

Review article

Mechanisms and approaches towards enhanced drought tolerance in cassava (*Manihot esculenta*)

Samwel K. Muiruri^{a,b}, Valentine O. Ntui^{a,1}, Leena Tripathi^{a,2}, Jaindra N. Tripathi^{a,*,3}

^a International Institute of Tropical Agriculture, Nairobi, Kenya

^b Kenyatta University, Department of Plant Sciences, Kenya

ARTICLE INFO

Keywords:

Cassava
Climate change
Drought-resilient crop
Genomics
Genetic engineering
Genome editing

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is cultivated in tropical and subtropical regions for its edible tuberous roots and minimally for its leaves. It provides food and revenue to over eight hundred million people particularly in Africa. Generally, cassava is drought-tolerant, and sheds leaves in drought conditions resulting in significantly lower yields. Cassava drought management strategies need to focus on maximizing the utilization of molecular tools for crop establishment and yield. Developing strategies to produce cassava cultivars with drought tolerance is vital to extending crop yield under limited rainfall. In this review, recent progress applying molecular genetics, genomics, genetic engineering, and genome editing are reviewed.

1. Introduction

Cassava (*Manihot esculenta* Crantz) is thought to have been domesticated around 8000 years in South America and brought by traders to West Africa in the 16th century [1]. Together with 98 other species including the rubber producing *Manihot glaziovii*, cassava belongs to Euphorbiaceae family, genus *Manihot* [2–5]. It is a highly heterozygous crop existing as polyploid or diploid with 36 chromosomes in the later [6] and is ranked third from rice and maize in human consumption. Additionally, it serves as feed for animals and is commercially used in the production of starch and biodegradable plastics. The crop is propagated through stem cuttings with a production range of 5000–20,000 cuttings per hectare depending on the cultivar's nature of growth and cropping system [7]. As a crop, cassava is among the most drought-tolerant and can also tolerate nutrient-depleted and acidic soils. Cassava productivity stands at 308 million tons over an area of 27.8 million hectares. Nigeria is one of the major producers with about 20% of total global production, other major growers are Angola, Brazil, China, Democratic Republic of the Congo, Ghana, Indonesia, Philippines and Mozambique, Viet Nam, and Thailand [8]. Cassava is extensively cultivated within the Tropics in regions 1500–2000 m above sea level. The range of temperature for cassava cultivation is 25–29 °C, with

rainfalls from 1000 to 15,000 mm annually [9].

Drought as plant abiotic stress limits growth resulting in low productivity [10]. Plants have adapted to water-deficit through multiple mechanisms including biochemical, morphological, physiological, and molecular. Four mechanisms have been postulated to govern plant's resistance to drought stress: drought tolerance (DT), avoidance (DA), escape (DE) and recovery (DR) [11]. Drought avoidance is a state in which plants modify their morphological features to retain a significantly greater tissue water content and regular physiological functions despite low soil water content [12]. Dehydration avoidance is achieved primarily through the closure of stomata, accumulation of wax, reduction in vegetative development, including leaf quantity and size, and improving uptake of water by the development of elaborate root system. Drought escape on the other hand is where plants modify their life cycles or period of growth before the beginning of drought, either naturally or artificially. Drought recovery is the plant's ability to recover its growth and vigor after being severely harmed by drought. Drought tolerance refers to ability of a plant to continue functioning physiologically in the face of severe drought through regulating stress-responsive genes and signaling pathways [11]. The increasing scarcity and competition for water resources call for the development of drought-tolerant/resistant cultivars as a sustainable way of extending agriculture to low rainfall

* Corresponding author.

E-mail address: j.tripathi@cgiar.org (J.N. Tripathi).

¹ <https://orcid.org/0000-0003-4709-8087>.

² <https://orcid.org/0000-0001-5723-4981>.

³ <https://orcid.org/0000-0002-6366-917X>.

<https://doi.org/10.1016/j.cpb.2021.100227>

Received 30 June 2021; Received in revised form 27 October 2021; Accepted 29 October 2021

Available online 2 November 2021

2214-6628/© 2021 The Authors.

Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

areas and those without an appropriate irrigation system. Studies on the physiology of cassava with known drought tolerance have indicated that cassava results in the rapid closure of stomata under both limited water in the atmosphere and in the soil, a result of which is the protection of the leaf against dehydration [29]. Cassava is certainly tolerant to drought, but, at the cost of tuber yield [13]. Underwater stress, cassava frequently sheds its leaves resulting in significantly reduced productivity [14]. The first 3–4 months after planting is a critical period in irrigated cassava system [15,16]. Moisture stress, during these first months of leaf formation, root initiation, and tuberization can result to the death of the plants or reduce the yield of storage root by up to 60% [17,18]. For example, in Uganda, up to 84.27% of mortality of cassava plants due to early period of drought stress was recorded [16,19]. A 30% yield reduction of cassava cultivated in Kerala was observed due to late monsoons and planting followed by a period of drought [15].

Other abiotic factors including marginal soils, traditional planting methods, and ambiguous farming practices also affect cassava productivity under a changing climate. In addition, post-harvest losses resulting from post-harvest physiological deterioration (PPD), defined as the discoloration resulting in root tubers turning blue/black [20], is another challenge. The PPD is a major problem in cassava production occurring 2–3 days after harvesting tubers even though this may vary with varieties, storage conditions, and weather [21]. Additionally, Cassava tubers in some genotypes have poor nutritional content and are laden with cyanogenic compounds [22]. These constraints impact yield and root quality significantly and are a serious economic burden to farmers with limited resources. In view of the importance of cassava as food, there is a need to develop strategies that will increase its productivity under drought conditions. This article overviews recent progress and potential applications of molecular genetics and genomics in enhancing resilience to drought. We also explore alternative strategies like genetic engineering and genome editing that can complement conventional breeding in cassava improvement for drought.

2. Breeding cassava for changing climate

Climate change involves a shift towards conditions worse than the prevailing ones. These changes affect crop growth and results in pest and disease introductions [23]. The cumulative effects of climate change on cassava cultivation vary with the geographical locations [24]. A study to predict climate change impacts on cassava production observed that it may be positively impacted by climate change with a – 3.7% to + 17.5% change across Africa [25]. Despite these positive projections, several regions of reduced productivity are expected within Africa and globally due to increased temperature as well as pest and disease dynamics. Breeding cassava that can overcome these challenges is a sure and long-term way of ensuring productivity.

3. Breeding for enhanced tolerance to drought

Increased drought and drought-like conditions are projected to affect cassava productivity the most heading into 2030 [25]. In a changing climate, breeding for tolerance is the most sustainable and effective approach to enhanced productivity [26,27]. Breeding for improved productivity under changing climate complements similar efforts for enhanced productivity under favorable conditions and does not equate to the neglect of the latter [28]. The generation of drought-tolerant varieties can result in enhanced productivity in cassava growing areas [27]. The second important trait in breeding for drought tolerance is an extensive fine root system which allows cassava to utilize water in deep soil layers (below two meters) [25,29]. The major limitation in cassava improvement against drought is the identification of germplasm with known and proven tolerance [27].

