Review

Engineering Abiotic Stress Tolerance in Crop Plants through CRISPR Genome Editing

Mehboob-ur Rahman 1,*, Sana Zulfiqar 1, Muhammad Ahmad Raza 1, Niaz Ahmad 1 and Baohong Zhang 2, *

1 Plant Genomics and Molecular Breeding Laboratory, National Institute for Biotechnology and Genetic Engineering College, Pakistan Institute of Engineering and Applied Sciences (NIBGE-C, PIEAS), Faisalabad 38000, Pakistan
2 Department of Biology, East Carolina University, Greenville, NC 27858, USA
* Correspondence: mehboob_pbd@yahoo.com (M.-u.R.); zhangb@ecu.edu (B.Z.)

Abstract: Environmental abiotic stresses challenge food security by depressing crop yields often exceeding 50% of their annual production. Different methods, including conventional as well as genomic-assisted breeding, mutagenesis, and genetic engineering have been utilized to enhance stress resilience in several crop species. Plant breeding has been partly successful in developing crop varieties against abiotic stresses owning to the complex genetics of the traits as well as the narrow genetic base in the germplasm. Irrespective of the fact that genetic engineering can transfer gene(s) from any organism(s), transgenic crops have become controversial mainly due to the potential risk of transgene-outcrossing. Consequently, the cultivation of transgenic crops is banned in certain countries, particularly in European countries. In this scenario, the discovery of the CRISPR tool provides a platform for producing transgene-free genetically edited plants—similar to the mutagenized crops that are not extensively regulated such as genetically modified organisms (GMOs). Thus, the genome-edited plants without a transgene would likely go into the field without any restriction. Here, we focused on the deployment of CRISPR for the successful development of abiotic stress-tolerant crop plants for sustaining crop productivity under changing environments.

Keywords: CRISPR; drought; salinity; heat; heavy metals; field crops; sustainable agriculture

1. Introduction

The process of crop domestication, which started at the dawn of human civilization, has led to the development of high-yielding crop varieties. These crop varieties have played a significant role in the transformation of every aspect of human society. However, these crops cannot withstand the changing environmental conditions and therefore these crops are undergoing significant yield losses. This phenomenon has recently been witnessed in Pakistan where the early onset of high-temperature regimes followed by heavy rains have almost entirely damaged the crops, especially rice and cotton. The rainfall has not only affected the standing crops but its knockdown effects are also extending this cropping season as well. For instance, it is feared that the next crop—wheat, a staple food crop in the country—would not be sown in many flooded regions in this growing season. The other abiotic stresses including drought, salinity, temperature, and heavy metal toxicity [1] are also gradually converting the cultivated lands into barren soils. Thus, all abiotic stresses in the face of changing climatic conditions are threatening global food security and are a major obstacle in realizing the UN’s target of a 70–100% increase in crop productivity by 2050.

Abiotic stresses can result in significant yield losses. For instance, drought alone can cause a 50–70% reduction in crop yield for different crops [2]. For example, due to drought stress, 40% yield losses were reported in maize [3], 21% in wheat [3], 50% in rice [4], 27–40% in chickpea [5], 42% in soybean [6], and 68% in cowpea [7]. Salinity stress is the second most devastating menace that not only reduces crop productivity but also deteriorates...
fertile lands [8]. About one-fifth of the agricultural irrigated land is affected by excessive salts [9,10]. Poor quality irrigation water together with changing climatic conditions and the excessive use of chemicals including fertilizers and pesticides continue to add new acreage under salinity stress. It was estimated that the excessive use of chemicals including fertilizers and pesticides will cause 50% of the cultivated lands to be saline by 2050 [11]. The third most important factor is the increase in temperature that may depress crop production substantially. For example, every 1 °C increase in atmospheric temperature reduces wheat yield by 6% [12], rice yield by 10–20% [13], and 21–31% corn yield [14]. This means that developing stress-tolerant crops could increase the crop yield. It has been a widely accepted fact that developing resilient crop varieties that can withstand the impact of abiotic stresses including changing climatic conditions is the only option for harvesting sustainable crop productions.

Although conventional breeding as well as molecular techniques and genetic engineering contributed significantly to producing biotic stress-resilient crop varieties [15], however, limited success was achieved in addressing abiotic stresses owing to the complex genetics involved in resistance mechanisms. New genetic tools including genome editing are being used extensively for developing resilient crops but stringent regulations for cultivating such crops remained a major hurdle in the spread of genome-edited crops [16,17]. Improvements in the genome editing assay including clustered regularly interspaced short palindromic repeat (CRISPR) have caused it to be possible to edit the genome very precisely [18–20]. Genome editing can be used for studying novel traits and targeting the improvement of traits for mitigating the impact of abiotic stresses [21,22]. In this review, we will discuss the role of CRISPR-Cas9 in the development of stress-resilient crops for addressing nutrient use efficiency, drought, salinity, temperature, tolerance to environmental pollutants, and heavy metal toxicity.

2. Genome Editing Machinery

Genome editing tools use a special class of nucleases that can modify a specific nucleotide(s) in the genome(s) by introducing target-specific double-stranded breaks. Four different types of nucleases including meganucleases (MegNs), zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/associated proteins (Cas) [23] have been reported [1]. Meganucleases, ZFNs, and TALENs can induce DSBs at the target site by DNA-protein interaction. However, their utility in genome editing is relatively laborious and time-consuming [24]. On the other hand, the deployment of CRISPR for editing genomes is relatively easy, efficient, and economical [25]. CRISPR-Cas9 was derived from the bacterial immune defense that targets the invading viruses [26,27]. Being a single effector molecule system, CRISPR/Cas9 belongs to class 2-type II and is by far the most widely used system for editing genomes precisely [28,29].

The CRISPR/Cas9 comprises Cas9 and single guide RNA (sgRNA). The sgRNA is categorized into two components viz. CRISPR-RNA (crRNA) and trans-activating RNA (tracrRNA). The crRNA is a 20-nucleotide-long complementary sequence to target the sequence of interest, pre-crRNA, which joins with tracrRNA to produce a double-stranded RNA [30]. The RNase III activates the pre-crRNA for converting to mature crRNA [31]. The Cas9 nuclease has six domains: two recognition domains (REC I and REC II) essential for binding sgRNA and DNA, two nuclease domains (HNH and RuvC) for the cleavage of the complementary and non-complementary strands at the target region, the protospacer adjacent motif (PAM) interaction (PI) domain, and the bridge helix domain for the initiation of nuclease activity (Figure 1) [32,33].

The Cas enzymes had many orthologues that recognize different and specific PAM sequences (Table 1). For example, Streptococcus pyogenes Cas9 (spCas9) recognizes the NGG sequence where N could be any nucleotide [30,34–36]. Different Cas proteins require the PAM position at the different directions of the target sequence. Certain Cas proteins require
PAM at 5′ (such as Cas12), while the majority of the Cas require PAM at the 3′ site of the target DNA.

Figure 1. The structure of Cas9 protein—the major component of the CRISPR/Cas9 system.

Table 1. Different CRISPR/Cas systems, classes, and types.

| Name                  | Cas | Organism                  | Type  | PAM * | PAM Location | References |
|-----------------------|-----|---------------------------|-------|-------|--------------|------------|
| SpCas9                | Cas9| Streptococcus pyogenes    | Type II| NGG   | 3′           | [30]       |
| SaCas9                | Cas9| Streptococcus aureus      | Type II| NNGRT | 3′           | [31,32]    |
| FnCas9                | Cas9| Francisella novicida      | Type II| NG    | 3′           | [40]       |
| NmCas9                | Cas9| Neisseria meningitidis    | Type II| NNNNGATT | 3′         | [41]       |
| CcCas9                | Cas9| Campylobacter jejuni      | Type II| NNNRYYAC and NNNNACAC | 3′ | [42]       |
| StlCas9               | Cas9| Streptococcus thermophilus| Type II| NNAGAW | 3′           | [33,34]    |
| StTCas9               | Cas9| Streptococcus thermophilus| Type II| NGNG   | 3′           | [29]       |
| Mb2Cas12a-RVR Cas12   | Cas9| Acidaminococcus sp.       | Type V | TTV   | 5′           | [44,45]    |
| LbCas12a              | Cas9| Lachnospiraceae bacterium | Type V | TTYV  | 5′           | [45,46]    |
| FnCas12a              | Cas9| Francisella novicida      | Type V | TTTN or YTN | 5′      | [45]       |
| LsCas13 **            | Cas9| Leptotrichia shahii       | Type VI| YG    | 3′           | [47]       |
| AsCas12a              | Cas9| Archaea                   | Type V | Thymine rich PAM sequences | 3′ | [48]       |
| SpCas9-VQR            | Cas9| Modified FnCas9          | Type II| YG    | 3′           | [49]       |
| SpCas9-EQR            | Cas9| Engineered SpCas9         | Type II| NGA   | 3′           | [50,51]    |
| SpCas9-NG             | Cas9| Engineered SpCas9         | Type II| NG    | 3′           | [52]       |
| SpCas9-VRER           | Cas9| Engineered SpCas9         | Type II| NGCG  | 3′           | [52]       |
| GeoCas9               | Cas9| Geobacillus               | Type II| NNNNCRAA | 3′    | [48]       |
| SaCas9-KKH            | Cas9| Engineered SaCas9         | Type II| NNNRRT | 3′           | [56]       |
| SpCas9-HF             | Cas9| Engineered SaCas9         | Type II| NGG   | 3′           | [57,58]    |
| eSpCas9               | Cas9| Engineered SpCas9         | Type II| NGG   | 3′           | [57,58]    |
| xSpCas9               | Cas9| Engineered SpCas9         | Type II| NGG   | 3′           | [57,58]    |
| Sniper-Cas9           | Cas9| Engineered SpCas9         | Type II| NG    | 3′           | [59]       |
| tCas9                 | Cas9| Engineered SpCas9         | Type II| NGG   | 3′           | [56]       |
| HypCas9               | Cas9| Engineered SpCas9         | Type II| NGG   | 3′           | [60]       |
| Cas9-NRNH             | Cas9| Engineered SpCas9         | Type II| NGNG  | 3′           | [61]       |
| pSpCas9               | Cas9| Engineered SpCas9         | Type II| NRG   | 3′           | [62]       |
| SpRY                  | Cas9| Engineered SpCas9         | Type II| NRYN  | 3′           | [63]       |
| ScCas9                | Cas9| Streptococcus canis       | Type II| NG    | 3′           | [65]       |
| LbCas12a-RR           | Cas12| Engineered LbCas12a       | Type V | TYYC, CCC | 5′    | [66,67]    |
Table 1. Cont.

| Name | Cas | Organism | Type | PAM * | PAM Location | References |
|------|-----|----------|------|-------|--------------|------------|
| LbCas12a-RVR | Cas12 | Engineered LbCas12a | Type V | TATV | 5' | [66,67] |
| FnCas12a-RVR | Cas12 | Engineered FnCas12a | Type V | TATG | 5' | [66] |
| enLbCas12a | Cas12 | Engineered LbCas12a | Type V | TTTV | 5' | [46] |
| ttlBbCas12a | Cas12 | Engineered LbCas12a | Type V | TTTV | 5' | [46,68] |
| AacCas12b | Cas12 | Engineered LbCas12a | Type V | TTTV | 5' | [65,69] |
| AaCas12b | Cas12 | Acidaminococcus sp. | Type V | VTTV | 5' | [69] |
| BhCas12b-v4 | Cas12 | Bacillus thermoamylovorans | Type V | ATTN | 5' | [71] |
| BhCas12b | Cas12 | Bacillus hisashii | Type V | ATTN | 5' | [70] |
| BvCas12b | Cas12 | Engineered Cas12a | Type V | TTTV | 5' | [72] |
| BsCas12a | Cas12 | Engineered Cas12a | Type V | TTTV | 5' | [72] |
| Mb2Cas12a | Cas12 | Engineered Cas12a | Type V | TTV | 5' | [72] |
| BoCas12a | Cas12 | Engineered Cas12a | Type V | TTV | 5' | [72] |
| MbCas12a | Cas12 | Engineered Cas12a | Type V | TTTV, TTV, TYCV, CCCV, CTCV | 5' | [72] |
| Mb2Cas12a-RVR | Cas12 | Engineered Cas12a | Type V | TTV | 5' | [72] |

* “N” represents any nucleotide. “R” represents A or G. “H” represents A, C, or T. “Y” represents C or T. “W” represents A or T in PAM sequence. ** Cas13 targets RNA sequences. *** Cas14 targets the single stranded DNA sequence that is why it does not require PAM.

