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Cell signaling during drought and/or cold stress in cassava

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Abstract

Cassava (*Manihot esculenta* Crantz) is a root crop significant in food security and various bio-industrial applications such as animal feed, modified starch, and biofuels. Drought and cold stress are two major factors limiting cassava production qualitatively and quantitatively, for which plants have evolved mechanisms to overcome the impact of these two stressors. In recent years, significant progress has been achieved in understanding the response mechanism of cassava plants to stress signals to tolerate the above stresses. In this review, core stress-signaling pathways, including transcription factor (TF)-related regulatory networks, plant hormone signaling, reactive oxygen species (ROS) scavenging, and non-coding RNA (ncRNA) and alternative splicing (AS) that modify gene expression levels in response to drought and/or cold stress in cassava, are summarized. Understanding these stress signaling and responses will increase our ability to improve the crops tolerance to multiple stresses for agricultural sustainability and food security for the growing world population.

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INTRODUCTION

Agriculture is essential in supplying fiber, fuel, and food for the rapidly growing population globally. In recent decades, the world population has increased tremendous pressure on agricultural crop production systems[1,2]. Moreover, climate change, such as drought and cold, have resulted in abiotic stresses, posing threats to crop production worldwide. Under drought and cold stresses, crops suffer various degrees of damage and huge yield losses[2]. Therefore, food security is a growing challenge facing humankind worldwide. In this respect, the improvement and expansion of crop varieties suited to grow under limited water resources and extreme temperatures are the keys to ensuring food security.

Cassava (*Manihot esculenta* Crantz) is an indispensable food and cash crop for resource-limited farmers in tropical and subtropical regions worldwide[3]. It represents an essential source of calories for more than one billion people, making it important for food security and economic development[4]. With multiple applications, cassava is used for human food or animal feed and as an industrial raw material, mainly owing to its low-cost, multi-purpose starch[5,6]. Although cassava tolerates low-fertility soil conditions and presents high productivity of starchy roots, this crop could be one of the optimum alternatives to provide food for the rapidly growing world population in the future[7,8]. However, drought and cold stressors lessen the yields of hardy crops like cassava. Currently, advancements in genomics, transcriptomics, and proteomics have been made in understanding the mechanisms of cassava evolution, root development, and abiotic and biotic stress tolerance[9–14]. This review focuses on recent advances in exploring cellular signaling networks of cassava plants against cold and drought stress and provides guidance for future research, which is expected to accelerate the production of drought/cold-tolerant varieties through genetic transformation or molecular breeding.

Physiological changes of cassava plants against drought and cold stress

Climate change leads to more frequent and/or extreme drought events in many agricultural regions globally. It has been reported that water deficiency caused drought stress is the major environmental stress limiting crop productivity, leading to over 70% of potential agriculture yield losses worldwide[15]. Overall, drought stress results in suppressed plant growth, reduced photosynthetic rates, accelerated leaf senescence, and intensified oxidative damage in plants[16] (Fig. 1a). The cassava growth cycle, especially the earlier stages (within 30–150 d after planting), is typically interrupted by drought, thus depressing growth, development, and economic yield[17,18]. The physiological responses of cassava plants to drought stress have been reported[19,20]. Cassava plants have evolved diverse mechanisms, such as drought avoidance in response to water stress. Once exposed to dry air and/or soils, cassava plants conserve water by closing stomata, restricting new leaf formation, and leaf drooping and defoliation, further decreasing the leaf canopy (as reflected in the production of fewer and smaller leaves) to reduce plant's overall water usage, and enhancing water uptake by increasing root length under prolonged water stress[19]. Upon recovering from drought stress, cassava can rapidly form new leaves[21]. At the same time, a range of small molecule compounds such as proline, soluble sugars, lignin, and reactive oxygen species (ROS) are