Cassava productivity in drought, poorly aerated and low nutrient soil conditions is greatly diminished, and well-aerated, watered, and fertilized soils are a prerequisite to improved productivity [30]. However,

major breeding efforts are already in place to develop cultivars that can maintain relatively high productivity under drought conditions [31]. Extensive efforts have therefore gone into characterizing existing germplasm with the aim of identifying the most suited cultivars to integrate into breeding. A wide range of genomic resources (germplasm) exists in national research organizations in Africa (Tanzania, Mozambique, and Nigeria), Asia (Thailand), and South America (Brazil) [32]. A huge germplasm collection also exists at the International Institute of Tropical Agriculture and the International Center for Tropical Agriculture with 2000 and 6000 accessions respectively. In South America, the North and South-Eastern regions of Brazil with limited rainfall (250 and 600 mm per year) for example are regions with a high diversity of drought-tolerant cultivars [31]. In these regions of Brazil, wide variations in productivity under-watered and drought experimental and field conditions have been observed [27,30,31]. The Brazilian cultivars were classified as tolerant or susceptible [27]. Similar studies have been conducted in Africa and Asia under field conditions and even using *in vitro* assays to identify cultivar variation to drought tolerance [26,33,34]. The major goal in these studies is classifying the performance of existing germplasm under drought conditions for breeding purposes. The variable response to drought in cassava genotypes is attributed to prolonged opening of stomata in the less tolerant varieties leading to more water loss [28]. The physiological and morphological screening for drought response is the first level in identifying appropriate breeding lines in germplasms for breeding purposes. Accessions with observed tolerance to drought are then integrated into breeding programs.

4. Cassava molecular drought response mechanisms

Identification of the molecular mechanisms for drought response of any crop lays a strong basis for downstream breeding efforts. Plants respond to drought by avoiding, escaping, drought tolerating and recovering or a combination of these methods. These four different mechanisms entail a complex interaction of proteins coded by multiple genes which are either upregulated or downregulated to enhance drought response. Cassava basically relies on avoidance by closing the stomata as well as developing extensive root network system [26]. However, with the availability of cassava genome sequences [83], further light is being shed into the exact mechanism of cassava drought response. To identify putative drought enhanced genes, over 18,000 Expressed Sequence Tags (ESTs) under conditions of drought and water were generated resulting in over 8000 unique gene clusters [35]. The unique gene clusters had representative genes with osmo-protective functions, heat-shock and other oxidative stress response and signal transduction proteins. In another study, 60-mer oligonucleotide Agilent microarrays representing about 20,000 genes were used in expression profiling of three cassava cultivars under drought conditions [36]. The oligo microarray study in three cassava genotypes observed a total of 168 genes as being upregulated. Among these 168 genes, some were associated with response to biotic and abiotic stress. In yet another study involving a susceptible and drought-tolerant variety, four drought-responsive candidate genes *MeALDH*, *MeZFP*, *MeMSD*, and *MeRD28* were exclusively upregulated during drought in the drought-tolerant variety [37]. The *MeMSD* and *MeALDH* genes are homologues of *Pisum sativum* Manganese Superoxide Dismutase and *Arabidopsis thaliana* ALDH7B4 respectively, both known to be reactive oxygen species (ROS) quenchers [37]. The genes *MeZFP* and *MeRD28* on the other hand are cassava homologues of *Oryza sativa* Japonica zinc finger protein ZFP252 and *A. thaliana* RD28 which play a role in osmotic adjustments [37].

Long non-coding RNAs (lncRNAs) are a class of regulators that play a role in drought response regulation [38,39] and are involved in cassava drought response [40,41]. Two lncRNAs were observed to enhance tolerance in autotetraploid cassava by increasing the stomata density [40]. This is a clear indication of possible additive roles in duplicated

copies of the lncRNAs in the autotetraploid cultivars. In a second study in cassava, about 318 lncRNAs identified as drought/cold-responsive were associated with biosynthesis of secondary metabolite, transduction of hormone signals, and sucrose metabolism [41]. In additional studies comparing transcription response to cold and drought, RNA sequencing was done at different time-point exposures [42]. This study observed exclusive response to drought by abiotic stress and ethylene metabolism related genes whereas those associated with photosynthesis, cell wall, and carbohydrate metabolism among other pathways were cold and drought responsive [42]. Overall, these studies indicate that cassava, like other crops employs a broad range of molecular mechanisms including ROS scavenging, osmotic regulation, and metabolic downregulation with an aim of preserving cell integrity.

5. Genetics and genomics research on drought tolerance in cassava

Drought response in cassava just like most of other plant traits are encoded by many genes and are therefore quantitative, multifactorial, and polygenic traits [43]. Efforts in cassava have therefore used molecular markers to narrow down to the genomic regions associated with some of these traits.

A limited number of studies have used isoenzymes as markers and for generating genetic maps. Three isoenzymes in combination with 132, 30 and 3 RFLPs, RAPDs, and 3 micro-satellites respectively, were used to generate a genetic map in a female heterozygous parent from an intra-specific cross [45]. In India, several isoenzyme markers were used to identify duplicates in cassava germplasm [46]. Similar studies using isoenzymes have been conducted in farmer's fields to identify genotypes [47]. Genome-wide analyses of eighteen glutaredoxins (GRXs) in the cassava genome were observed to be drought-induced in two cassava cultivars [48]. Additionally, a total of 91 Class III Peroxidase (POD) enzymes that are linked to drought response were identified in cassava [49].

Simple molecular (DNA) markers have extensively been used in different traits including drought [27,41,42,50–52]. The major goal of these studies is to easily link drought as a trait to the genomic regions involved. To identify cassava diversity and drought-associated alleles, one hundred and seven abiotic-related EST-SSRs were used in 134 cassava genotypes [53]. This study identified 53 markers that were closely associated with drought response and concluded that they could be used in drought tolerance marker-assisted selection. In another study aimed at evaluating the effects of drought on genetic parameters of 47 cassava genotypes, significant differences of genetic nature were observed [27].

The advancement in genomics has enhanced the use of genomic resources to identify and map variant alleles in cassava in a high-throughput manner. In a study evaluating multiple traits including drought tolerance stability index in 49 cassava genotypes, a total of 62 Single Nucleotide Polymorphisms (SNPs) in the 18 cassava chromosomes were identified through genome-wide association studies (GWAS) [54]. The 62 nucleotides obtained were mapped to different transcripts, some of which have known drought-associated functions. Transcriptome analysis has been used to physiologically investigate polyethylene glycol (PEG) drought-induced profiles [55]. In this PEG-induced drought study, novel pathways dependent and independent of Abscisic acid (ABA) were elucidated [55]. Together, these studies highlight a deeper understanding of mechanisms involved in cassava drought response and create valuable genetic resources for breeding. Additionally, multiple markers have been used in characterizing drought in cassava and these have been highlighted in Table 1.

6. Conventional cassava breeding and its challenges

Conventional cassava breeding is time-intensive with a minimum of six years required to reach cultivar trial and even longer to release. The breeding time is lengthened by variation in plant performance

Table 1

Summary of selected molecular and genomic studies on resistance to drought in cassava.