The Cas9 endonuclease is activated upon binding with mature gRNA that induces conformational changes to it. Upon binding with target DNA, Cas induces double-stranded break 3 nucleotide upstream to the PAM sequence (5' NGG 3'). These breaks will be repaired either through non-homologous end joining (NHEJ) or the homologous directed repair (HDR) pathway [73], depending on the presence of the DNA template with homology to the flanking position of the DSB. During the cut and repair mechanism, some nucleotides are removed or added to the original sequence, which may alter the protein structure or completely abolish its function [74]. Genome editing can knock out or overexpress an individual gene based on different repair mechanisms (Figure 2) [75–78]. Several studies have shown the utility of genome editing assays especially CRISPR/Cas9 for improving tolerance to various stresses including drought, salinity, heat, heavy metals, etc. (Table 2).

Figure 2. The schematic illustration of the CRISPR-based genome editing for improvement in resilience against various abiotic stresses.
Table 2. Crop improvement with tolerance to abiotic stress by using CRISPR genome editing.

| Crop Species | Targeted Gene | Function | Phenotype | References |
|--------------|---------------|----------|-----------|------------|
| Rice         | DERF1, PMS3, MSH1, MYB5, SPP | Amino acid synthesis and drought tolerance | DT | [79] |
| Rice         | SRL1, SRL2    | Regulate leaf rolling | DT | [80] |
| Rice         | ERA1          | Regulates ABA signaling and dehydration response | DT | [81] |
| Tomato       | GID1a         | Gibberellin (GAs) receptor | DT | [82] |
| Tomato       | LDL10        | Involved in jasmonic acid (JAs) -mediated stress response | DT | [83] |
| Maize        | ARGOS8       | Involved in ethylene response | DT | [84] |
| Maize        | abh2         | Abscisic acid 8′-hydroxylase mediates stomatal opening | DT | [85] |
| Rapeseed     | A6.RGA       | DELLA protein, negative regulator of gibberellin signaling | DT | [86] |
| Maize        | STL1         | Dirigent protein localized to the Casparian strip | ST | [87] |
| Tomato       | ABG1         | Homeodomain-leucine Zipper (HD-ZIP) TF | ST | [88] |
| Tomato       | HpyPPI       | Negative regulator of salt stress | ST | [89] |
| Soybean      | AITR         | Regulation of ABA signaling | ST | [90] |
| Rice         | SPL10        | Regulate trichome development | ST | [91] |
| Rice         | RAV2         | Function in the regulation of developmental processes | ST | [92] |
| Rice         | RR9, RR10    | Negatively regulate cytokinin signaling | ST | [93] |
| Rice         | DMT           | Involved in stomata development | ST | [94] |
| Rice         | SOS1         | Na⁺/H⁺ antiporter mediating Na⁺ transport | ST | [95] |
| Rice         | GI            | Circadian clock component | ST | [96] |
| Rice         | mHILH024     | Basic helix-loop-helix TF involved in growth and stress responses | ST | [97] |
| Rice         | KR22         | Involved in cytokinin signaling | ST | [98] |
| Rice         | PQT3         | E3 ubiquitin ligase | ST | [99] |
| Rice         | miR535       | Involved in Salinity stress regulation | ST | [100] |
| Rice         | HSJ1         | Chloroplast development and protection | HT | [101] |
| Tomato       | MAPK3        | Negative regulator of heat stress | HT | [102] |
| Rice         | PYL1         | Regulatory component of Abscisic acid | HT | [103] |
| Rice         | PYL4, PYL6   | Involved in fruit development | CT | [104] |
| Rice         | MYB30        | Negative regulator of cold stress | CT | [105] |
| Rice         | Nramp5       | Role in Cadmium translocation | HMT | [106] |
| Rice         | HAK1         | Transportation of Cesium | HMT | [107] |
| Rice         | ARM1         | Regulation of Arsenic response | HMT | [108] |
| Rice         | ALS          | Involved in herbicide tolerance | HerT | [109-110] |
| Watermelon   | ALS          | Involved in herbicide tolerance | HerT | [111] |
| Maize        | ALS          | Involved in herbicide tolerance | HerT | [112] |

"DT" Drought tolerance, "ST" Salinity tolerance, "HT" Heat tolerance, "CT" Cold tolerance, "HMT" Heavy metal tolerance, "HerT" Herbicide Tolerance.

3. CRISPR for Improving Drought Tolerance in Crop Plants

Drought, aggravated by climate change effects such as uneven rainfall patterns and increasing temperature, is becoming a threat to sustainable agriculture in many parts of the world. Tolerance to drought stress is a complex quantitative trait that is attributed to multiple physiological and biochemical processes [113]. Many efforts were performed to tailor these genes as well as to add new genes by adopting transgenic approaches. The transgenic crops were not commercialized due to the marginal impact of the transgene(s) in conferring drought tolerance and strict regulatory policies for the release of GM crops in the environment. After the discovery of genome editing tools, experiments are being designed to edit the genes involved in drought tolerance pathways for increasing the public acceptance of genome-edited crops [114]. Many studies have reported the conferring of drought tolerance in plants through CRISPR. For instance, downregulating the expression of DERF1, PMS3, MSH1, MYB5, and SPP regulatory genes using CRISPR/Cas9 have shown to result in drought tolerance enhanced in rice [79]. Mutation induced in Arabidopsis OST2 structural gene through deploying CRISPR/Cas9 demonstrated drought tolerance [115].

In another study, CRISPR/Cas9-mediated knockout of the miR169a gene in Arabidopsis resulted in a significant improvement of drought tolerance [116]. Similarly, activation of the vacuolar H⁺-pyrophosphatase (AVP1) regulatory gene with CRISPR/Cas9 resulted in drought tolerance improving in Arabidopsis [117]. Likewise, activation of the abscisic acid-responsive element binding gene (AREB1) by CRISPR/Cas9 exhibited enhanced drought tolerance in Arabidopsis [118]. Recently, the silencing of the trehalase (TRE1) gene through CRISPR/Cas9 demonstrated drought tolerance in Arabidopsis thaliana [119]. Also, the editing in the STL1 structural gene conferred improved drought tolerance in A. thaliana. In maize, editing of the ARGOS8 gene—the negative regulator of ethylene response—through CRISPR/Cas9 enhanced drought tolerance [84]. Also, suppressing the
expression of the abscisic acid hydroxylase 2 (\textit{abh2}) gene improved drought tolerance in maize [85]. Among oil seed crops, CRISPR/Cas9 was deployed to edit the \textit{A6.RGA} gene, which showed significant enhancement in drought tolerance in rapeseed [86]. In rice, the CRISPR/Cas9-mediated knockout of \textit{SRL1}, \textit{SRL2}, and \textit{ERA1} genes improved drought tolerance [80,81]. Multiple genes were also edited in tomato plants through CRISPR/Cas9 assay for improving drought tolerance. For instance, the gibberellin insensitive dwarf1 (\textit{GID1}) gene [82] and the \textit{LBD40} gene were edited [83]. In wheat, the \textit{SAL1} gene negative regulator of drought tolerance was edited through multiplex CRISPR/Cas9 assay that improves drought tolerance at the seedling stage [120]. Cotton’s ability to withstand drought can be improved by CRISPR/Cas genome editing of the \textit{HB12} gene [121].

4. CRISPR for Improving Salt Tolerance in Crop Plants

To meet the increasing world food demand, the UN has estimated that 70–100% of crop production should be increased by the end of 2050. However, at the same time, increasing crop cultivation has led to reduced soil fertility and salinization, which are quite unsuitable for crop growth and cultivation. Soil salinization occurs due to the accumulation of excessive soluble salts in the crop root zone, which hinders water absorption by roots. Consequently, plants exhibit osmotic stress along with nutritional imbalance that pose detrimental effects on plant morphology, plant biochemistry, and biomass and ultimately result in irreversible damage to plants [122–124]. Salt stress/salinity also increases the level of reactive oxygen species (ROS); resultantly, the cellular as well as metabolic activities of plants are badly affected [125,126]. The toxic impact of ROS is lipid peroxidation and membrane deterioration, as well as DNA and protein damage [127]. Salt stress hinders the photosynthetic machinery and transpiration by reducing chlorophyll content and stomatal conductance and impairing the chloroplast and photosystem II development [128]. In addition, it lowers the soil and leaf water potential; reduces plant turgor pressure by affecting water relations and ends up with osmotic stress [129]. Consequently, plants suffer a reduced leaf area, reduced photosynthesis, less production of biomass, poor seed germination, and reduced crop yield [130–132].

