]. Over the past 10 years, a number of different CRISPR-based tools have been developed, allowing editing at almost any desired location in the genome. Some examples include DNA base editors [73], epigenetic modifiers [74, 75], prime editors [76, 77], and transcription regulators [78, 79]. Fusion of various additional molecules with partially disrupted (nickase Cas9, nCas9) or nuclease-deficient (dead Cas9, dCas9) Cas9 has been used as a vehicle to deliver the CRISPR fusion protein to the target genomic site. RNA-targeting Cas proteins also enable a variety of RNA manipulations beyond simple RNA editing, such as RNA degradation, detection of ribonucleic acids and pathogens, single RNA base editing, and live imaging of RNA, which can be read in more detail in recently published reviews [72, 75]. Plants cope with stress through a range of finely tuned mechanisms, which involve both protein-coding genes and non-coding regions of the plant genome, along with various epigenetic

mechanisms realized through the control of DNA packaging. The CRISPR-based tools described in this section can exploit the full range of molecular mechanisms mediated by these genomic elements.

4.2 Editing genes associated with transport and signaling of ABA and CKs

Major thriving areas of research include gene discovery (allele mining, investigation of cryptic genes) and introgression of new traits to achieve the desired goal—biotic/abiotic stress-resilient crops. Today, there is already a fairly large pool of works devoted to editing genes associated with the transport and signaling of ABA and CKs [75, 80]. Editing and transgenesis have helped to establish the functions of a number of genes associated with the ABA signaling pathway and their participation in the response to stress [81, 82]. For example, the enzymes SAPK1 and SAPK2 belonging to the SnRK2 family are members of the ABA signaling pathway in rice. Loss-of-function mutants of SAPK2 generated by CRISPR/Cas9 were insensitive to ABA [81]. The SAPK2 mutants displayed high sensitivity to dehydration and ROS, highlighting the role of SAPK2 in drought stress, the same as how CRISPR-edited OsERA1 mutant lines displayed enhanced tolerance to drought stress [83]. Another example is the work with histone acetyltransferase (HAT) enzyme that relaxes chromatin folding and promotes enhanced gene expression fused with dCas9 protein. Tools for gene activation and epigenetic modification combined with the CRISPR system made it possible to create the dCas9-HAT system, which increased the expression of AREB1 and as a result increased the resistance of Arabidopsis plants to drought [84]. CRISPR/Cas9 was successfully used to create new alleles of the OST2 gene in Arabidopsis, and as a result, edited plant lines carrying the new alleles exhibited an enhanced response to stress due to changes in stomatal closure under drought stress [85]. A number of genes have been shown to be involved in the negative regulation of plant responses to salinity and other abiotic stresses. Reducing the expression level of the RR22 gene, which encodes a type B response regulator (ARR B) involved in CK signaling, using the CRISPR/Cas9 system, made it possible to increase the tolerance of rice plants to soil salinity [86]. Additional examples of negative regulators research using editing tools include work in Arabidopsis and rice. Editing of the C/VIF1 gene encoding the fructosidase inhibitor protein 1 showed that it is a regulator of the response to ABA and is involved in the development of salt tolerance [87]. Editing of the RR9 and RR10 genes in rice, encoding proteins involved in the CK signaling pathway and associated with response regulators type A (ARR A), allowed to establish their function as negative regulators in response to salinity [88]. As recent work on AITR family genes has shown, targeting mutations in genes with redundant or unclear functions using CRISPR editing systems can help elucidate their role in plant stress biology [89, 90].

As it can be seen, various genome editing tools have been successfully used to study genes associated with plant stress resistance and to create stress-tolerant plants belonging not only to model plant species but also to plant species important for agriculture. The ever-expanding set of CRISPR tools allows you to make changes to any process occurring in a plant cell and thereby regulate the growth, development, and all life processes of plants, through precise and effective genetic engineering. Consistent changes and grouping of genes responsible for resistance to various types of stress, both biotic and abiotic, can help in the development of new lines for plant breeding. Accelerated identification of new genes, as well as the creation of gene-edited crops that do not fall under the regulatory requirements developed for transgenic plants, could be a step toward the next Green Transformation.