Number/type of marker	Major observations	Ref.
18166/EST	8577 unique gene clusters identified (5383 singletons and 3194 clusters)	[35]
60-mer oligonucleotide Agilent microarray	1300 drought stress up-regulated genes identified	[36]
Quantitative RT-PCR	4 drought tolerance candidate genes identified	[37]
Transcriptome analysis	2372 long noncoding RNAs (lncRNAs) and 86 autotetraploid-specific lncRNAs recognized	[40]
RNA-sequencing study	A catalogue of 682 high-confidence lncRNAs	[41]
RNA-sequencing study	Multiple differentially expressed genes identified	[42]
RNA-sequencing study	18 CC-type GRXs identified to be drought induced, Six CC-Type GRXs induced by exogenous ABA	[48]
RNA-sequencing study	91 cassava POD genes (MePODs) identified	[49]
104/expressed sequence tags—simple sequence repeat (EST-SSR)	53 markers that were significantly associated with drought-related traits recognized	[53]
Genome-wide association study (GWAS)	62 single nucleotide polymorphisms (SNPs) were identified	[54]
RNA-sequencing study	Identification of novel pathways in ABA-dependent and ABA-independent regulatory networks underlying PEG-induced dehydration response	[55]

depending on the physiology of the vegetative cutting and mostly by delayed and non-synchronized flowering of cassava breeding lines [56].

Induced flowering has been made possible following the identification of a *Flowering locus T (FT)* that produces a systemic flowering signal called florigen, which allows plants to transition to flowering [57]. Antagonistic to *FT* is the *Terminal Flower 1 locus (TFL1)* which inhibits flowering. Homologues of *A. thaliana FT (AtFT)* and *TFL1* have since been identified and shown to be critical flowering genes in other plants species including tomato, rice, potato, tobacco. Early flowering has been observed to be induced by either overexpressing the *FT* genes or by silencing *FT* antagonist *TFL1* demonstrated in crops like strawberry [57]. In cassava early flowering has equally been induced by heterologous expression of *AtFT gene* either by over-expressing it from 35S promoter as well from an Alcohol inducible promoter [58]. The early induced flowers were observed to not only be fertile but also resulted in viable seeds upon fertilization [59]. Early flowering in cassava has also been achieved through other approaches including grafting and hormonal treatment.

7. Biotechnologies for enhancing tolerance to drought

Despite the successes in developing drought-tolerant through conventional breeding in several other crops (Table 2), cassava, has lagged behind due to limited availability of germplasm, a narrow genetic material pool and limitation in sexually compatible germplasm, poor flowering, polyploidy, vegetative propagation, and heterozygosity, which hinder desirable agronomic trait transfer. Overcoming the limitations of conventional breeding in developing drought-resilient cassava calls for complementary approaches like genetic engineering and genome editing (Fig. 1).

8. Genetic engineering for drought tolerance: lessons from other crops

Genetic engineering approaches offer a pathway to developing resistance to drought in cassava. The Isopentenyl transferase (IPT) enzyme is key in cytokinin biosynthesis where it catalyzes the rate-

Table 2
Genetic engineering and genome editing (CRISPR/Cas9) studies on drought resistance in the selected plants.

Plant	Engineering system	Target gene	Observations	Ref.
Arabidopsis	CRISPR/Cas9	<i>OST2</i>	Stomatal response	[77]
Arabidopsis	CRISPR/dCas9	<i>AREB1</i>	ABA signaling-mediated drought tolerance	[84]
Arabidopsis	RNAi	<i>CBP80</i> (<i>ABH1</i>)	stomatal closing and reduced wilting during	[66, 69, 71]
Arabidopsis	RNAi	<i>CBP20</i>	Increased water-deficit tolerance during drought stress	[66]
Cassava	Overexpression	<i>IPT</i>	Increased water retention	[60]
Maize	CRISPR/Cas9	<i>ARGOS8</i>	Drought tolerance	[79]
Potato	RNAi	<i>CBP80</i>	Increased tolerance to drought	[71]
Rice	CRISPR/Cas9	<i>OsSAPK2</i>	ABA signaling-mediated drought tolerance	[67]
Rice	CRISPR/Cas9	<i>OsERA1</i> ,	Leaf rolling	[81]
Tobacco	Overexpression	<i>IPT</i>	Increased water retention	[61]
Tobacco	Overexpression	<i>IPT</i>	Delayed leaf senescence	[62]
Tomato	CRISPR/Cas9	<i>SINPR1</i>	Drought resistance	[80]
Tomato	CRISPR/Cas9	<i>SIMAPK3</i>	ABA dependent kinase signaling	[82]

limiting step in forming isopentenyl AMP, a precursor is synthesis of isoprenoid cytokinins [60]. Overexpression of *ipt* gene is associated with a delay in tobacco leaf senescence [61] and an increase in plant water retention [62]. Similarly, *ipt* gene driven by *Arabidopsis* senescence specific (SAG12) promoter transformed into cassava accession TMS 60444 resulted in increased water retention and a stay green phenotype under water stress [60]. This indicates that genetic engineering could be used to develop drought tolerant cassava.

The ABA stress hormone in a plant under drought results in closure of stomata which reduces further loss of water through transpiration. The closure of stomata works in a feed-back mechanism where availability of water, again leads to a drop in ABA levels and re-opening of the stomata. Many transcription factors (TFs) and genes encoding signaling factors (SFs) in the ABA signaling cascade play major roles in plant drought response [62]. The TFs activate downstream genes resulting in stress adaptation where both repressors as well as activators are engaged in drought tolerance [63]. The involvement of TFs like dehydration responsive binding protein (DREB), ABA-responsive element binding protein1 (AREB1), ABA-responsive binding factor 2 (ABF2), *bZIP* encoding and *MYB* encoding genes has been reported. The over-expression of these TFs in plants has demonstrated enhanced drought tolerance [62].

Signal transduction systems play critical roles in plant drought response with involvement of factors like ABA and MAPK kinases, farnesyltransferases, osmotic-stress-activated kinases such as the SNF1-related protein kinase 2 family (SnRK2) and Calcineurin B-like protein-interacting protein kinases CIPKs [64,65]. Some of the factors that act as regulators of ABA, have been identified with their suppression or activation resulting in enhanced drought tolerance [62].

The inactivation cap-binding protein 80 of CBP80 in ABA-hypersensitive *A. thaliana* resulted in stomata closure leading to reduced wilting under drought conditions [66,67]. Arabidopsis cap-binding protein 20 (CBP20) null mutants were hypersensitive to ABA during germination and were tolerant to drought stress [68]. In potato, silencing of CBP80 by RNAi resulted to increased tolerance to drought [69]. Similarly, the manipulation of TFs and SFs in cassava might help cassava breeders to develop drought stress tolerant cultivars.