Salinity tolerance is conferred by a series of molecular as well as physiological mechanisms in plants [133]. Genome editing and genetic engineering tools have been deployed to target genes involved in ion transport for regulating osmotic adjustment under salt stress [134]. The overexpression of \textit{SOS1} (salt overly sensitive 1) increased the salinity tolerance in Arabidopsis [135]. Similar to \textit{SOS1}, overexpression of \textit{HvHKT2;1} (subfamily II HKT transporter from Hordeum vulgare) led to increased translocation of Na+, which resulted in enhanced salinity tolerance in barley [136]. In another study, editing in the \textit{OsRR22} gene encoding response regulator (type-B) expressed high tolerance to salinity in rice [98]. In addition, the \textit{PARAQUAT TOLERANCE 3} mutants (\textit{OsPQT3}) developed through CRISPR/Cas9 conferred a high degree of salinity tolerance in rice [99]. The role of \textit{OsmiR535} in salt stress tolerance was explored by deploying genome editing tools and it was suggested that the knockout of \textit{OsmiR535} through CRISPR/Cas9 could improve salinity tolerance in rice. Moreover, a homozygous five bp deletion in the coding sequence of \textit{OsmiR535} could serve as a potential target for improving salinity tolerance in rice [100]. Another study demonstrated the potential application of CRISPR/Cas9 by manipulating the hybrid proline-rich protein 1 (\textit{HyPRP1}) gene—a negative regulator of salt stress in tomato. The knockout of \textit{SlHyPRP1} negative-response domain(s) enhanced salinity tolerance at seedling as well as vegetative stages in tomato plants [89]. A gene cluster containing (\textit{ACQOS}; AT5G46520) and (NLRs; AT5G46510) is involved in osmotic stress tolerance. The role of \textit{ACQOS} was investigated by inducing small insertion/deletion mutations through CRISPR-Cas9, which suggested that \textit{ACQOS} was linked with salt stress resistance directly in Arabidopsis [137]. Although, limited reports are available on the potential implications of the CRISPR/Cas9 system toward the enhancement of salinity tolerance, there is no doubt that the CRISPR/Cas system is a promising tool in improving salinity tolerance in different crops.
5. CRISPR/Cas9 for Mitigating the Impact of Heat Stress

The optimum temperature for plant growth and development is 15–24 °C [138–140]. Heat stress is explained as the 10–15 °C increase in temperature above the ambient temperature, which is required for normal growth and development. High heat stress emerged as a serious issue responsible for huge yield losses and is expected to exacerbate in the future [141]. Heat stress poses extremely negative effects on plants during all growth stages, from germination to harvesting [142,143]. Heat stress not only aggravates the mortality rate of plants but also deteriorates their quality [144,145]. Plants restrict their growth, metabolism, and cellular activities above the normal temperature. Heat stress also affects the plant’s phenology including its photosynthetic machinery, respiration, and sink/source machinery and impairs photosystem II resulting in reduced production [146–148].

Prolonged exposures to extreme temperatures may lead to irreversible changes in plants such as cellular destruction. Plants respond to heat stress by wilting, fruit senescence, bolting, and leaf damage [149]. It causes several molecular, biochemical, and physiological changes that can adversely affect plant growth and productivity and may result in visual symptoms including leaf burn and discoloration [150]. Mostly, reproductive growth was highly affected under heat stress as temperatures ≥30 °C may lead to pollen shedding, poor pollen viability, poor germination, and growth of pollen [151].

Heat stress affects the physiological processes of plants in several ways. It increases the membrane fluidity, leading to a series of reactions that alter metabolisms and impair cellular machinery [152]. Furthermore, other cellular processes such as protein degradation and cytoskeleton are also influenced by heat stress [153]. Climate change, prolonged heat waves, and global warming are among the leading cause of heat stress [154]. Heat stress severely limits the productivity of crop plants. For instance, it is speculated that each one-degree rise in temperature reduces wheat production by more than 6% [12,155]. Therefore, strategies to mitigate the devastating effects of heat stress are urgently required as global warming is worsening day by day.

The deletion of heat sensitive albino1 (OsHSA1) gene in rice exhibited more sensitivity to heat but had a faster greening phenotype as compared to the wild type. It was demonstrated that HSA1 plays important roles in chloroplast development at early stages and functions in protecting chloroplasts under heat stress at later stages [101]. The OsHSA1 encodes a fructokinase-like protein that is involved in chloroplast protection and development during different growth stages in rice. Mutants generated through CRISPR/Cas9 in tomato Slcpk28 showed an increased accumulation of ROS and protein oxidation and decreased the activity of antioxidant enzymes including ascorbate peroxidase under heat stress [156]. The CRISPR/Cas9-mediated knockout of SIMAPK3 expressed improved heat stress tolerance by decreasing ROS accumulation and up-regulating the expression of genes encoding heat shock proteins (HSPs) and heat stress transcription factors (HSFs) [102].

Brassinosteroids (BRs) are plant hormones involved in conferring tolerance to abiotic stresses in plants [157]. In tomato plants, the BZR1 gene serves as a key regulator of the BR response. Heat-stress-induced damage was exacerbated in the Δbzr1 mutants and BR-induced heat stress tolerance was lost through the respiratory burst of oxidase homolog (RBOH1)-dependent ROS signaling, which is regulated by feronia homologs [158]. The knockout of OsNAC006 by CRISPR/Cas9 exhibited an increased level of H$_2$O$_2$ and superoxide radicals (O$_2^-$) as well as decreased chlorophyll content and antioxidant enzymes. It indicates that Osna006 may be involved in heat stress tolerance by mediating the process of photosynthesis and limiting the activity of antioxidant enzymes, triggered in response to oxidative stress under high temperatures [159]. The gene knock-out by CRISPR/Cas9 in genes encoding the abscisic acid receptor (PYL1/4/6) has shown considerable high-temperature tolerance in rice [103]. To address the issues such as global warming and climate change, the identification of targets for improving heat stress tolerance as well as the development of heat tolerant varieties are necessary. Phytochrome (PHY) could be an important target in this respect as PHYB has been identified as a thermo-sensor [160,161]. The PHY mutants expressed improved tolerance to high temperatures in Arabidopsis [162].
and tomato plants [163]. This mutant information is important for the definition of targets of genome editing.

High temperatures result in higher respiration in pollen grains, which can lead to the elimination of respiratory substrates and decreased mitochondrial activity, ultimately resulting in pollen abortion and poor fruit setting [164,165]. Parthenocarpy (fruit development without pollination/fertilization) is an important target for seedless fruit development due to its fertilization independence, consumers’ preference, and good quality of fruit [166,167]. During the screening of an ethyl-methanesulfonate (EMS) mutated population in tomato under heat stress, a mutant capable of generating high-quality seedless fruit was selected. Following the CRISPR/Cas9 gene knockout revealed that the seedless phenotype was caused by a mutation in the tomato SIAGAMOUSLIKE 6 (SIAGL6) gene encoding MADS-box. Hence, mutations in SIAGL6 increased heat stress in tomato plants. Moreover, these mutants exhibited facultative parthenocarpy without any pleiotropic effect, which was comparable in both shape and weight to the wild-type fruits (seeded) [21]. Aux/IAA9 (IAA9) is responsible for fruit development in tomato and represses parthenocarpy. CRISPR/Cas9-mediated mutant plants showed fruit development without fertilization and mutants were also heritable in successive generations [168]. Another hormone DELLA is a negative regulator of gibberellin signaling, the loss-of-function mutations in SIDEHLA exhibited high gibberellin sensitivity and a parthenocarpic phenotype [169,170]. All these findings suggested that the CRISPR/Cas9 system enhanced parthenocarpy in tomato plants. Other members of Solanaceae, such as peppers and eggplants, can also be improved by deploying the genome editing tool CRISPR/Cas9. Also, the overexpression of ZmWRKY106 enhances drought and heat tolerance in transgenic maize plants by regulating the expression of stress-related genes, reducing ROS content, and by increasing the activities of antioxidant enzymes [171]. Heat shock proteins (HSPs) are molecular chaperones involved in cellular survival by transporting, folding, and degrading other proteins under heat stress [172]. The overexpression of HSP70 genes conferred increased resistance to abiotic stresses including high-temperature stress [173,174]. Moreover, the overexpression of HSP40 enhanced thermo-tolerance in transgenic Arabidopsis [175].

6. CRISPR/Cas9 for Mitigating the Impact of Cold Stress

In recent years, climate change has become the core problem threatening global food security [176]. In addition to heat waves, extremely cold temperatures have also been recorded in different ecological regions of the world [177]. Cold- or low-temperature stress may be divided into freezing stress (<0 °C) and chilling stress (0–15 °C), which adversely affects crop growth and production [178–180]. Excessive cold temperatures halt plant growth as it causes mechanical injury and dysfunction of metabolic activities [181]. Cold stress poses negative effects on the biochemical, physiological, and molecular activities of plants during their growth and development. Cold exposure, especially during winter, severely affects the photosynthetic potential and the plant anatomy [182,183]. Cold stress during the seedling stage may lead to poor germination and emergence. Prolonged exposures cause stunted growth, leaf chlorosis, poor source–sink relations, and nutrient localization [184]. The major impact of cold stress in plants is membrane rigidification, which aggravates other downstream processes in response to cold stress. Moreover, it disturbs the stability of the protein and expression, as well as impairs the activities of several enzymes including ROS-scavenging enzymes. Resultantly, the photosynthetic capacity of plant cells is questioned along with membrane damage and the formation of secondary structures in RNA that restrict its expression [185]. Low temperatures can injure crop species affecting their growth, productivity, and survival [186].

Various physiological and biochemical processes in plants are regulated by proline-rich proteins involved in growth and stress tolerance in plants. The knockout of OsPRP1 (encodes proline-rich protein) by CRISPR/Cas9 enhanced the cold tolerance ability in rice [187]. In addition, the CRISPR/Cas9-mediated knockout of OsMYB30, characterized as a cold-responsive gene in rice, exhibited a higher cold tolerance than that of wild-
type rice [104]. The CRISPR/Cas9-mediated ΔAtcbf single, double, and triple mutants in Arabidopsis elucidated that three tandemly arranged CCAAT-binding factor (CBF) genes such as CBF1, CBF2, and CBF3 have been involved in cold acclimatization. The cold-acclimated Atcbf triple mutants exhibited a highly sensitive response under cold stress compared to that of the wild type. Under prolonged exposures to chilling temperatures, the expression of CBF genes was suddenly enhanced. Resultantly, CBF proteins activate the transcription of downstream cold-responsive genes to improve the freezing tolerance in plants [188]. The CBF1 is the only cold-inducible gene in tomato plants and its increased expression resulted in salicylic acid and hydrogen peroxide-induced cold tolerance in tomato plants [189]. The CBF1 mutants generated by the CRISPR/Cas9 exhibited greater electrolyte leakage and malondialdehyde (MDA) levels than wild-type plants in tomato plants, indicating that the knockout of CBF1 can increase cold-stress-induced membrane damage [190]. Plant annexins and phospholipid-binding proteins are involved in the regulation of plant development and stress tolerance. The OsAnn3-knockout mutants developed by CRISPR/Cas9 showed enhanced relative electrical conductivity as compared to wild-type plants, which proved that OsAnn3 can perform a role in cold tolerance in rice [191]. Several cis-regulatory elements in the rice promoter region OsAnn5 are common promoter elements. However, some elements are unique to OsAnn5, including recognition sites for MYB, dehydration-responsive elements, and light-responsive elements, showing that several transcription factors regulate the expression of OsAnn5 in rice. The elimination of OsAnn5 function through CRISPR/Cas9 significantly increased the survival rates at the seedling stage under cold stress in rice demonstrating that OsAnn5 regulates cold stress tolerance at the seedling stage in rice [192].