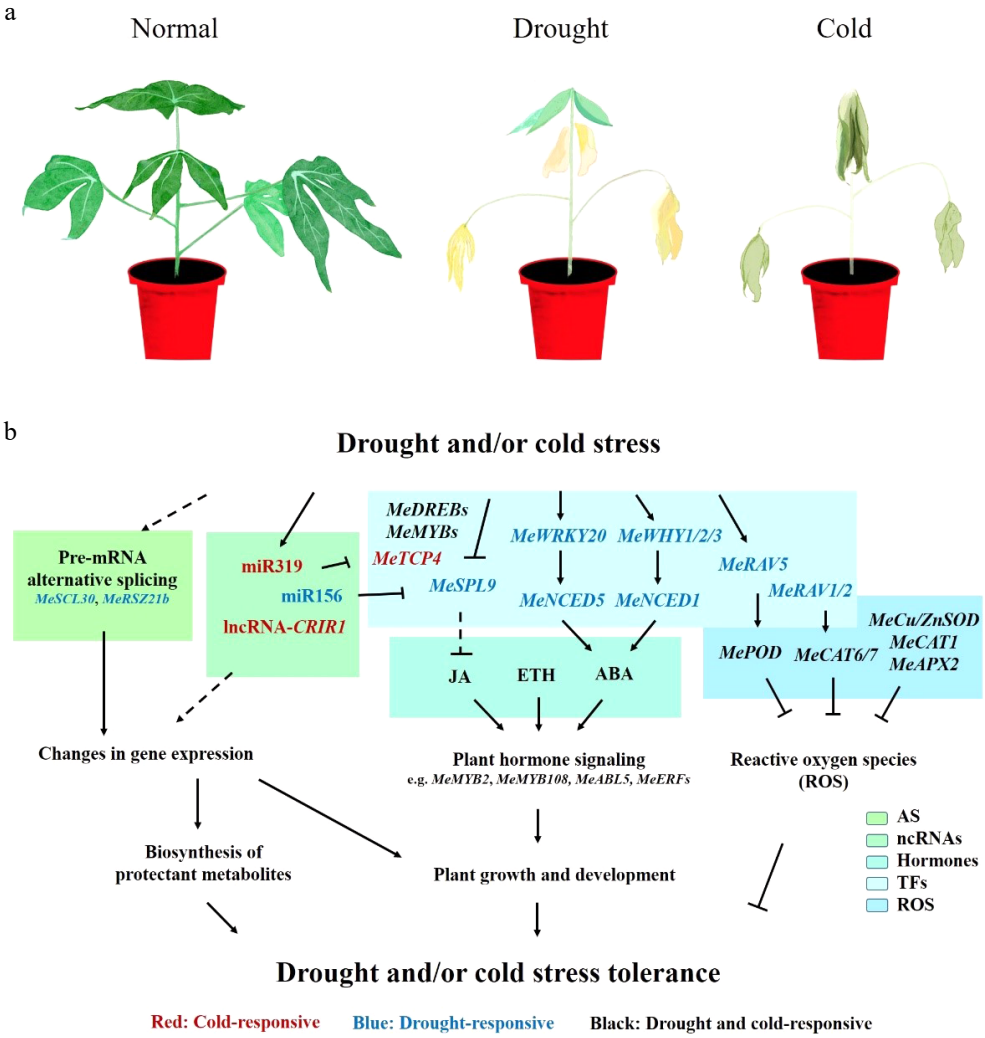

Fig. 1 A simplified diagrammatic representation of (a) physiological phenotypes and (b) cell signaling of cassava plants under drought and/or cold stress. AS, alternative splicing; ncRNAs, non-coding RNAs; TFs, transcription factors; ROS, reactive oxygen species; JA, jasmonates; ETH, ethylene; ABA, abscisic acid.

accumulated to maintain the cellular water content under drought stress[22]. Plant hormones, e.g., abscisic acid (ABA), jasmonates (JA), and ethylene (ETH), play an essential role in plant drought stress signaling as well as the control of leaf development and senescence, stomatal movements, and root growth[21]. Several studies showed that ABA and JA levels were strongly increased in cassava plants under drought conditions[23–25].