9. Strategies for development of drought-resistant cassava through gene editing

In recent years, genome editing has become the tool of choice in plant modification. Techniques like clustered regularly interspaced

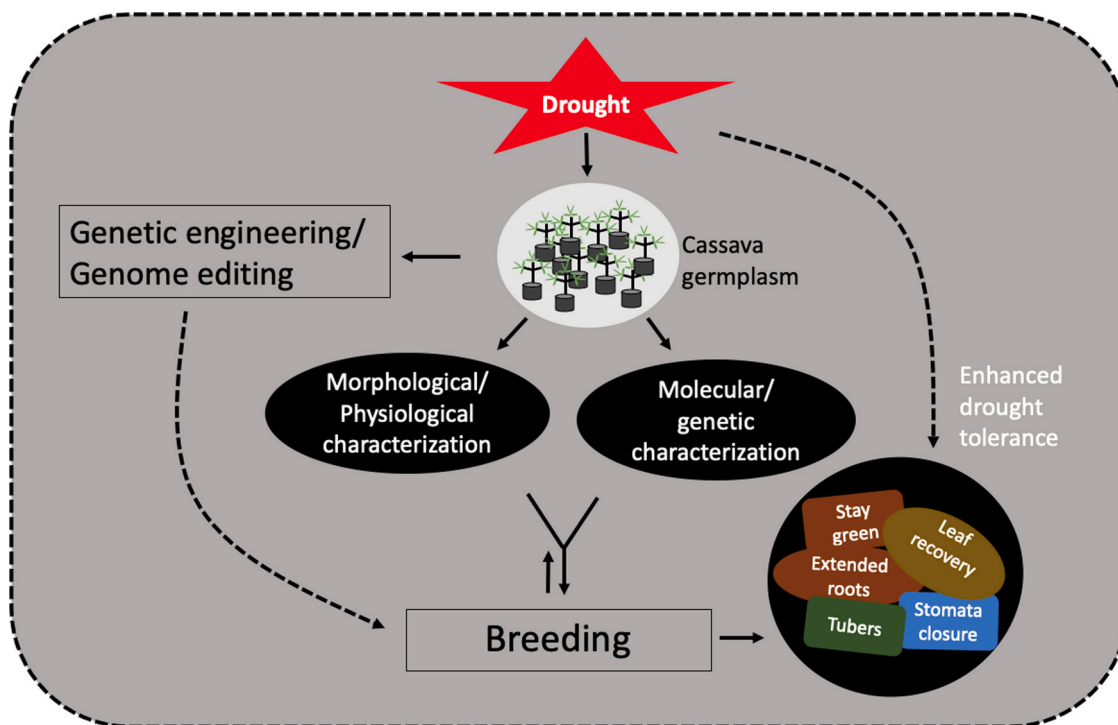


Fig. 1. Schematic representation of the main approaches used for drought improvement in cassava. The aspects involved in cassava improvement against drought include screening for drought tolerance of farmer preferred cultivars in cassava germplasm. Among others, aspects like stay green (leaf retention), tuberization during drought, drought recovery and enhanced stomata closure are bred. Cassava cultivars can be improved through genetic engineering and genome editing.

short palindromic repeats and associated protein (CRISPR/Cas), meganucleases, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) have been developed to achieve effective genome editing [70]. Among these nucleases, CRISPR/Cas9, is the most effective editing tool in plants due to its being simple, flexible, highly efficient and easy to multiplex [70–72].

The CRISPR/Cas9 technology comprises of the Cas9 nuclease and gRNA (guide RNA). The Cas9 protein recognizes DNA target(s) because of gRNA-DNA pairing in gRNA's 5' leading sequence. Additionally, Cas9 also recognizes the PAM (protospacer adjacent motif) resulting in edits up-stream of it. The common PAM nucleotides are NGG or NAG with N being any nucleotide base. Usually, Cas9 shows more affinity to NGG than NAG. The gRNA directs Cas9 to precisely cut DNA resulting in double stranded breaks (DSB). The breaks are consequently repaired by endogenous mechanisms of homology-directed repair (HDR) and non-homologous end joining (NHEJ) resulting in a user-desired mutation or genetic outcome. The error-prone NHEJ repair results in random insertions and deletions (SNPs, indels) that consequently lead to in-frame shift mutations and gene knockouts [73]. The HDR pathway offers more repair precision of the DSB resulting in gene knock-in, replacement or insertion of target DNA sequences.

Gene editing is classified into three site-directed nucleases (SDN) types based on the repair types involved [74]. In the SDN1 type, NHEJ is involved resulting in random genomic mutations a consequence of which target gene is knocked out. In SDN2, a repair template with homology to the DSB site is used as a reference for repair of DSB via HDR leading in substitution of nucleotides or targeted indels. When the HDR repair template is larger than the region to be repaired, then this is SDN3 and results in targeted insertion of foreign genes.

The ability of CRISPR/Cas9 to create sequence-specific double stranded breaks in DNA or RNA makes it an excellent tool to engineer drought resistance in crops. Recently, CRISPR/Cas variants with different editing strategies, such as Cas12a (Cpf1), Cas13 and CRISPR activation (CRISPRa or dCas) have been used in plant editing [75].

Use of genome editing in enhancing plant tolerance to drought has been demonstrated in several plant species [76] (Table 2). A modified CRISPR-Cas9 combining a Cas9 and a truncated sgRNA (tru-sgRNA) was used to disrupt the *Open Stomatal 2 (OST2)* gene in Arabidopsis. The OST2 is a known plasma membrane H⁺ ATPase that influences how stomata responds in Arabidopsis. Evaluation of *ost2_crispr* mutants under drought stress conditions showed enhanced stomatal closure and reduced water loss suggesting that disruption of the OST2 locus was found to facilitate drought tolerance by enhancing stomatal response [77]. Higher grain yield under drought stress is positively enhanced by ethylene signaling genes and negatively regulated by organ size (ARGOS) auxin regulated genes [78]. The maize ARGOS8 CRISPR/Cas9 induced mutant had increased grain yield under drought stress and no yield losses under well-irrigated conditions [79]. To establish the role of *non-expresser of pathogenesis related gene 1 (NPR1)* in tomato drought tolerance, CRISPR/Cas9 was used to induce mutation [80]. The *NPR1* is a plant defense regulator with limited information on its role in drought tolerance. Transgenic tomato *S1NPR1* mutants experienced loss of function and exhibited enhanced drought susceptibility and a wider stomatal aperture relative to the wild type (WT) tomatoes [81]. The Arabidopsis *Enhanced Response to ABA1 (ERA1)* codes for the β -subunit of farnesyltransferase and is known to regulate ABA signaling as well as dehydration response. The *ERA1* rice homologue mutant lines obtained by CRISPR/Cas9 editing, displayed enhanced sensitivity to ABA tolerance to drought [82]. In microorganisms, trehalose a non-reducing disaccharide of two D-glucose units functions as an osmo-protectant against stresses like limited water, oxidation, osmotic shock, freezing, salinity and radiation [83]. *Arabidopsis thaliana* with mutations in the trehalase substrate-binding domain exhibited enhanced tolerance to drought [82]. Overexpression of AREB1 through by activating its promoter using CRISPRa positively regulates drought stress response [84]. The many approaches used in other plants can be harnessed to further

enhance drought tolerance in cassava especially drought susceptible varieties.