7. CRISPR for Improving Plant Tolerance to Climate Change

Climate change coupled with environmental pollution renders detrimental effects on the growth, development, phenology, and production potential of crop plants. Drastic changes in global environmental conditions have led to the development of climate-resilient phytoremediation methods. These approaches are of huge importance due to the current situation of the environmental crisis. Soil contamination with heavy metals and metalloids is one of the major harmful effects of environmental pollution worldwide. Some of the metal ions are carcinogenic pollutants with a long half-life and are non-degradable in the environment. Therefore, enhancing the adaptive potential of plants to the changing environmental conditions is a major concern regarding phytoremediation practice. Genome modification using artificial nucleases has the potential to enhance phytoremediation.

Recently, the CRISPR-Cas9-based gene editing approach has been extensively used for the phytoremediation of heavy metals. These modifications facilitate to control and stabilize the harmful effects of environmental pollutants on various crop plants. CRISPR-Cas9-based gene editing offers exciting options for photo technologies such as phytoremediation [193]. Several phytoremediators have been sequenced such as Thlaspi caerulescens (hyper-accumulator for Ni, Zn, and Cd), Arabidopsis halleri (hyper-accumulator for Zn and Cd), Hirschfeldia incana (for controlling Pb), Brassica juncea, and Pteris vittata [194–197]. Phytoremediation is the most effective, environmentally friendly, and cost-effective approach for the remediation of toxic metals and plant pollutants. Gene editing has the potential to modify the efficacy of phytoremediation for metal uptake, metal transport, and sequestration. Many genes including metal transporter, a metal chelator, phytochelatin, and metallothionein have been transferred to plants to enhance metal uptake and sequestration. For instance, the CRISPR-Cas9-based genome modification in a metal transporter gene OsNRAMP5 resulted in low Cd-accumulation without affecting the yield in indica rice [105]. Moreover, transgenic plants developed by CRISPR-Cas9 genome editing demonstrated an increased ability to tolerate, detoxify, or accumulate heavy metals thereby promoting phytoremediation [198]. The overexpression of metallothioneins encoding genes (MT1, MT2, and MTA1) led to the increased potential of accumulating Cu, Zn, and Cd in Arabidopsis and tobacco [199,200]. Similarly, the overexpression of ATP Sulphurylase and Selenocysteine
Methyltransferase gene in *B. juncea* led to enhanced tolerance towards Selenium [201]. The expression of *BcMT1* and *BcMT2* metallothionein genes from *B. campestris* to Arabidopsis resulted in increased tolerance to Cu and Cd [202]. CRISPR has opened the way of phytoremediation for many plants, such as maize and poplar, which were considered capable but not yet investigated due to the complex architecture of their genome.

8. CRISPR for Improving Plant Tolerance to Herbicides and Heavy Metals

Heavy metals (As, Ni, Mn, Co, Cu, Zn, etc.) have been accumulated in soils due to several anthropogenic activities which exert negative impacts on plant growth by disturbing the cellular membranes, photosynthetic ability, and cellular respiration that ultimately limits crop productivity [203,204]. Moreover, heavy metals produce hydrogen peroxide (H$_2$O$_2$), free radicals such as hydroxyl radicals (OH), and superoxide radicals (O$^{-}$), which lead to oxidative stress [205]. In addition, the continuous application of herbicides used for eradicating weeds has produced herbicide resistance in several plants [206,207].

Several genes play a role in improving the tolerance to heavy metals in plants [208]. For instance, γ-glutamyl acyltransferase mutants expressed defensive properties against heavy metal toxicity, due to enhanced accumulation of glutathione. Hence, the development of mutants using CRISPR-Cas9 would be beneficial to cope with heavy metal stress in plants. Recently, *oxy1* CRISPR-mediated mutants in Arabidopsis showed increased resistance to Cadmium (Cd) [209]. In rice, several transporter genes (*OsNramp1, OsCd1*, and *OsNramp5*) are involved in the absorption of Cd by the roots [210]. Manipulation in the expression of these genes through CRISPR/Cas9 has resulted in minimizing the concentration of Cd in rice. Mutants of *OsLCT1* and *OsNramp5* generated through CRISPR/Cas9 has resulted in reduced levels of Cd in rice [105]. Likewise, *OsARM1* regulates the expression of Arsenic (As) linked genes in rice crops. The knock-out mutants of the *OsARM1* by CRISPR produced tolerance to As [209]. The *OsHAK1* gene controls the uptake and translocation of Cesium (Cs$^{+}$) in rice. The CRISPR-Cas system was deployed to reduce the uptake of radioactive Cs by the rice plants. The knock-out mutants of *OsHAK1* exhibited a significant reduction in 137 Cs$^{+}$ content levels in roots [106]. The *OsPRX2* is known to limit ROS production under K$^{+}$ limiting conditions. The overexpression of *Os-PRX2* produced K$^{+}$ deficiency tolerance by closing the stomata in rice [211]. The CRISPR/Cas9-mediated knock-out mutants in *OsARM1* expressed an improved tolerance to arsenic in rice plants [107].

The development of herbicide tolerance in crop plants is one of the major targets for increasing crop production. Currently, genome editing-based on the CRISPR-Cas9 system has been used to develop herbicide-tolerant crops as an effective weed control strategy [212,213]. The recombination of acetolactate synthase generated using CRISPR/Cas9 produced herbicide resistance rice [110]. CRISPR/Cas9-based mutants in the *ALS* gene significantly enhanced herbicide tolerance in watermelons [111]. In addition, Herbicide resistant maize plants were developed using the same approach targeting *ALS1* and *ALS2* genes [112]. Herbicide tolerance traits have been incorporated in rice by exploiting CRISPR-based editing in *OsALS1* [108,109]. The knock-out mutants in the *OsALS* gene of rice depicted strong herbicide tolerance potential by conferring resistance to imazapic and imazethapyr [109]. Recently, the CRISPR-Cas9-based targeted mutations in EPSPS (5-Enolpyruvylshikimate-3-phosphate synthase, PDS (phytoene desaturase), and ALS [37] conferred herbicide resistance in tomato plants [214].

9. Conclusions and Future Direction

Traditional crop improvement approaches, including molecular breeding, mutagenesis, and transgenesis are time-consuming and expensive. Additionally, they are not specific in bringing intended change to crop plants. For example, molecular breeding is limited by species-specific barriers and is often inflicted by linkage drag, which brings with it many unwanted characteristics. The elimination of these so-called unwanted genomic chunks requires intensive backcrossing, which makes the procedure of developing new varieties quite challenging. On the other hand, genetic transformation allows the engineering of
those traits that do not even exist in the plant gene pool. However, the use of GM crops has become so controversial that many countries in the world have completely banned the cultivation of GM crops. Consequently, the power of the “gene revolution” faded away before delivering. The advent of new genome editing tools such as CRISPR offers hope to address the issues associated with GM crops. If the selectable marker as well as the gene coding for Cas9 are removed from the plant genome, it would become similar to the one developed by non-genetic engineering tools. Therefore, an increasing number of countries are allowing the cultivation of transgene-free genetically edited crops. However, the major limitation to the application of CRISPR technology to improving field crops would be the scarcity of functionally characterized gene(s) involved in the agronomic traits [25]. The scarcity of validated targets would be one of the major bottlenecks in unlocking the CRISPR potential for developing climate-smart stress-resilient crops. Nevertheless, we have seen that CRISPR is being increasingly employed in field crops to help address climate issues. The development of abiotic stress-tolerant and heavy metal stress-tolerant plants through the manipulation of cis-, and trans-regulatory elements, resistance (R) genes, and susceptibility (S) genes will allow their open-field cultivation as genome-edited plants do not differ genetically from their unedited counterparts except for the desired genetic change at a specific location on the genome. Using multiplex genome editing would allow for the development of genome-edited crops engineered for tolerance against multiple traits in a single transformation event. Therefore, it is expected that genome editing will become the technology of choice for developing desired genetically and epigenetically [215,216] biotech crops for different purposes particularly to address the food shortage problems as well as to fight climate change more effectively.

Author Contributions: Conceptualization, M.-u.R. and B.Z.; writing—original draft preparation, S.Z. and M.A.R.; writing—review and editing, M.-u.R., N.A. and B.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: M.-u.R. is grateful to Pakistan Science Foundation (PSF/NSFC-AGR/P-NIBGE(12) and Punjab Agriculture Research Board (Punjab, PARB-20-206), Pakistan, for funding research in his lab. N.A. is supported by the International Centre for Genome Engineering and Biotechnology (ICGEB), Italy.

Conflicts of Interest: The authors declare that no conflict of interest exists.

References

1. Zhang, B.; Rahman, M.-u. Targeted Breeding in Cotton Using CRISPR/Cas9 Genome Editing. In Cotton Precision Breeding; Rahman, M.-u., Zafar, Y., Zhang, T., Eds.; Springer International Publishing: Cham, Switzerland, 2021; pp. 313–327.

2. Kumar, S. Abiotic stresses and their effects on plant growth, yield and nutritional quality of agricultural produce. Int. J. Food Sci. Agric. 2020, 4, 367–378. [CrossRef]

3. Daryanto, S.; Wang, L.; Jacinthe, P.-A. Global Synthesis of Drought Effects on Maize and Wheat Production. PLoS ONE 2016, 11, e0156362. [CrossRef] [PubMed]

4. Daryanto, S.; Wang, L.; Jacinthe, P.-A. Global synthesis of drought effects on cereal, legume, tuber and root crops production: A review. Agric. Water Manag. 2017, 179, 18–33. [CrossRef]

5. Mafakheri, A.; Siosemardeh, A.; Bahrampour, B.; Struik, P.C.; Sohrabi, Y. Effect of Drought Stress on Yield, Proline and Chlorophyll Contents in Three Chickpea Cultivars. Aust. J. Crop Sci. 2010, 4, 580–585.

6. Maleki, A.; Nadert, A.; Nasert, R.; Fathi, A.; Bahamin, S.; Maleki, R. Physiological performance of soybean cultivars under drought stress. Bull. Environ. Pharmacol. Life Sci. 2013, 2, 38–44.