Cold stress, including freezing (< 0 °C) and chilling (0–15 °C) stress, causes tremendous changes in the physiology, biochemistry, and development of plants, especially the geographical distribution[16]. Freezing and chilling temperatures exert preliminary effects on cell membrane fluidity and enzyme activities, thereby impacting various cellular processes[16]. They also chronically influence the abundance of RNA and protein at the transcriptional or translational level[26]. Because cassava plants grow natively in tropical regions, they are highly sensitive to low temperatures and cannot survive long under freezing conditions[27]. Cassava seedlings exposed to cold stress (e.g., temperatures below 15 °C but above 0 °C) cease growth with dehydrated leaves. Under prolonged exposure to stress, the whole plant exhibits obvious phenotypic damages, including loss of strength in immature stems, softening and downward bending of petioles, and low photosynthetic rate[11] (Fig. 1a). In addition, exposure to cold will also increase the levels of proline, malondialdehyde (MDA), soluble sugars, and ROS in cassava plants[11]. Conversely, the content of chlorophyll significant for the absorption and conversion of light energy is reduced under cold stress[12].

Stress-responsive mechanisms of cassava plant under drought and cold stresses

Recent advances showed that cassava plants have developed various mechanisms to cope with drought and/or cold stresses, including changes at physiological and molecular levels, altering the expression level of stress-associated genes and leading to the formation of various protectant metabolites[11,28,29]. These genes and metabolites play significant roles in stress tolerance via detrimental cellular change prevention, water retention of plant cells, cellular membrane stabilization, and protein or RNA structure protection under drought and cold stresses. Among them, the primary features are described in the following sections.

Transcription factors (TFs) and their central regulatory roles in plant stress signaling

As the molecular switches for controlling downstream target gene expression by promoting/suppressing messenger RNA (mRNA) transcription, TFs regulate to a large extent plant growth and biotic/abiotic stress responses. To date, a great deal of TFs of different families, e.g., *myeloblastosis* (*MYB*), *basic helix-loop-helix* (*bHLH*), *growth-regulating factor* (*GRF*), *WRKY*, *dehydration responsive element binding* (*DREB*), *APETALA2/ethylene responsive factor* (*AP2/ERF*), *basic leucine zipper* (*bZIP*), *homeodomain leucine zipper* (*HD-ZIP*), *NAC*, and *ABRE-binding factor* (*ABF*), have been identified to be associated with the drought and/or cold stress response in cassava plants based on genome-wide analysis[28,30–37]. DREBs play a central role in improving drought and cold stress tolerance in various plant species by binding a DRE/CRT cis-element in the promoter regions of target genes[38]. Two DREB homologous genes, *i.e., MeDREB1A* and *MeDREB1D*, are functionally characterized in cassava[39,40]. *MeDREB1A* expression is extremely responsive to cold and significantly induced by polyethylene glycol (PEG) and ABA treatments. *MeDREB1A* overexpression in transgenic *Arabidopsis* and cassava plants enhance their cold tolerance[39]. Similarly, *MeDREB1D* overexpression also confers tolerance to cold and drought stresses in transgenic *Arabidopsis*[40]. Increasing evidence showed that TF-mediated stress adaptive signaling was intimately linked to primary cellular metabolism, ROS metabolism, and hormone signaling pathways. For example, a drought stress-responsive TF MeRAV5 promoted the activities of peroxidase (MePOD) and lignin-related cinnamyl alcohol dehydrogenase 15 (MeCAD15) to affect the accumulation of H_2O_2 and endogenous lignin, respectively, which were important in drought stress resistance of cassava[22]. Modified tolerance to cold stress of *MeTCP4*-overexpressed plants was attributed to *MeTCP4*-mediated cellular protection against toxic ROS[41]. RNAi-driven repression of the ABA-responsive MYB TF, namely *MeMYB2*, resulted in drought and low temperature tolerance in transgenic cassava and allowed the identification of target genes, including other MYB and WRKY TFs[42]. Based on this concept, the drought-responsive *MeWRKY20* and *MeWHY1/2/3* controlled the cellular accumulation of ABA via inducing the expression of ABA biosynthetic genes, *MeNCED5* and *MeNCED1*, respectively, thereby enhancing the drought tolerance of wild-type cassava plants[24,43]. As another example, the drought-responsive TF *SQUAMOSA promoter binding protein-like 9* (*MeSPL9*) was a repressor of anthocyanin and JA formation and showed negative functions in drought stress resistance. Additionally, bZIP TF *MeABL5* responsive to ABA and JA positively regulated *MeCWINV3* expression and might participate in robust resistance to abiotic stress in cassava[44]. These findings indicate the importance of TFs in drought and cold stresse tolerance in cassava.