10. Conclusions

Climate changes are anticipated to impact cassava productivity in major ways in growing regions within African and globally. Drought is projected to remain a challenge in cassava productivity as it will affect tuber yield. Additionally, enhanced drought will change pest and disease dynamics resulting in high drought-associated pests like cassava green mites (CGM). The challenging conventional breeding approach in cassava requires complementation with techniques like genetic engineering and genome editing. Integration of such biotechnologies requires clear understanding of mode of action and possible target genes. The advancement in genome editing technology allows the possibility of tweaking existing genes in susceptible varieties to make them drought tolerant or resistant. Some of the mapping studies have so far been able to dissect into the respective QTLs and identified specific genes. However, studies on the roles of individual genes identified have not been accomplished for almost all the target traits. The potential genes for genetic engineering as well as targets for genome editing in cassava have been explored here mainly based on evidence from other crops and homologs of similar targets that need to be characterized in cassava. Overall, a multifaceted approach could be the most successful strategy for addressing cassava drought challenges in a current changing climate.

CRedit authorship contribution statement

JNT is responsible for the original concept. SMK, VNT and JNT contributed to writing. SMK, JNT, VNT and LT reviewed and edited the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors wish to thank the CGIAR Roots, Tubers and Banana Program, The Royal Society and The African Academy of Sciences for funding under Grant no. FLR\R1\201370.

References

- [1] O. Iwuagwu, The spread of cassava (manioc) in Igboland, south-east Nigeria: a reappraisal of the evidence, *Agric. Hist. Rev. Publ. Br. Agric. Hist. Soc.* 60 (1) (2012) 60–76.
- [2] A.C. Allem, The origin of *Manihot esculenta* crantz, *Genet. Resour. Crop Evol.* 41 (1994) 133–150, <https://doi.org/10.1007/BF00051630>.
- [3] N.M.A. Nassar, D.Y.C. Hashimoto, S.D.C. Fernandes, Wild Manihot species: botanical aspects, geographic distribution and economic value, *Genet. Mol. Res.* 7 (1) (2008) 16–28, <https://doi.org/10.4238/vol7-1-gmr389>.
- [4] G. Léotard, A. Duputié, F. Kjellberg, E.J.P. Douzery, C. Debain, J.-J. de Granville, D. McKey, Phylogeography and the origin of cassava: new insights from the northern rim of the Amazonian basin, *Mol. Phylogenet. Evol.* 53 (2009) 329–334, <https://doi.org/10.1016/j.ympev.2009.05.003>.
- [5] K.M. Olsen, B.A. Schaal, Insights on the evolution of a vegetatively propagated crop species, *Mol. Ecol.* 16 (2007) 2838–2840, <https://doi.org/10.1111/j.1365-294X.2007.03359.x>.
- [6] M.A. El-Sharkawy, Drought tolerant cassava for Africa, Asia and Latin America: breeding projects work to stabilize productivity without increasing pressures on limited natural resources, *BioScience* 43 (7) (1993) 441–451, <https://doi.org/10.2307/1311903>.
- [7] B.A. Keating, G.L. Wilson, J.P. Evenson, Effects of length, thickness, orientation, and planting density of cassava (*Manihot esculenta* Crantz) planting material on subsequent establishment, growth and yield, *East Afr. Agric. For. J.* 53 (1988) 145–149.
- [8] FAOSTAT, Statistical Database of the Food and Agricultural Organization of the United Nations, 2019. (<http://www.fao.org>), (Assessed May 2021).