4.3 AREB/ABF and CKX gene families as potential targets for editing

CKX genes, which are key regulators of the level of CKs in plant cells and, accordingly, can influence the homeostasis of CKs in the cell, have long attracted the attention of researchers as providing ample opportunities for improving crops. Most studies investigating the function of CKX genes have been carried out using RNAi-based silencing or overexpression of CKX genes. Overexpression of AtCKX7 in the model plant results in shorter primary roots [91]. Overexpressing the AtCKS2 gene in oilseed *Brassica napus* increased the root-to-shoot ratio [92]. A number of studies have shown that reducing the expression level of CKX genes in some cases can lead to increased crop yields. For example, in barley, cotton, rice, and Arabidopsis, downregulation of CKX family genes through RNAi-based silencing or various genome editing systems, or with the help of mutations, has resulted in increased seed number and/or seed weight [93–96]. Also, in a number of works on editing genes of the OsCKX family in rice, it was shown that OsCKX genes serve as a link between CK and other plant hormones, in particular ABA [97, 98]. The perspectives of utilization of genome editing technologies to improve crop performance were discussed recently [80, 99]. The findings support the critical role of CKs in a variety of model plants.

There are significantly fewer studies on the AREB/ABF family. There is work to increase ABF2 expression using dCas9-HAT [84], but most of the research has been done on T-DNA-induced mutations in Arabidopsis obtained in the early 2000s [100–102]. In Arabidopsis, three members of the AREB/ABF family that respond to water stress and participate in the ABA signaling pathway, ABF2, ABF4 and ABF3, are the master transcription factors that co-regulate ABF-dependent ABA signaling and require ABA for full activation [100]. At the same time, the incomplete functional redundancy of ABF transcription factors gives reason to expect that differential manipulations of ABF can be used to create plants with the desired mode of ABA signaling, for example, to reduce trade-offs between ABA-induced stress tolerance and productivity.

Over the past few years, experimental evidence has been obtained on changes in DNA regions located at some distance from the site of T-DNA integration [103, 104]. This prompted a reconsideration of the relevance of using such mutations to identify the functions of genes of interest, since the manifestation of a mutation caused by the insertion of foreign DNA into the region of the gene under study and causing the loss of its function (knockout) can be masked by other insertions in regions remote from the region of the target gene. The development of new genome editing tools using CRISPR/Cas9 makes it possible to specifically make changes only in the target gene and obtain new series of knockouts for genes of interest. This work firstly examines the possibility of editing genes of the ABF family encoding the AREB1/ABF2, AREB2/ABF4, and ABF3 transcription factors using *Arabidopsis thaliana* as an example, taking into account the possible participation of other genes included in the network of regulation of abscisic acid biosynthesis. Secondly, the possibility of multiplex editing of CKX1 and CKX4 genes of *Arabidopsis thaliana* to establish their role in the response of plants to abiotic stress. Crossing the resulting mutants will make it possible to establish the details of the interaction between ABA and CKs.

5. Conclusion

The development and improvement of molecular biology methods by the beginning of the twenty first century stimulated the creation of modern tools that make it

possible to modify plant genomes by targeted changes in the functioning of genes of interest. This opens up great opportunities for researchers to modify genes involved in the control of complex traits in plants, such as resistance to water deficiency. The use of genome editing to knockout individual genes that control plant response to various stress conditions, including water deficiency, will reveal the role of both regulatory genes encoding transcription factors for ABA biosynthesis and genes that provide interconnections between the signaling pathways of various phytohormones, in particular, the relationship between ABA and CKs.

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Conflict of interest

The authors declare no conflict of interest.

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