Non-coding RNAs acting as novel agents in plant abiotic stress signaling

Non-coding RNAs (ncRNA), such as microRNAs (miRNAs, 20–24 nt) and long non-coding RNAs (lncRNAs, > 200 nt), have been increasingly essential bioactive molecules regulating plant growth, biotic and abiotic stress responses in various species[45]. Generally, they interact with DNA, RNA, and proteins to control gene expression at the transcriptional, post-transcriptional, and translational levels[46]. Unlike lncRNAs, miRNAs are highly conserved in the evolution of plant species from monocots to dicots[46]. Individual plant species harbor conserved miRNAs and species-specific miRNAs[46]. For example, 85 conserved miRNAs of 23 families have been identified in four *Euphorbiaceous* species, including cassava, jatropha, castor bean, and rubber tree[47]. Among them, miR156, miR397, and miR399 are up-regulated under dehydration stress, while miR164 and miR398 are induced by chilling treatments in cassava[48,49]. The targets of these miRNAs have been verified and functionally characterized, such as miR156-targeted *MeSPL9* and miR319-targeted *MeTCP4*[25,41,50–52]. Likewise, a series of novel miRNAs, e.g., miR2118, novel-52, and novel-54, have been identified in the deep-sequencing and EST database[49,53–55]. The expression of the miRNAs is promoted or suppressed under drought and chilling stresses[48,56].

Recently, lncRNAs have been proved to be key regulators of gene expression in various biological processes of plants, and a great number of lncRNAs have been identified in cassava[57–59]. For instance, based on the analysis of strand-specific RNA-seq (ssRNA-seq) data, Li et al.[60] presented the first reference catalog of 682 high-confidence lncRNAs from cassava shoots under drought, cold, and control conditions. Among them, 69 lncRNAs were confirmed as responsive to both cold and drought stresses[60]. Suksamran et al. indicated that stress-induced lncRNAs might participate in the post-transcriptional regulation of stress-responsive TFs such as nuclear factor Y, zinc-finger, and WRKY gene families[61]. Furthermore, 652 intergenic lncRNAs and 181 antisense lncRNAs have been identified in cassava leaves and roots, 124 of which were drought-responsive[59]. In addition, Ding et al. found 185 lncRNAs differentially expressed under PEG or melatonin (MT) treatment versus the control condition[62]. The trans-regulatory co-expression network revealed that MT-responsive lncRNAs were mainly involved in cell wall modification, cytochrome P450, and tetrapyrrole synthesis; in contrast, PEG-responsive lncRNAs mainly participated in hormone metabolism, calcium signaling, and the RNA regulation of transcription[59,62]. Notably, 86 auto-tetraploid-specific lncRNAs were identified to be differentially expressed in drought-stressed leaves. Trans-regulatory network analysis showed that these lncRNAs were associated with galactose metabolism, brassinosteroid biosynthesis, and pentose phosphate pathway[58]. Although plenty of abiotic stress-related lncRNAs have been investigated in cassava, their biological functions still remain to be determined. Recently, a novel *cold-responsive intergenic lncRNA 1* (*CRIR1*) was characterized as a positive regulator of plant responses to cold stress. It can regulate a number of cold stress-related genes in a *CBF*-independent pathway and directly interact with cold shock protein 5 (MeCSP5), which may improve the translation efficiency of mRNAs[57]. Similarly, Dong et al. identified a novel lncRNA, namely *drought-induced intergenic lncRNA* (*DIR*), which could enhance proline accumulation and drought tolerance in transgenic cassava[63]. Collectively, these recent research advances highlight the importance of ncRNAs for drought and cold adaptation in plants.