- [9] I.C. Onwueme, Cassava in Asia and the Pacific, in: R.J. Hillock, J.M. Thresh, A. C. Bellotti (Eds.), *Cassava: Biology, Production and Utilization*, CABI Publishing Oxon, UK and New York, USA, 2002, pp. 55–65.
- [10] R.K. Joshi, S.S. Bharat, R. Mishra, Engineering drought tolerance in plants through CRISPR/Cas genome editing, *3 Biotech* 10 (2020) 400, <https://doi.org/10.1007/s13205-020-02390-3>.
- [11] Y. Fang, L. Xiong, General mechanisms of drought response and their application in drought resistance improvement in plants, *Cell. Mol. Life Sci.* 72 (2015) 673–689, <https://doi.org/10.1007/s00018-014-1767-0>.
- [12] L.J. Luo, Breeding for water-saving and drought-resistance rice (WDR) in China, *J. Exp. Bot.* 61 (2010) 3509–3517, <https://doi.org/10.1093/jxb/erq185>.
- [13] S. Daryanto, L. Wang, P.A. Jacinthe, Drought effects on root and tuber production: a meta-analysis, *Agric. Water Manag.* 176 (2016) 122–131, <https://doi.org/10.1016/j.agwat.2016.05.019>.
- [14] M.A. El-Sharkawy, Global warming: causes and impacts on agroecosystems productivity and food security with emphasis on cassava comparative advantage in the tropics/subtropics, *Photosynthetica* 52 (2014) 161–178, <https://doi.org/10.1007/s11099-014-0028-7>.
- [15] J. George, C.R. Mohankumar, G.M. Nair, C.S. Ravindran, Cassava agronomy research and adoption of improved practices in India: major achievements during the past 30 years, in: *Proceedings of the 6th Regional Workshop on Cassava's Potential in Asia in the 21st Century: Present Situation and Future Research and Development Needs*, Ho Chi Minh City, Vietnam, 2001, pp. 279–99.
- [16] L.F. Turyagyenda, E.B. Kizito, Y. Baguma, D. Osiru, Evaluation of Ugandan cassava germplasm for drought tolerance, *IJACS* 5 (2013) 212–226, <https://hdl.handle.net/20.500.11951/87>.
- [17] D.J. Connor, J. Palta, Response of cassava to water shortage III. Stomatal control of plant water status, *Field Crops Res.* 4 (1981) 297–311, [https://doi.org/10.1016/0378-4290\(81\)90080-0](https://doi.org/10.1016/0378-4290(81)90080-0).
- [18] A.A.C. Alves, Cassava botany and physiology, in: R.J. Hillocks, J.M. Thresh, A. C. Bellotti (Eds.), *Cassava: Biology, Production and Utilization*, 2002, pp. 67–89, <https://doi.org/10.1079/9780851995243.0067>.
- [19] A.V.V. Koundinya, Breeding for drought tolerance in cassava" in training manual on "genetic improvement of tropical tuber crops through conventional and biotechnological approach, in: *Genetic Improvement of Tropical Tuber Crops through Conventional and Biotechnological Approach*, ICAR-Central Tuber Crops Research Institute, Thiruvananthapuram, 2018.
- [20] H. Buschmann, M.X. Rodriguez, J. Tohme, J.R. Beeching, Accumulation of hydroxycoumarins during post-harvest deterioration of tuberous roots of Cassava (*Manihot esculenta* Crantz), *Ann. Bot.* 86 (2000) 1153–1160, <https://doi.org/10.1006/anbo.2000.1285>.
- [21] K. Reilly, R. Gómez-Vásquez, H. Buschmann, J. Tohme, J.R. Beeching, Oxidative stress responses during cassava post-harvest physiological deterioration, *Plant Mol. Biol.* 56 (2004) 625–641, <https://doi.org/10.1007/s11103-005-2271-6>.
- [22] D.G. Barceloux, Cyanogenic foods (cassava, fruit kernels, and cycad seeds), *Dis. Mon.* 55 (2009) 336–352, <https://doi.org/10.1016/j.disamonth.2009.03.010>.
- [23] A. Scheben, Y. Yuan, D. Edwards, Advances in genomics for adapting crops to climate change, *Curr. Plant Biol.* 6 (2016) 2–10, <https://doi.org/10.1016/j.cpb.2016.09.001>.
- [24] Y. Elad, I. Pertot, Climate change impacts on plant pathogens and plant diseases, *J. Crop Improv.* 28 (2014) 99–139, <https://doi.org/10.1080/15427528.2014.865412>.
- [25] A. Jarvis, J. Ramirez-Villegas, B.V.H. Campo, C. Navarro-Racines, Is cassava the answer to African climate change adaptation? *Trop. Plant Biol.* 5 (2012) 9–29, <https://doi.org/10.1007/s12042-012-9096-7>.
- [26] C. Orek, W. Gruijssem, M. Ferguson, H. Vanderschuren, Morpho-physiological and molecular evaluation of drought tolerance in cassava (*Manihot esculenta* Crantz), *Field Crops Res.* 255 (2020), 107861, <https://doi.org/10.1016/j.fcr.2020.107861>.
- [27] E.J. de Oliveira, S. de T. Aidar, C.V. Morgante, A.R. de M. Chaves, J.L. Cruz, M.A. Coelho Filho, Genetic parameters for drought-tolerance in cassava, *Pesqui. Agropecu. Bras.* 50 (2015) 233–241, <https://doi.org/10.1590/S0100-204x2015000300007>.
- [28] M.A. El-Sharkawy, Drought-tolerant cassava for Africa, Asia, and Latin America, *Bioscience* 43 (1993) 441–451, <https://doi.org/10.2307/1311903>.
- [29] M.A. El-Sharkawy, Cassava biology and physiology, *Plant Mol. Biol.* 56 (2004) 481–501, <https://doi.org/10.1007/s11103-005-2270-7>.
- [30] L.M. de Carvalho, H.W.L. de Carvalho, I.R. de Oliveira, M.A.S. Rangel, V. da S. Santos, Produtividade e tolerância à deficiência hídrica de cultivares de mandioca nos tabuleiros costeiros do Nordeste, *Cienc. Rural* 46 (2016) 796–801, <https://doi.org/10.1590/0103-8478cr20151035>.
- [31] E.J. de Oliveira, C.V. Morgante, S. de Tarso Aidar, A.R. de Melo Chaves, R. P. Antonio, J.L. Cruz, M.A.C. Filho, Evaluation of cassava germplasm for drought tolerance under field conditions, *Euphytica* 213 (2017) 188, <https://doi.org/10.1007/s10681-017-1972-7>.
- [32] E. Okogbenin, T.L. Setter, M. Ferguson, R. Mutegi, H. Ceballos, B. Olasanmi, M. Fregene, Phenotypic approaches to drought in cassava: review, *Front. Physiol.* 4 (2013) 1–15, <https://doi.org/10.3389/fphys.2013.00093>.
- [33] O.L. Jolayemi, J.T. Opabode, Responses of cassava (*Manihot esculenta* Crantz) varieties to *in vitro* mannitol-induced drought stress, *J. Crop Improv.* 32 (2018) 566–578, <https://doi.org/10.1080/15427528.2018.1471431>.
- [34] Z. Shan, X. Luo, M. Wei, T. Huang, A. Khan, Y. Zhu, Physiological and proteomic analysis on long-term drought resistance of cassava (*Manihot esculenta* Crantz), *Sci. Rep.* 8 (2018) 1–12, <https://doi.org/10.1038/s41598-018-35711-x>.