7. Farooq, M.; Gogoi, N.; Barthakur, S.; Barooowa, B.; Bharadwaj, N.; Alghamdi, S.S.; Siddique, K.H.M. Drought Stress in Grain Legumes during Reproduction and Grain Filling. J. Agron. Crop Sci. 2017, 203, 81–102. [CrossRef]

8. Gong, Z.; Xiong, L.; Shi, H.; Yang, S.; Herrera-Estrella, L.R.; Xu, G.; Chao, D.-Y.; Li, J.; Wang, P.-Y.; Qin, F. Plant abiotic stress response and nutrient use efficiency. Sci. China Life Sci. 2020, 63, 635–674. [CrossRef]
9. Van Zelm, E.; Zhang, Y.; Testerink, C. Salt tolerance mechanisms of plants. *Annu. Rev. Plant Biol.* **2020**, *71*, 403–433. [CrossRef]
10. Morton, M.J.; Avrila, M.; Al-Tamimi, N.; Saade, S.; Pailles, Y.; Negrao, S.; Tester, M. Salt stress under the scalpel—dissecting the genetics of salt tolerance. *Plant J.* **2019**, *97*, 148–163. [CrossRef]
11. Jamil, A.; Riaz, S.; Ashraf, M.; Foolad, M. Gene expression profiling of plants under salt stress. *Crit. Rev. Plant Sci.* **2011**, *30*, 435–458. [CrossRef]
12. Asseng, S.; Ewert, F.; Martre, P.; Rötter, R.P.; Lobell, D.B.; Cammarano, D.; Kimball, B.A.; Ottman, M.J.; Wall, G.W.; White, J.W.; et al. Rising temperatures reduce global wheat production. *Nat. Clim. Chang.* **2015**, *5*, 143–147. [CrossRef]
13. Wang, Y.; Wang, L.; Zhou, J.; Hu, S.; Chen, H.; Xiang, J.; Zhang, Y.; Zeng, Y.; Shi, Q.; Zhu, D.; et al. Research Progress on Heat Stress of Rice at Flowering Stage. *Rice Sci.* **2019**, *26*, 1–10. [CrossRef]
14. Yang, H.; Huang, T.; Ding, M.; Lu, D.; Lu, W. High Temperature during Grain Filling Impacts on Leaf Senescence in Waxy Maize. *Agron. J.* **2017**, *109*, 906–916. [CrossRef]
15. Nongpiur, R.C.; Singla-Pareek, S.L.; Pareek, A. Genomics Approaches For Improving Salinity Stress Tolerance in Crop Plants. *Curr. Genom.* **2016**, *17*, 343–357. [CrossRef] [PubMed]
16. Shukla, M.; Al-Butaiean, K.T.; Trivedi, M.; Tiwari, R.K. Status of research, regulations and challenges for genetically modified crops in India. *GM Crops Food* **2018**, *9*, 173–188. [CrossRef]
17. Raman, R. The impact of Genetically Modified (GM) crops in modern agriculture: A review. *GM Crops Food* **2017**, *8*, 195–208. [CrossRef]
18. Ahmad, N.; Awan, M.J.A.; Mansoor, S. Improving editing efficiency of prime editor in plants. *Trends Plant Sci.* **2022**. [CrossRef]
19. Zong, Y.; Wang, Y.; Li, C.; Zhang, R.; Chen, K.; Ran, Y.; Qiu, J.L.; Wang, D.; Gao, C. Precise base editing in rice, wheat and maize with a Cas9-cytidine deaminase fusion. *Nat. Biotechnol.* **2017**, *35*, 438–440. [CrossRef]
20. Lu, Y.; Zhu, J.-K. Precise Editing of a Target Base in the Rice Genome Using a Modified CRISPR/Cas9 System. *Mol. Plant* **2017**, *10*, 523–525. [CrossRef]
21. Klap, C.; Yeshayahu, E.; Bolger, A.M.; Arazi, T.; Gupta, S.K.; Shabtai, S.; Usadel, B.; Salts, Y.; Barg, R. Tomato facultative parthenocarpy results from SlAGAMOUS-LIKE 6 loss of function. *Plant Biotechnol. J.* **2017**, *15*, 634–647. [CrossRef]
22. Dalla Costa, L.; Malnoy, M.; Gribaudo, I. Breeding next generation tree fruits: Technical and legal challenges. *Crit. Rev. Plant Sci.* **2017**, *36*, 906–916. [CrossRef]
23. Lopez, D.C.; Sword, G.A. The endophytic fungal entomopathogens *Beauveria bassiana* and *Purpureocillium lilacinum* enhance the growth of cultivated cotton (*Gossypium hirsutum*) and negatively affect survival of the cotton bollworm (*Helicoverpa zeae*). *Biol. Control* **2015**, *89*, 53–60. [CrossRef]
24. Gao, C. Genome engineering for crop improvement and future agriculture. *Cell* **2021**, *184*, 1621–1635. [CrossRef] [PubMed]
25. Ahmad, N.; Rahman, M.u.; Mukhtar, Z.; Zafar, Y.; Zhang, B. A critical look on CRISPR-based genome editing in plants. *J. Cell Physiol.* **2020**, *235*, 666–682. [CrossRef]
26. Makarova, K.S.; Haft, D.H.; Barrangou, R.; Brouns, S.J.J.; Charpentier, E.; Horvath, P.; et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science* **2013**, *339*, 589–604. [CrossRef] [PubMed]
27. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* **2012**, *337*, 816–821. [CrossRef]
28. Horvath, P.; Barrangou, R. CRISPR/Cas, the Immune System of Bacteria and Archaea. *Science* **2010**, *327*, 167–170. [CrossRef]
29. Mali, P.; Yang, L.; Esvelt, K.M.; Aach, J.; Guell, M.; DiCarlo, J.E.; Norville, J.E.; Church, G.M. RNA-Guided Human Genome Engineering via Cas9. *Science* **2013**, *339*, 823–826. [CrossRef]
30. Cong, L.; Ran, F.A.; Cox, D.; Lin, S.; Barretto, R.; Habib, N.; Hsu, P.D.; Wu, X.; Jiang, W.; Marraffini, L.A.; et al. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science* **2013**, *339*, 586–593. [CrossRef]
31. Nishimasu, H.; Cong, L.; Yan, W.X.; Ran, F.A.; Zetsche, B.; Li, Y.; Kurabayashi, A.; Ishitani, R.; Zhang, F.; Nureki, O. Crystal structure of Cas9 in Complex with Guide RNA and Target DNA. *Cell* **2014**, *156*, 935–949. [CrossRef] [PubMed]
32. Jinek, M.; Jiang, F.; Taylor, D.W.; Sternberg, S.H.; Kaya, E.; Ma, E.; Anders, C.; Hauer, M.; Zhou, K.; Lin, S.; et al. Structures of Cas9 Endonucleases Reveal RNA-Mediated Conformational Activation. *Science* **2014**, *343*, 1247997. [CrossRef] [PubMed]
33. Gasiunas, G.; Barrangou, R.; Horvath, P.; Siksnys, V. Cas9–crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, E2579–E2586. [CrossRef] [PubMed]
34. Zhang, D.Q.; Zhang, Z.Y.; Unver, T.; Zhang, B.H. CRISPR/Cas: A powerful tool for gene function study and crop improvement. *J. Adv. Res.* **2021**, *29*, 207–221. [CrossRef]
35. Zhang, Y.; Malzahn, A.A.; Sretenovic, S.; Qi, Y. The emerging and unexploited potential of CRISPR technology in plant science. *Nat. Plants* **2019**, *5*, 778–794. [CrossRef]
36. Wolter, F.; Klemm, J.; Puchta, H. Efficient in planta gene targeting in Arabidopsis using egg cell-specific expression of the Cas9 nuclease of *Staphylococcus aureus*. *Plant J.* **2018**, *94*, 735–746. [CrossRef]
37. Steinert, J.; Schiml, S.; Fauser, F.; Puchta, H. Highly efficient heritable plant genome engineering using Cas9 orthologues from *Streptococcus thermophilus* and *Staphylococcus aureus*. *Plant J.* **2015**, *84*, 1295–1305. [CrossRef]
38. Nishimasu, H.; Cong, L.; Yan, W.X.; Ran, F.A.; Zetsche, B.; Li, Y.; Kurabayashi, A.; Ishitani, R.; Zhang, F.; Nureki, O. Crystal structure of Staphylococcus aureus Cas9. *Cell* **2015**, *162*, 1113–1126. [CrossRef]
40. Hirano, H.; Gootenberg, J.S.; Horii, T.; Abudayeh, O.O.; Kimura, M.; Hsu, P.D.; Nakane, T.; Ishitani, R.; Hatada, I.; Zhang, F. Structure and engineering of Francisella novicida Cas9. *Cell* 2016, 164, 950–961. [CrossRef]

41. Lee, C.M.; Cradick, T.J.; Bao, G. The *Neisseria meningitidis* CRISPR-Cas9 system enables specific genome editing in mammalian cells. *Mol. Ther.* 2016, 24, 645–654. [CrossRef]

42. Kim, E.; Koo, T.; Park, S.W.; Kim, D.; Kim, K.; Cho, H.-Y.; Song, D.W.; Lee, K.J.; Jung, M.H.; Kim, S. In vivo genome editing with a small Cas9 orthologue derived from *Campylobacter jejuni*. *Nat. Commun.* 2017, 8, 14500. [CrossRef] [PubMed]

43. Kleinstiver, B.P.; Prew, M.S.; Tsai, S.Q.; Topkar, V.V.; Nguyen, N.T.; Zheng, Z.; Gonzales, A.P.; Li, Z.; Peterson, R.T.; Yeh, J.-R.J. Engineered CRISPR-Cas9 nucleases with altered PAM specificities. *Nature* 2015, 523, 481–485. [CrossRef] [PubMed]

44. Bernabé-Orsí, J.M.; Casas-Rodrigo, I.; Minguet, E.G.; Landolfi, V.; Garcia-Carpintero, V.; Gianoglio, S.; Vázquez-Vilar, M.; Granell, A.; Orzaez, D. Assessment of Cas12a-mediated gene editing efficiency in plants. *Plant Biotechnol. J.* 2019, 17, 1971–1984. [CrossRef]

45. Malzahn, A.A.; Tang, L.; Lee, K.; Ren, Q.; Sretenovic, S.; Zhang, Y.; Chen, H.; Kang, M.; Bao, Y.; Zheng, X.; et al. Application of CRISPR-Cas12a temperature sensitivity for improved genome editing in rice, maize, and *Arabidopsis*. *BMC Biol.* 2019, 17, 9. [CrossRef] [PubMed]

46. Schindele, P.; Puchta, H. Engineering CRISPR/LbCas12a for highly efficient, temperature-tolerant plant gene editing. *Plant Biotechnol. J.* 2020, 18, 1118–1120. [CrossRef]

47. García-Doval, C.; Jinek, M. Molecular architectures and mechanisms of Class 2 CRISPR-associated nucleases. *Curr. Opin. Struct. Biol.* 2017, 47, 157–166. [CrossRef] [PubMed]

48. Harrington, L.B.; Paez-Espino, D.; Staahl, B.T.; Chen, J.S.; Ma, E.; Kyrpides, N.C.; Doudna, J.A. A thermostable Cas9 with increased lifetime in human plasma. *Nat. Commun.* 2018, 9, 1424. [CrossRef]

49. Acharya, S.; Ansari, A.; Hirano, S.; Paul, D.; Rauthan, R.; Kumar, M.; Phutela, R.; Sarkar, S.; Gulati, S.; Mahato, S. Engineered PAM-flexible FnCas9 variants for robust and specific genome editing and diagnostics. *Res. Sq.* 2021; in press.