Plant hormones: key regulators of abiotic stress responses

Plant hormones function as central integrators in maintaining the balance between plant growth and stress tolerance[64]. In cassava, increasing evidence has proved a convergence and

crosstalk of ABA, JA, and ETH responses with the abiotic stress signaling pathways[29]. As reviewed by Sah et al.[65], ABA was the most important hormone conferring abiotic stress tolerance in crop plants. An increased level of endogenous ABA has been observed in drought-stressed cassava seedlings, and exogenous ABA application to cassava plants could increase their adaptive responses to water stress[24,43]. Many genes, including *KUP*, *MYB*, *NAC*,*GRF*, *WRKY*, *GRX* and *CIPK*, were induced or repressed by ABA treatment[33,35,66–70]. Among them, TF MeWRKY20 has been reported to directly activate the expression of ABA synthesis gene *NCED5* (9-*cis*-epoxycarotenoid dioxygenase), which was facilitated by 90 kDa heat shock protein (MeHSP90), and to regulate drought resistance by modulating ABA biosynthesis[24]. In cassava, six NCEDs were found to be increased under drought stress[10,71]. Recently, Yan et al. showed that MeWHY1/2/3 directly targeted the PB element of the *MeNCED1* promoter and promoted *MeNCED1* transcription to activateABA biosynthesis[43].

In cassava plants, the endogenous JA concentrations increased rapidly after drought stress, and external JA application has also been revealed to improve drought tolerance by closing stomata and preventing water loss. Drought and/or cold stress could induce the expression of a range of JA biosynthesis or responsive genes, such as *LOXs* (*lipoxygenases*), *JAZs*, and *MYCs*[25,29]. In addition, numerous TFs associated with drought and cold stresses, such as *MeARC5* (*accumulation and replication of chloroplasts 5*), *MeFtsZ2-1* (*filamentous temperature-sensitive protein Z 2-1*), and *MeMYB108*, were expressed following JA treatment[72–74]. Li et al.[25] demonstrated that MeSPL9 down-regulated JA biosynthetic genes and played a negative regulatory role in drought tolerance in cassava. Endogenous ETH was also induced by water stress and was involved in cassava leaf abscission by enhancing ROS accumulation in the cassava leaf pulvinus-petiole abscission zones, where it has been shown that GST and ERF genes are also highly expressed under both ETH and drought treatments[34,75]. Interestingly, a crosstalk of ABA and ETH signaling was found in plants under drought stress recently. For example, *MeGRXC15*-overexpressed *Arabidopsis* plants were more resistant to drought stress, and *MeGRXC15* might affect various TF expressions involved in ABA and ET signaling pathways[69]. As another example, Wang et al. found that *MeMYB108* was induced by ABA, JA, and ETH treatments, and *MeMYB108* overexpression significantly reduced the rate of drought-induced leaf abscission under drought[74]. Taken together, these findings strongly suggest the modulating role of hormone pathways in abiotic stress tolerance in cassava plants.

Protection against reactive oxygen species (ROS)

Oxidative damage is a major feature of crop plants exposed to abiotic stresses. ROS in the form of hydrogen peroxide, superoxide, and nitric oxide are produced under drought and cold conditions and lead to cellular damage via oxidation or membrane injury. In plants, ROS homeostasis is maintained by an antioxidative system composed of non-enzymes (ascorbate, α-tocopherol, carotenoid, and glutathione) and ROS-scavenging enzymes, including ascorbate peroxidase (APX), superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR)[76]. Up-regulation of ROS metabolism genes in cassava leaves or abscission zone cells under drought and/or cold stress has been reported[11,12,77]. MeCAT1- or peroxidase (MePOD)-silenced plants displayed drought sensitivity in cassava through virus-induced gene silencing, indicating their importance for drought stress response[22,24]. MeCAT1 activity could be positively regulated by the MeHSP90, which was essential for drought resistance in cassava[24]. Xu et al. reported that transgenic cassava with increased expression of MeCAT1 and MeCu/ZnSOD represented improved resistance to drought and cold stresses[78]. Similarly, the coupled expression of MeCu/ZnSOD and MeAPX2 could simultaneously activate the antioxidant defense mechanisms and thereby enhance cassava tolerance to oxidative and cold stresses, as observed by higher levels of SOD, catalase, and ascorbate-glutathione cycle enzymes, as well as lower levels of MDA content[79]. Recently, the regulatory functions of TFs, namely MeRAV1/2 and RAV5, in ROS detoxification by targeting *MeCAT6/7* and *MePOD* suggested a function of TFs in linking ROS scavenging to drought and oxidative stress-induced signaling[22,80]. Additionally, according to the transcriptional profiling studies of *Arabidopsis* plants overexpressing *MeTCP4* and *MeDREB1D*, *MeTCP4* and *MeDREB1D* induced a member of the ROS-scavenging genes, respectively, under drought and/or cold stress, leading to increased tolerance to abiotic stresses[40,41]. These studies strongly suggest the involvement of the ROS signaling pathway in drought adaptation in cassava plants.