- [35] Y. Lokko, J.V. Anderson, S. Rudd, A. Raji, D. Horvath, M.A. Mikel, R. Kim, L. Liu, A. Hernandez, A.G.O. Dixon, I.L. Ingelbrecht, Characterization of an 18,166 EST dataset for cassava (*Manihot esculenta* Crantz) enriched for drought-responsive genes, *Plant Cell Rep.* 26 (2007) 1605–1618, <https://doi.org/10.1007/s00299-007-0378-8>.
- [36] Y. Utsumi, M. Tanaka, T. Morosawa, A. Kurotani, T. Yoshida, K. Mochida, A. Matsui, Y. Umamura, M. Ishitani, K. Shinozaki, T. Sakurai, M. Seki, Transcriptome analysis using a high-density oligomicroarray under drought stress in various genotypes of cassava: an important tropical crop, *DNA Res.* 19 (2012) 335–345, <https://doi.org/10.1093/dnares/dss016>.
- [37] L.F. Turyagyenda, E.B. Kizito, M. Ferguson, Y. Baguma, M. Agaba, J.J.W. Harvey, D.S.O. Osiru, Physiological and molecular characterization of drought responses and identification of candidate tolerance genes in cassava, *AoB Plants* 5 (2013) 007, <https://doi.org/10.1093/aobpla/plt007>.
- [38] U.C. Jha, H. Nayyar, R. Jha, M. Khurshid, M. Zhou, N. Mantri, K.H.M. Siddique, Long non-coding RNAs: emerging players regulating plant abiotic stress response and adaptation, *BMC Plant Biol.* 20 (2020) 1–20, <https://doi.org/10.1186/s12870-020-02595-x>.
- [39] W. Zhang, Z. Han, Q. Guo, Y. Liu, Y. Zheng, F. Wu, W. Jin, Identification of maize long non-coding RNAs responsive to drought stress, *PLoS One* 9 (2014), <https://doi.org/10.1371/journal.pone.0098958>.
- [40] L. Xiao, X.H. Shang, S. Cao, X.Y. Xie, W.D. Zeng, L.Y. Lu, S.B. Chen, H.B. Yan, Comparative physiology and transcriptome analysis allows for identification of lncRNAs imparting tolerance to drought stress in autotetraploid cassava, *BMC Genomics* 20 (2019) 1–15, <https://doi.org/10.1186/s12864-019-5895-7>.
- [41] S. Li, X. Yu, N. Lei, Z. Cheng, P. Zhao, Y. He, W. Wang, M. Peng, Genome-wide identification and functional prediction of cold and/or drought-responsive lncRNAs in cassava, *Sci. Rep.* 7 (2017), <https://doi.org/10.1038/srep45981>.
- [42] C. Zeng, Z. Ding, F. Zhou, Y. Zhou, R. Yang, Z. Yang, W. Wang, M. Peng, The discrepant and similar responses of genome-wide transcriptional profiles between drought and cold stresses in Cassava, *Int. J. Mol. Sci.* 18 (2017) 2668, <https://doi.org/10.3390/ijms18122668>.
- [43] B.C.Y. Collard, M.Z.Z. Jahufer, J.B. Brouwer, E.C.K. Pang, An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts, *Euphytica* 142 (2005) 169–196, <https://doi.org/10.1007/s10681-005-1681-5>.
- [44] M. Fregene, F. Angel, R. Gomez, F. Rodriguez, P. Chavarriaga, W. Roca, J. Tohme, M. Bonierbale, A molecular genetic map of cassava (*Manihot esculenta* crantz), *Theor. Appl. Genet.* 95 (1997) 431–441, <https://doi.org/10.1007/s001220050580>.
- [45] G.O. Sumarani, S.V. Pillai, P. Harisankar, S. Sundaresan, Isozyme analysis of indigenous cassava germplasm for identification of duplicates, *Genet. Resour. Crop Evol.* 51 (2004) 205–209, <https://doi.org/10.1023/B:GRES.0000020862.61748.26>.
- [46] A.A. Efishue, Isozyme-based genetic fingerprinting of *Manihot* sp, *Niger. J. Biotechnol.* 26 (2013) 1–10.
- [47] M.B. Ruan, Y.L. Yang, K.M. Li, X. Guo, B. Wang, X.L. Yu, M. Peng, Identification and characterization of drought-responsive CC-type glutaredoxins from cassava cultivars reveals their involvement in ABA signalling, *BMC Plant Biol.* 18 (2018) 1–17, <https://doi.org/10.1186/s12870-018-1528-6>.
- [48] C. Wu, X. Ding, Z. Ding, W. Tie, Y. Yan, Y. Wang, H. Yang, W. Hu, The class III peroxidase (POD) gene family in cassava: identification, phylogeny, duplication, and expression, *Int. J. Mol. Sci.* 20 (2019) 1–17, <https://doi.org/10.3390/ijms20112730>.
- [49] S. Zhang, X. Chen, C. Lu, J. Ye, M. Zou, K. Lu, S. Feng, J. Pei, C. Liu, X. Zhou, P. Ma, Z. Li, C. Liu, Q. Liao, Z. Xia, W. Wang, Genome-wide association studies of 11 agronomic traits in cassava (*Manihot esculenta* crantz), *Front. Plant Sci.* 9 (2018) 1–15, <https://doi.org/10.3389/fpls.2018.00503>.
- [50] T.L. Setter, M.A. Fregene, Recent advances in molecular breeding of cassava for improved drought stress tolerance, in: *Adv. Mol. Breed. Toward Drought Salt Toler. Crop.*, Springer Netherlands, Dordrecht, 2007, pp. 701–711, https://doi.org/10.1007/978-1-4020-5578-2_28.
- [51] S. Li, X. Yu, Z. Cheng, X. Yu, M. Ruan, W. Li, M. Peng, Global gene expression analysis reveals crosstalk between response mechanisms to cold and drought stresses in cassava seedlings, *Front. Plant Sci.* 8 (2017) 1–18, <https://doi.org/10.3389/fpls.2017.01259>.
- [52] B. Wang, X. Guo, P. Zhao, M. Ruan, X. Yu, L. Zou, Y. Yang, X. Li, D. Deng, J. Xiao, Y. Xiao, C. Hu, X. Wang, X. Wang, W. Wang, M. Peng, Molecular diversity analysis, drought related marker-trait association mapping and discovery of excellent alleles for 100-day old plants by EST-SSRs in cassava germplasm (*Manihot esculenta* Crantz), *PLoS One* 12 (2017) 1–23, <https://doi.org/10.1371/journal.pone.0177456>.
- [53] P.P. dos Santos Silva, M.B.E. Sousa, E.J. de Oliveira, C.V. Morgante, C.R.S. de Oliveira, S.L. Vieira, J.C. Borel, Genome-wide association study of drought tolerance in cassava, *Euphytica* 217 (2021) 60, <https://doi.org/10.1007/s10681-021-02800-4>.
- [54] L. Fu, Z. Ding, B. Han, W. Hu, Y. Li, J. Zhang, Physiological investigation and transcriptome analysis of polyethylene glycol (Peg)-induced dehydration stress in Cassava, *Int. J. Mol. Sci.* 17 (2016) 283, <https://doi.org/10.3390/ijms17030283>.
- [55] O.S. Adeyemo, P.T. Hyde, T.L. Setter, T.L. Identification of FT family genes that respond to photoperiod, temperature and genotype in relation to flowering in cassava (*Manihot esculenta*, Crantz), *Plant Reprod.* 32 (2019) 181–191, <https://doi.org/10.1007/s00497-018-00354-5>.
- [56] F. Turck, F. Fornara, G. Coupland, Regulation and identity of Florigen: FLOWERING LOCUS T moves center stage, *Annu. Rev. Plant Biol.* 59 (2018) (2008) 573–594, <https://doi.org/10.1146/annurev-arplant.59.032607.092755>.
- [57] O.S. Adeyemo, P. Chavarriaga, J. Tohme, M. Fregene, S.J. Davis, T.L. Setter, Overexpression of Arabidopsis FLOWERING LOCUS T (FT) gene improves floral