50. Yamamoto, A.; Ishida, T.; Yoshimura, M.; Kimura, Y.; Sawa, S. Developing Heritable Mutations in *Arabidopsis thaliana* Using a Modified CRISPR/Cas9 Toolkit Comprising PAM-Alterned Cas9 Variants and gRNAs. *Plant Cell Physiol.* 2019, 60, 2255–2262. [CrossRef]

51. Hu, X.; Meng, X.; Liu, Q.; Li, J.; Wang, K. Increasing the efficiency of CRISPR-Cas9-VQR precise genome editing in rice. *Plant Biotechnol. J.* 2018, 16, 292–297. [CrossRef]

52. García-Doval, C.; Jinek, M. Molecular architectures and mechanisms of Class 2 CRISPR-associated nucleases. *Curr. Opin. Struct. Biol.* 2017, 47, 157–166. [CrossRef] [PubMed]

53. Qin, R.; Li, J.; Liu, X.; Xu, R.; Yang, J.; Wei, P. SpCas9-NG self-targets the sgRNA sequence in plant genome editing. *Nat. Plants* 2020, 6, 197–201. [CrossRef] [PubMed]

54. Zhong, Z.; Sretenovic, S.; Ren, Q.; Yang, L.; Bao, Y.; Qi, C.; Yuan, M.; He, Y.; Liu, S.; Liu, X.; et al. Improving Plant Genome Editing with High-Fidelity xCas9 and Non-canonical PAM-Targeting Cas9-NG. *Mol. Plant* 2019, 12, 1027–1036. [CrossRef] [PubMed]

55. Endo, M.; Mikami, M.; Endo, A.; Kaya, H.; Itoh, T.; Nishimasu, H.; Nureki, O.; Toki, S. Genome editing in plants by engineered CRISPR–Cas9 recognizing NG PAM. *Nat. Plants* 2019, 5, 14–17. [CrossRef]

56. Qin, R.; Li, J.; Li, H.; Zhang, Y.; Liu, X.; Xiao, Y.; Zhang, X.; Wei, P. Developing a highly efficient and wildly adaptive CRISPR-SaCas9 toolset for plant genome editing. *Plant Biotechnol. J.* 2019, 17, 706. [CrossRef]

57. Xu, L.; Zhao, H.; Wan, R.; Liu, Y.; Xu, Z.; Tian, W.; Ruan, W.; Wang, F.; Deng, M.; Wang, J. Identification of vacuolar phosphate efflux transporters in land plants. *Nat. Plants* 2019, 5, 84–94. [CrossRef]

58. Zhang, D.; Zhang, H.; Li, T.; Chen, K.; Qiu, J.-L.; Gao, C. Perfectly matched 20-nucleotide guide RNA sequences enable robust genome editing using high-fidelity SpCas9. *Genome Biol.* 2017, 18, 191. [CrossRef]

59. Casini, A.; Olivieri, M.; Petris, G.; Montagna, C.; Reginato, G.; Maule, G.; Lorenzin, F.; Prandi, D.; Romanel, A.; Demicheli, F.; et al. A highly specific SpCas9 variant is identified by in vivo screening in yeast. *Nat. Biotechnol.* 2020, 38, 1328–1336. [CrossRef]

60. Casini, A.; Olivieri, M.; Petris, G.; Montagna, C.; Reginato, G.; Maule, G.; Lorenzin, F.; Prandi, D.; Romanel, A.; Demicheli, F.; et al. A highly specific SpCas9 variant is identified by in vivo screening in yeast. *Nat. Biotechnol.* 2020, 38, 1328–1336. [CrossRef]

61. Casini, A.; Olivieri, M.; Petris, G.; Montagna, C.; Reginato, G.; Maule, G.; Lorenzin, F.; Prandi, D.; Romanel, A.; Demicheli, F.; et al. A highly specific SpCas9 variant is identified by in vivo screening in yeast. *Nat. Biotechnol.* 2020, 38, 1328–1336. [CrossRef]

62. Xu, W.; Song, W.; Yang, Y.; Wu, Y.; Lv, X.; Yuan, S.; Liu, Y.; Yang, J. Multiplex nuclease editing by high-fidelity Cas9 variants with improved efficiency in rice. *BMC Plant Biol.* 2019, 19, 511. [CrossRef]

63. Li, J.; Xu, R.; Qin, R.; Liu, X.; Kong, F.; Wei, P. Genome editing mediated by SpCas9 variants with broad non-canonical PAM compatibility in plants. *Mol. Plant Biol.* 2021, 14, 352–360. [CrossRef] [PubMed]

64. Vicencio, J.; Sánchez-Bolaños, C.; Moreno-Sánchez, I.; Brena, D.; Vejnar, C.E.; Kukhtar, D.; Ruiz-López, M.; Cots-Ponjoan, M.; Rubio, A.; Melero, N.R.; et al. Genome editing in animals with minimal PAM CRISPR-Cas9 enzymes. *Nat. Commun.* 2022, 13, 2601. [CrossRef] [PubMed]

65. Ren, Q.; Sretenovic, S.; Liu, S.; Tang, X.; Huang, L.; He, Y.; Liu, L.; Guo, Y.; Zhong, Z.; Liu, G.; et al. PAM-less plant genome editing using a CRISPR–SpyR toolbox. *Nat. Plants* 2021, 7, 25–33. [CrossRef] [PubMed]

66. Wang, M.; Xu, Z.; Gosavi, G.; Ren, B.; Cao, Y.; Kuang, Y.; Zhou, C.; Spetz, C.; Yan, F.; Zhou, X.; et al. Targeted base editing in rice with CRISPR/SceCas9 system. *Plant Biotechnol. J.* 2020, 18, 1645–1647. [CrossRef]

67. Zhong, Z.; Zhang, Y.; You, Q.; Tang, X.; Ren, Q.; Liu, S.; Yang, L.; Wang, Y.; Liu, X.; Liu, B.; et al. Plant Genome Editing Using FnCpf1 and LbCpf1 Nucleases at Redefined and Altered PAM Sites. *Mol. Plant* 2018, 11, 999–1002. [CrossRef] [PubMed]

68. Li, S.; Zhang, X.; Wang, W.; Guo, X.; Wu, Z.; Du, W.; Zhao, Y.; Xia, L. Expanding the Scope of CRISPR/Cpf1-Mediated Genome Editing in Rice. *Mol. Plant* 2018, 11, 995–998. [CrossRef]
Ogata, T.; Ishizaki, T.; Fujita, M.; Fujita, Y. CRISPR/Cas9-targeted mutagenesis of Strecker, J.; Jones, S.; Koopal, B.; Schmid-Burgk, J.; Zetsche, B.; Gao, L.; Makarova, K.S.; Koonin, E.V.; Zhang, F. Engineering of CRISPR-Cas12b for human genome editing. Nat. Commun. 2019, 10, 212. [CrossRef]

Wada, N.; Osakabe, K.; Osakabe, Y. Expanding the plant genome editing toolbox with recently developed CRISPR-Cas systems. Plant Physiol. 2022, 188, 1825–1837. [CrossRef]

Zhang, Y.; Ren, Q.; Tang, X.; Liu, S.; Malzahn, A.A.; Zhou, J.; Wang, J.; Yin, D.; Pan, C.; Yuan, M.; et al. Expanding the scope of plant genome engineering with Cas12aorthologs and highly multiplexable editing systems. Nat. Commun. 2021, 12, 19444. [CrossRef] [PubMed]

O’Driscoll, M.; Jeggo, P.A. The role of double-strand break repair—Insights from human genetics. Nat. Rev. Genet. 2006, 7, 45–54. [CrossRef] [PubMed]

Chang, H.H.Y.; Pannunzio, N.R.; Adachi, N.; Lieber, M.R. Non-homologous DNA end joining and alternative pathways to double-strand break repair. Nat. Rev. Mol. Cell Biol. 2017, 18, 495–506. [CrossRef] [PubMed]

Zhang, D.; Zhang, B. SpRY: Engineered CRISPR/Cas9 Harnesses New Genome-Editing Power. Trends Genet. 2020, 36, 546–548. [CrossRef] [PubMed]

Chen, S.; Zhang, N.; Zhang, Q.; Zhou, G.; Tian, H.; Hussain, S.; Ahmed, S.; Wang, T.; Wang, S. Genome editing to integrate seed size and abiotic stress tolerance traits in Arabidopsis reveals a role for DPA4 and SOD7 in the regulation of inflorescence architecture. Int. J. Mol. Sci. 2019, 20, 2695. [CrossRef]

Hsu, P.D.; Lander, E.S.; Zhang, F. Development and applications of CRISPR-Cas9 for genome engineering. Cell 2014, 157, 1262–1278. [CrossRef]

Doudna, J.A.; Charpentier, E. The new frontier of genome engineering with CRISPR-Cas9. Science 2014, 346, 1258096. [CrossRef]

Zhang, H.; Zhang, J.; Wei, P.; Zhang, B.; Gou, F.; Feng, Z.; Mao, Y.; Yang, L.; Zhang, H.; Xu, N.; et al. The CRISPR/Cas9 system produces specific and homozygous targeted gene editing in rice in one generation. Plant Biotechnol. J. 2014, 12, 797–807. [CrossRef]

Liao, S.; Qin, X.; Luo, L.; Han, Y.; Wang, X.; Usman, B.; Nawaz, G.; Zhao, N.; Liu, Y.; Li, R. CRISPR/Cas9-induced mutagenesis of semi-rolled leaf1, 2 confers curled leaf phenotype and drought tolerance by influencing protein expression patterns and ROS scavenging in rice (Oryza sativa L.). Agronomy 2019, 9, 728. [CrossRef]

Ogata, T.; Ishizaki, T.; Fujita, M.; Fujita, Y. CRISPR/Cas9-targeted mutagenesis of OsERA1 confers enhanced responses to abscisic acid and drought stress and increased primary root growth under nonstressed conditions in rice. PLoS ONE 2020, 15, e0243376. [CrossRef]

Illouz-Eliaz, N.; Nissan, I.; Nir, I.; Ramon, U.; Shohat, H.; Weiss, D. Mutations in the tomato gibberellin receptors suppress xylem proliferation and reduce water loss under water-deficit conditions. J. Exp. Bot. 2020, 71, 3603–3612. [CrossRef] [PubMed]

Liu, L.; Zhang, J.; Xu, J.; Li, Y.; Guo, L.; Wang, Z.; Zhang, X.; Zhao, B.; Guo, Y.-D.; Zhang, N. CRISPR/Cas9 targeted mutagenesis of SlLBD40, a lateral organ boundaries domain transcription factor, enhances drought tolerance in tomato. Plant Sci. 2020, 310, 110683. [CrossRef] [PubMed]

Shi, J.; Gao, H.; Wang, H.; Lafitte, H.R.; Archibald, R.L.; Yang, M.; Hakimi, S.M.; Mo, H.; Habben, J.E. ARGOS 8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. Plant Biotechnol. J. 2017, 15, 207–216. [CrossRef] [PubMed]

Liu, S.; Li, C.; Wang, H.; Wang, S.; Yang, S.; Liu, X.; Yan, J.; Li, B.; Beatty, M.; Zastrow-Hayes, G. Mapping regulatory variants controlling gene expression in drought response and tolerance in maize. Genome Biol. 2020, 21, 163. [CrossRef]