RNA alternative splicing in plant response to drought and/or cold stress

Alternative splicing (AS), the differential processing of exons and introns in pre-mRNAs to generate multiple transcript isoforms for one gene, remarkably enhances the adaptability of plants under stresses via increasing the diversity of transcriptomes and proteins[81]. Generally, AS can perform two main molecular functions: (1) AS expand the complexity of proteome by producing two or more protein isoforms, which may present different functional properties. (2) AS regulates mRNA level by disrupting the main open reading frame (ORF) of the gene, creating truncated protein isoforms and/or triggering nonsense-mediated mRNA decay[82]. The five major AS events include intron retention (IR), mutually exclusive exons (MXE), exon skipping (ES), alternative 5′ splice sites (A5SS; alternative donor site), and alternative 3′ splice sites (A3SS; alternative acceptor site)[83], among which IR is the most common type in plants[84]. A number of transcriptomic and single-gene studies have investigated that AS participate in response of plants to environmental stimuli, especially drought and cold stresses[85–87]. Vast numbers of stress-related splicing factors and regulators of cassava underwent different types of AS events, and modulated gene expression under cold stress[88]. However, very few studies have elucidated the upstream regulatory mechanisms of AS during the response to stress in cassava. SR proteins play important roles in both AS and constitutive by regulating the recruitment of splicing machinery to splice sites[89]. Weng et al.[90] found that *MeSCL30* overexpression in *Arabidopsis* enhanced drought tolerance by maintaining ROS homeostasis and increasing drought-responsive gene expression. Similarly, the overexpression of *MeRSZ21b*, the RSZ subgroup of the SR family, improved drought tolerance through modulating ABA-dependent signaling in *Arabidopsis*[91]. Remarkably, increasing evidence in model plants has shown that ABA signaling is widely regulated at the AS level[81]. ABA regulates the splicing of *HAB1*, a key

gene of ABA signaling, by mediating SR45 expression, thereby adjusting salt and drought stresses[92]. However, the interaction of ABA signaling and AS-related proteins were less explored in cassava. In the future, novel splicing factors and their target mRNAs acting in the ABA pathway should be investigated to improve our understanding of how AS modulates abiotic stress responses in plants. Taken together, these findings validate the importance of AS and provided novel insights into the manipulation of AS-related genes to enhance the resistance of cassava plants to abiotic stresses.

Concluding remarks and future perspectives

In conclusion, recent research advances have preliminarily elucidated a complex molecular signaling network to explain how cassava plants regulate adaptation to drought and/or cold stresses. More importantly, molecular signaling components of plant adaptation to both stresses have been linked to TFs, ncRNAs, ROS, AS, and hormone-derived pathways (Fig. 1b). The cassava genome resequencing and various reverse genetics strategies for generating knockout mutants are expected to contribute to the identification of more signaling components, thereby getting a clearer picture of drought and/or cold stress signaling networks. Further studies on physiological and molecular mechanisms of abiotic stress tolerance are critical for characterisation of a number of genes associated with stress adaptation. Furthermore, plant biotechnology, marker-assisted selection, genomic selection and inbreeding techniques could be employed to improve abiotic stress tolerant of cassava and other crops.

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Conflict of interest

The authors declare that they have no conflict of interest.

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