- development in cassava (*Manihot esculenta*, Crantz), *PLoS One* 12 (2017) 1–15, <https://doi.org/10.1371/journal.pone.0181460>.
- [59] S. Bull, A. Alder, C. Barsan, M. Kohler, L. Hennig, W. Gruissem, H. Vanderschuren, FLOWERING LOCUS T triggers early and fertile flowering in glasshouse cassava (*Manihot esculenta* Crantz), *Plants* 6 (2017) 22, <https://doi.org/10.3390/plants6020022>.
- [60] P. Zhang, W.Q. Wang, G.L. Zhang, M. Kaminek, P. Dobrev, J. Xu, W. Gruissem, Senescence-inducible expression of isopentenyl transferase extends leaf life, increases drought stress resistance and alters cytokinin metabolism in cassava, *J. Integr. Plant Biol.* 52 (2010) 653–669, <https://doi.org/10.1111/j.1744-7909.2010.00956.x>.
- [61] S. Gan, R.M. Amasino, Inhibition of leaf senescence by autoregulated production of cytokinin, *Science* 270 (1995) 1986–1988, <https://doi.org/10.1126/science.270.5244.1986>.
- [62] R.M. Rivero, M. Kojima, A. Gepstein, H. Sakakibara, R. Mittler, S. Gepstein, E. Blumwald, Delayed leaf senescence induces extreme drought tolerance in a flowering plant, *Proc. Natl. Acad. Sci. USA* 104 (2007) 19631–19636, <https://doi.org/10.1073/pnas.0709453104>.
- [63] Y. Sakuma, K. Maruyama, F. Qin, Y. Osakabe, K. Shinozaki, K. Yamaguchi-Shinozaki, Dual function of an Arabidopsis transcription factor DREB2A in water-stress-responsive and heat-stress-responsive gene expression, *Proc. Natl. Acad. Sci. USA* 103 (2006) 18822–18827, <https://doi.org/10.1073/pnas.0605639103>.
- [64] R.F. Ahmed, M. Irfan, H.A. Shakir, M. Khan, L. Chen, Engineering drought tolerance in plants by modification of transcription and signalling factors, *Biotechnol. Bioinform. Equip.* 34 (2020) 781–789, <https://doi.org/10.1080/13102818.2020.1805359>.
- [65] J. Xu, S. Zhang, Mitogen-activated protein kinase cascades in signaling plant growth and development, *Trends Plant Sci.* 20 (2015) 56–64, <https://doi.org/10.1016/j.tplants.2014.10.001>.
- [66] A. Daszkowska-Golec, W. Wojnar, M. Rosikiewicz, I. Szarejko, M. Maluszynski, Z. Szweykowska-Kulinska, A. Jarmolowski, Arabidopsis suppressor mutant of ABH1 shows a new face of the already known players: ABH1 (CBP80) and ABI4-in response to ABA and abiotic stresses during seed germination, *Plant Mol. Biol.* 81 (1–2) (2013) 189–209, <https://doi.org/10.1007/s11103-012-9991-1>.
- [67] D. Lou, H. Wang, G. Liang, D. Yu, OsSAPK2 confers abscisic acid sensitivity and tolerance to drought stress in rice, *Front. Plant Sci.* 8 (2017) 993, <https://doi.org/10.3389/fpls.2017.00993>.
- [68] V. Hugouvieux, J.M. Kwak, J.I. Schroeder, An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis, *Cell* 106 (2001) 477–487, [https://doi.org/10.1016/S0092-8674\(01\)00460-3](https://doi.org/10.1016/S0092-8674(01)00460-3).
- [69] M. Kmiecik, C.G. Simpson, D. Lewandowska, J.W.S. Brown, A. Jarmolowski, Cloning and characterization of two subunits of *Arabidopsis thaliana* nuclear cap-binding complex, *Gene* 283 (2002) 171–183, [https://doi.org/10.1016/S0378-1119\(01\)00859-9](https://doi.org/10.1016/S0378-1119(01)00859-9).
- [70] I. Papp, L.A. Mur, A. Dalmadi, S. Dulai, C. Koncz, A mutation in the cap binding protein 20 gene confers drought tolerance to Arabidopsis, *Plant Mol. Biol.* 55 (2004) 679–686, <https://doi.org/10.1007/s11103-004-1680-2>.
- [71] M. Pieczynski, W. Marczewski, J. Hennig, J. Dolata, P. Piontek, A. Wyrzykowska, D. Krusiewicz, D. Strzelczyk-Zyta, D. Konopka-Postupolska, M. Krzeslowska, A. Jarmolowski, Z. Szweykowska-Kulinska, Down-regulation of CBP80 gene expression as a strategy to engineer a drought-tolerant potato, *Plant Biotechnol. J.* 11 (2013) 459–469, <https://doi.org/10.1111/pbi.12032>.
- [72] L. Tripathi, V.O. Ntui, J.N. Tripathi, Application of genetic modification and genome editing for developing climate smart banana, *Food Energy Secur.* e00168 (2019), <https://doi.org/10.1002/fes3.168>.
- [73] A. Scheben, F. Wolter, J. Batley, H. Puchta, D. Edwards, Towards CRISPR/Cas crops—bringing together genomics and genome editing, *New Phytol.* 216 (2017) 682–698, <https://doi.org/10.1111/nph.14702>.
- [74] V.O. Ntui, J.N. Tripathi, L. Tripathi Robust CRISPR/Cas9 mediated genome editing tool for banana and plantain (*Musa* spp.), *Curr. Plant Biol.* 21 (2020), 100128, <https://doi.org/10.1016/j.cpb.2019.100128>.
- [75] A. Razzaq, F. Saleem, M. Kanwal, G. Mustafa, S. Yousaf, H.M. Imran-Arshad, M. K. Hameed, M.S. Khan, F.A. Joyia, Modern trends in plant genome editing: an inclusive review of the CRISPR/Cas9 toolbox, *Int. J. Mol. Sci.* 20 (2019) 4045, <https://doi.org/10.3390/ijms20164045>.
- [76] D. Modrzejewski, F. Hartung, T. Sprink, D. Krause, C. Kohl, R. Wilhelm, What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map, *Environ. Evid.* 8 (2019) 11, <https://doi.org/10.1186/s13750-018-0130-6>.
- [77] Y. Osakabe, T. Watanabe, S.S. Sugano, R. Ueta, R. Ishihara, K. Shinozaki, K. Osakabe, Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants, *Sci. Rep.* 6 (2016), 26685, <https://doi.org/10.1038/srep26685>.
- [78] R.K. Joshi, S. Sutar Bharat, R. Mishra, Engineering drought tolerance in plants through CRISPR/Cas genome editing, *3 Biotech* 10 (2020) 400, <https://doi.org/10.1007/s13205-020-02390-3>.
- [79] J. Shi, H. Gao, H. Wang, H.R. Lafitte, R.L. Archibald, M. Yang, S.M. Hakimi, H. Mo, J.E. Habben, ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions, *Plant Biotechnol. J.* 17 (2017) 207–216, <https://doi.org/10.1111/pbi.12603>.
- [80] R. Li, C. Liu, R. Zhao, L. Wang, L. Chen, W. Yu, S. Zhang, J. Shen, L. Shen, CRISPR/Cas9-mediated *SINPR1* mutagenesis reduces tomato plant drought tolerance, *BMC Plant Biol.* 19 (2019) 38, <https://doi.org/10.1186/s1287-0-018-1627-4>.
- [81] T. Ogata, T. Ishizaki, M. Fujita, Y. Fujita, CRISPR/Cas9-targeted mutagenesis of *OsERA1* confers enhanced responses to abscisic acid and drought stress and increased primary root growth under non-stressed conditions in rice, *PLoS One* 15 (2020), e0243376, <https://doi.org/10.1371/journal.pone.0243376>.
- [82] L. Wang, L. Chen, R. Li, R. Zhao, M. Yang, J. Sheng, L. Shen, Reduced drought tolerance by CRISPR/Cas9-mediated SIMAPK3 mutagenesis in tomato plants, *J. Agric. Food Chem.* 65 (2017) 8674–8682, <https://doi.org/10.1021/acs.jafc.7b02745>.
- [83] L. Nuñez-Muñozhtps, B. Vargas-Hernández, J. Hinojosa-Moya, R. Ruiz-Medrano, B. Xocostle-Cázares, Plant drought tolerance provided through genome editing of the trehalase gene, *Plant Signal. Behav.* 16 (2021) 4, <https://doi.org/10.1080/15592324.2021.1877005>.
- [84] J.F. Roca Paixão, F.X. Gillet, T.P. Ribeiro, C. Bournaud, I.T. Lourenço-Tessutti, D. D. Noriega, B. Paes de Melo, J. de Almeida-Engler, M.F. Grossi-de-Sa, Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a histone acetyl transferase, *Sci. Rep.* 9 (2019) 8080, <https://doi.org/10.1038/s41598-019-44571-y>.