Wu, J.; Yan, G.; Duan, Z.; Wang, Z.; Kang, C.; Guo, L.; Liu, K.; Tu, J.; Shen, J.; Yi, B. Roles of the Brassica napus DELLA protein BnaA6. RGA, in modulating drought tolerance by interacting with the ABA signaling component BnaA10. ABF2. Front. Plant Sci. 2020, 11, 577. [CrossRef]

Wang, Y.; Cao, Y.; Liang, X.; Zhuang, J.; Wang, X.; Qiu, F.; Jiang, C. A dirigent family protein confers variation of Casparian strip thickness and salt tolerance in maize. Nat. Commun. 2022, 13, 2222. [CrossRef]

Ding, F.; Qiang, X.; Jia, Z.; Li, L.; Hu, J.; Yin, M.; Xia, S.; Chen, B.; Qi, J.; Li, Q.; et al. Knockout of a novel salt responsive gene SIABIG1 enhance salinity tolerance in tomato. Environ. Exp. Bot. 2022, 200, 104903. [CrossRef]

Tran, M.T.; Doan, D.T.H.; Kim, J.; Song, Y.J.; Sung, Y.W.; Das, S.; Kim, E.J.; Son, G.H.; Kim, S.H.; Van Vu, T. CRISPR/Cas9-based precise excision of SlHyPRP1 domain(s) to obtain salt stress-tolerant tomato. Plant Cell Rep. 2021, 40, 999–1011. [CrossRef]

Wang, T.; Xun, H.; Wang, W.; Ding, X.; Tian, H.; Hussain, S.; Dong, Q.; Li, Y.; Cheng, Y.; Wang, C.; et al. Mutation of GmAITR Genes by CRISPR/Cas9 Genome Editing Results in Enhanced Salinity Stress Tolerance in Soybean. Front. Plant Sci. 2022, 11, 77958. [CrossRef]

Lan, T.; Zheng, Y.; Su, Z.; Yu, S.; Song, H.; Zheng, X.; Lin, G.; Wu, W. OsSPL10, a SBP-Box Gene, Plays a Dual Role in Salt Tolerance and Trichome Formation in Rice (Oryza sativa L.). G3: Genes Genomes Genet. 2019, 9, 4107–4114. [CrossRef]

Duan, Y.-B.; Li, J.; Qin, R.-Y.; Xu, R.-F.; Li, H.; Yang, Y.-C.; Ma, H.; Li, L.; Wei, P.-C.; Yang, J.-B. Identification of a regulatory element responsible for salt induction of rice OsRAV2 through ex situ and in situ promoter analysis. Plant Mol. Biol. 2016, 90, 49–62. [CrossRef] [PubMed]
93. Wang, W.-C.; Lin, T.-C.; Kieber, J.; Tsai, Y.-C. Response Regulators 9 and 10 Negatively Regulate Salinity Tolerance in Rice. *Plant Cell Physiol.* 2019, 60, 2549–2563. [CrossRef] [PubMed]

94. Santosh Kumar, V.V.; Verma, R.K.; Yadav, S.K.; Yadav, P.; Watts, A.; Rao, M.V.; Chinnusamy, V. CRISPR-Cas9 mediated genome editing of drought and salt tolerance (OsDST) gene in indica mega rice cultivar MTU1010. *Physiol. Mol. Biol. Plants* 2020, 26, 1099–1110. [CrossRef] [PubMed]

95. Lu, Y.; Tian, Y.; Shen, R.; Yao, Q.; Wang, M.; Chen, M.; Dong, J.; Zhang, T.; Li, F.; Lei, M.; et al. Targeted, efficient sequence insertion and replacement in rice. *Nat. Biotechnol.* 2020, 38, 1402–1407. [CrossRef]

96. Wang, X.; He, Y.; Wei, H.; Wang, L. A clock regulatory module is required for salt tolerance and control of heading date in rice. *Plant Cell Environ.* 2021, 44, 3283–3301. [CrossRef]

97. Alam, M.S.; Kong, J.; Tao, R.; Ahmed, T.; Alamin, M.; Atotali, S.S.; Abdelsalam, N.R.; Xu, J.-H. CRISPR/Cas9 Mediated Knockout of the OsbHLH024 Transcription Factor Improves Salt Stress Resistance in Rice (*Oryza sativa* L.). *Plants* 2022, 11, 1184. [CrossRef]

98. Zhang, A.; Liu, Y.; Wang, F.; Li, T.; Chen, Z.; Kong, D.; Bi, J.; Zhang, F.; Luo, X.; Wang, J. Enhanced rice salinity tolerance via CRISPR/Cas9-targeted mutagenesis of the OsRR22 gene. *Mol. Breed.* 2019, 39, 47. [CrossRef]

99. Alfaith, A.; Wu, J.; Jan, S.U.; Zhang, Z.S.; Xia, J.Q.; Xiang, C.B. Loss of rice PARAOQUAT TOLERANCE 3 confers enhanced resistance for biotic stresses and increases grain yield in field. *Plant Cell Environ.* 2020, 43, 2743–2754. [CrossRef]

100. Yue, E.; Cao, H.; Liu, B. OsMrR535, a potential genetic editing target for drought and salinity stress tolerance in *Oryza sativa*. *Plants* 2020, 9, 1337. [CrossRef]

101. Qi, Z.; Kang, S.; He, L.; Zhao, J.; Zhang, S.; Hu, J.; Zeng, D.; Zhang, G.; Dong, G.; Gao, Z. The newly identified heat-stress sensitive albino 1 gene affects chloroplast development in rice. *Plant Sci.* 2018, 267, 168–179. [CrossRef]

102. Yu, W.; Wang, L.; Zhao, R.; Sheng, J.; Zhang, S.; Li, R.; Shen, L. Knockout of SIMAPK3 enhances tolerance to heat stress involving ROS homeostasis in tomato plants. *BMC Plant Biol.* 2019, 19, 354. [CrossRef] [PubMed]

103. Miao, C.; Xiao, L.; Hua, K.; Zou, C.; Zhao, Y.; Bressan, R.A.; Zhu, J.-K. Mutations in a subfamily of asbcsic acid receptor genes promote rice growth and productivity. *Proc. Natl. Acad. Sci. USA* 2018, 115, 6058–6063. [CrossRef] [PubMed]

104. Zeng, Y.; Wen, J.; Zhao, W.; Wang, Q.; Huang, W. Rational improvement of rice yield and cold tolerance by editing the three genes *OsPln5b, G5*, and *OsMYB30* with the CRISPR-Cas9 system. *Front. Plant Sci.* 2020, 10, 1663. [CrossRef] [PubMed]

105. Tang, L.; Mao, B.; Li, Y.; Lv, Q.; Zhang, L.; Chen, C.; He, H.; Wang, W.; Zeng, X.; Shao, Y. Knockout of *OsNrrmp5* using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Sci. Rep.* 2017, 7, 14438. [CrossRef]

106. Nieves-Cordones, M.; Mohamed, S.; Tanoi, K.; Kobayashi, N.I.; Takagi, K.; Vernet, A.; Guiderdoni, E.; Pagnussat, G. CRISPR/Cas9 gene editing to modify abiotic stress responses in plants. *Curr. Issues Mol. Biol.* 2015, 17, 44–56. [CrossRef] [PubMed]

107. Wang, F.-Z.; Chen, M.-X.; Yu, L.-J.; Xie, L.-J.; Yuan, L.-B.; Qi, H.; Xiao, M.; Guo, W.; Chen, Z.; Yi, K. OsARM1, an R2R3 MYB transcription factor, is involved in regulation of the response to arsenic stress in rice. *Front. Plant Sci.* 2017, 8, 1868. [CrossRef]

108. Wang, F.; Xu, Y.; Li, W.; Chen, Z.; Wang, J.; Fan, F.; Tao, Y.; Jiang, Y.; Zhu, Q.-H.; Yang, J. Creating a novel herbicide-tolerance *OsALS* allele using CRISPR/Cas9-mediated gene editing. *Crop J.* 2019, 9, 305–312. [CrossRef]

109. Hu, X.; Li, S.; Ren, B.; Yan, F.; Spetz, C.; Li, X.; Zhou, X.; Zhou, H. Base-editing-mediated artificial evolution of *OsALS1* in plants to develop novel herbicide-tolerant rice germplasms. *Mol. Plant* 2020, 13, 565–572. [CrossRef]

110. Sun, Y.; Zhang, X.; Wu, C.; He, Y.; Ma, Y.; Hou, H.; Gao, X.; Du, W.; Zhao, Y.; Xia, L. Engineering herbicide-resistant rice plants through CRISPR/Cas9-mediated homologous recombination of acetolactate synthase. *Mol. Plant* 2016, 9, 628–631. [CrossRef]

111. Tian, S.; Jiang, L.; Cui, X.; Zhang, J.; Guo, S.; Li, M.; Zhang, H.; Ren, Y.; Gong, G.; Zong, M. Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. *Plant Cell Rep.* 2018, 37, 1353–1356. [CrossRef]

112. Svitashev, S.; Young, J.K.; Schwartz, C.; Gao, H.; Falco, S.C.; Cigan, A.M. Targeted mutagenesis, precise gene editing, and site-specific gene insertion in maize using Cas9 and guide RNA. *Plant Physiol.* 2015, 169, 931–945. [CrossRef] [PubMed]

113. Bhat, J.A.; Deshmukh, R.; Zhao, T.; Patil, G.; Deokar, A.; Shinde, S.; Chaudhary, J. Harnessing high-throughput phenotyping and genotyping for enhanced drought tolerance in crop plants. *J. Biotechnol.* 2020, 324, 248–260. [CrossRef] [PubMed]

114. Li, X.; Xu, S.; Fuhrmann-Aoyagi, M.B.; Yuan, S.; Iwama, T.; Kobayashi, M.; Miura, K. CRISPR/Cas9 Technique for Temperature, Drought, and Salinity Stress Responses. *Curr. Issues Mol. Biol.* 2022, 44, 2644–2662. [CrossRef] [PubMed]

115. Osakabe, Y.; Watanabe, T.; Sugano, S.S.; Ueta, R.; Ishihara, R.; Shinozaki, K.; Osakabe, K. Optimization of CRISPR/Cas9 genome editing to modify abiotic stress responses in plants. *Sci. Rep.* 2016, 6, 26685. [CrossRef]

116. Zhao, Y.; Zhang, C.; Liu, W.; Gao, W.; Liu, C.; Song, G.; Li, W.-X.; Mao, L.; Chen, B.; Xu, Y. An alternative strategy for targeted gene replacement in plants using a dual-sgRNA/Cas9 design. *Sci. Rep.* 2016, 6, 23890. [CrossRef]

117. Park, J.-J.; Demepwolf, E.; Zhang, W.; Wang, Z.-Y. RNA-guided transcriptional activation via CRISPR/dCas9 fusion with a Histone Acetyltransferase. *PLoS ONE* 2017, 12, e0179410. [CrossRef]

118. Roca Paixà, J.F.; Gillet, F.-X.; Ribeiro, T.P.; Bournaud, C.; Lourenço-Tessutti, I.T.; Noriega, D.D.; Melo, B.P.; de Almeida-Engler, J.; Grossi-de-Sa, M.F. Improved drought stress tolerance in Arabidopsis by CRISPR/dCas9 fusion with a Histone Acetyltransferase. *Sci. Rep.* 2019, 9, 8080. [CrossRef]

119. Nuñez-Muñoz, L.; Vargas-Hernández, B.; Hinojosoa-Moya, J.; Ruiz-Medrano, R.; Xoconostle-Cazares, B. Plant drought tolerance provided through genome editing of the trehalase gene. *Plant Signal. Behav.* 2021, 16, 1877005. [CrossRef]

120. Abdallah, N.A.; Elsharawy, H.; Abulela, H.A.; Thuilmony, R.; Abdellahi, A.A.; Elarabi, N.I. Multiplex CRISPR/Cas9-mediated genome editing to address drought tolerance in wheat. *GM Crops Food* 2022, 1–17. [CrossRef]
121. He, X.; Luo, X.; Wang, T.; Liu, S.; Zhang, X.; Zhu, L. GhHB12 negatively regulates abiotic stress tolerance in *Arabidopsis* and cotton. *Environ. Exp. Bot.* 2020, 176, 104087. [CrossRef]

122. Rahneshan, Z.; Nasibi, F.; Moghadam, A.A. Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera L.*) rootstocks. *J. Plant Interact.* 2018, 13, 73–82. [CrossRef]

123. Gharsallah, C.; Fakhfakh, H.; Grubb, D.; Gorsane, F. Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants* 2016, 8, plw055. [CrossRef] [PubMed]

124. Shrivastava, P.; Kumar, R. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saud. J. Biol. Sci.* 2015, 22, 123–131. [CrossRef] [PubMed]

125. Sahin, U.; Ekinci, M.; Ors, S.; Turan, M.; Yildiz, S.; Yildirim, E. Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical properties of cabbage (*Brassica oleracea var. capitata*). *Sci. Hortic.* 2018, 240, 196–204. [CrossRef]

126. Ali, Q.; Daud, M.; Haider, M.Z.; Ali, S.; Rizwan, M.; Aslam, N.; Noman, A.; Iqbal, N.; Shahzad, F.; Deeba, F. Seed priming by sodium nitroprusside improves salt tolerance in wheat (*Triticum aestivum*) L. by enhancing physiological and biochemical parameters. *Plant Physiol. Biochem.* 2017, 119, 50–58. [CrossRef]

127. El Ghazali, G.E. *Suada vermiculata* Forssk.: ex JF Gmel.: Structural characteristics and adaptations to salinity and drought: A review. *Int. J. Sci.* 2020, 9, 28–33. [CrossRef]

128. Pan, T.; Liu, M.; Kreslavski, V.D.; Zharmukhamedov, S.K.; Nie, C.; Yu, M.; Kuznetsov, V.V.; Allakhverdiev, S.I.; Shabala, S. Non-stomatal limitation of photosynthesis by soil salinity. *Crit. Rev. Environ. Sci. Technol.* 2021, 51, 791–825. [CrossRef]

129. Navada, S.; Vadstein, O.; Gaument, F.; Tveten, A.-K.; Spanu, C.; Mikkelsen, Ø.; Kolarevic, J. Biofilms remember: Osmotic stress priming as a microbial management strategy for improving salinity acclimation in nitrifying biofilms. *Water Res.* 2020, 176, 115732. [CrossRef]

130. Zhang, Q.; Dai, W. Plant response to salinity stress. In *Stress Physiology of Woody Plants*; Dai, W., Ed.; CRC Press: Boca Raton, FL, USA, 2019; pp. 155–173.

131. Huang, Y.; Guan, C.; Liu, Y.; Chen, B.; Yuan, S.; Cui, X.; Zhang, Y.; Yang, F. Enhanced growth performance and salinity tolerance in transgenic switchgrass via overexpressing vacuolar Na+ (K+)/H+ antiporter gene (PvNHX1). *Front. Plant Sci.* 2017, 8, 458. [CrossRef]

132. Atieno, J.; Li, Y.; Langridge, P.; Dowling, K.; Brien, C.; Berger, B.; Varshney, R.K.; Sutton, T. Exploring genetic variation for salinity tolerance in chickpea using image-based phenotyping. *Sci. Rep.* 2017, 7, 1300. [CrossRef]

133. Munnik, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 2008, 59, 651. [CrossRef] [PubMed]

134. Volkov, V. Salinity tolerance in plants. Quantitative approach to ion transport starting from halophytes and stepping to genetic and protein engineering for manipulating ion fluxes. *Front. Plant Sci.* 2015, 6, 873. [CrossRef] [PubMed]

135. Yue, Y.; Zhang, M.; Zhang, J.; Duan, L.; Li, Z. SOS1 gene overexpression increased salt tolerance in transgenic tobacco by maintaining a higher K+/Na+ ratio. *J. Plant Physiol.* 2012, 169, 255–261. [CrossRef] [PubMed]

136. Mian, A.; Oomen, R.J.; Isayenkov, S.; Sentenac, H.; Maathuis, F.J.; Very, A.A. Over-expression of an Na+-and K+-permeable HKT transporter in barley improves salt tolerance. *Plant J.* 2011, 68, 468–479. [CrossRef]

137. Kim, S.-T.; Choi, J.; Bae, S.-J.; Kim, J.-S. The functional association of ACQOS/VICTR with salt stress resistance in *Arabidopsis thaliana* was confirmed by CRISPR-Mediated mutagenesis. *Int. J. Mol. Sci.* 2021, 22, 11389. [CrossRef]

138. Marchin, R.M.; Backes, D.; Ossola, A.; Leishman, M.R.; Tjoelker, M.G.; Ellsworth, D.S. Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species. *Glob. Chang. Biol.* 2022, 28, 1133–1146. [CrossRef]

139. Lippmann, R.; Babben, S.; Menger, A.; Delker, C.; Quint, M. Development of wild and cultivated plants under global warming conditions. *Curr. Biol.* 2019, 29, R1326–R1338. [CrossRef]

140. Banerjee, A.; Roychoudhury, A. Small heat shock proteins: Structural assembly and functional responses against heat stress in plants. In *Plant Metabolites and Regulation under Environmental Stress*; Ahmad, P., Ahanger, M.A., Singh, V.P., Tripathi, D.K., Alam, P., Alyemeni, M.N., Eds.; Elsevier: Amsterdam, The Netherlands, 2018; pp. 367–376.

141. Harvey, L.D. *Global Warming*. Forssk.: ex JF Gmel.: Structural characteristics and adaptations to salinity and drought: A review.

142. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 2018, 69, 3590. [CrossRef] [PubMed]

143. Ali, M.G.; Ahmed, M.; Ibrahim, M.M.; El Baroudy, A.A.; Ali, E.F.; Shokr, M.S.; Majrashi, A.; Kheir, A. Optimizing sowing window, cultivar choice, and plant density to boost maize yield under RCP8.5 climate scenario of CMIP5. *Crit. Rev. Environ. Sci. Technol.* 2019, 49, 458.

144. Haider, S.; Raza, M.A.; Kheir, A.; Ahmed, M.; Kakar, K.M.; Ahmad, S. Modelling Climate Uncertainty and Adaptations for Soybean-Based Cropping System. *Int. J. Plant Prod.* 2022, 16, 235–250. [CrossRef]

145. Ali, M.G.; Ahmed, M.; Ibrahim, M.M.; El Baroudy, A.A.; Ali, E.F.; Shokr, M.S.; Aldosari, A.A.; Majrashi, A.; Kheir, A. Optimizing sowing window, cultivar choice, and plant density to boost maize yield under RCP8.5 climate scenario of CMIP5. *Int. J. Biometeorol.* 2022, 66, 971–985. [CrossRef]

146. Haider, S.; Raza, A.; Iqbal, J.; Shaukt, M.; Mahmood, T. Analyzing the regulatory role of heat shock transcription factors in plant heat stress tolerance: A brief appraisal. *Mol. Biol. Rep.* 2022, 49, 5771–5785. [CrossRef]

147. dos Santos, T.B.; Ribas, A.F.; de Souza, S.G.H.; Budzinski, I.G.F.; Domingues, D.S. Physiological Responses to Drought, Salinity, and Heat Stress in Plants: A Review. *Stress Physiology* 2022, 2, 113–135. [CrossRef]

148. Naik, K.; Mishra, S.; Srichandan, H.; Singh, P.K.; Sarangi, P.K. Plant growth promoting microbes: Potential link to sustainable agriculture and environment. *Biocatal. Agric. Biotechnol.* 2019, 21, 101326. [CrossRef]

149. Brestic, M.; Zivcak, M.; Hauptvogel, P.; Misheva, S.; Kocheva, K.; Yang, X.; Li, X.; Allakhverdiev, S.I. Wheat plant selection for high yields entailed improvement of leaf anatomical and biochemical traits including tolerance to non-optimal temperature conditions. *Photosynth. Res.* 2018, 136, 245–255. [CrossRef] [PubMed]
148. Maheswari, M.; Sarkar, B.; Vanaja, M.; Rao, M.S.; Rao, C.S.; Venkateswarlu, B.; Sikka, A. Climate Resilient Crop Varieties for Sustainable Food Production under Aberrant Weather Conditions; ICAR-Central Research Institute for Dryland Agriculture: Hyderabad, India, 2015.

149. Faizan, M.; Yu, F.; Rajput, V.D.; Minkina, T.; Hayat, S. Role of Brassinosteroids in Protein Folding Under High-Temperature Stress. In Brassinosteroids Signalling; Springer: Berlin/Heidelberg, Germany, 2022; pp. 259–268.

150. Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int. J. Mol. Sci. 2013, 14, 9643–9684. [CrossRef] [PubMed]

151. Fahad, S.; Hussain, S.; Saud, S.; Khan, F.; Hassan, S.; Amanullah; Nasim, W.; Arif, M.; Wang, F.; Huang, J. Exogenously Applied Plant Growth Regulators Affect Heat-Stressed Rice Pollens. J. Agron. Crop Sci. 2016, 202, 139–150. [CrossRef]

152. Thakur, M.P.; van Der Putten, W.H.; Apon, F.; Angelini, E.; Vreš, B.; Geisen, S. Resilience of rhizosphere microbial predators and their prey communities after an extreme heat event. Funct. Ecol. 2021, 35, 216–225. [CrossRef]

153. Hassan, M.U.; Chattha, M.U.; Khan, I.; Chattha, M.B.; Barbanti, L.; Aamer, M.; Iqbal, M.M.; Nawaz, M.; Mahmood, A.; Ali, A. Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—A review. Plant Biosyst. 2021, 155, 211–234. [CrossRef]

154. Trivedi, P.; Batista, B.D.; Bazany, K.E.; Singh, B.K. Plant–microbiome interactions under a changing world: Responses, consequences and perspectives. New Phytol. 2022, 234, 1951–1959. [CrossRef]

155. Abdelrahman, M.; El-Sayed, M.; Jogaiah, S.; Burritt, D.J.; Tran, L.-S.P. The “STAY-GREEN” trait and phytohormone signaling networks in plants under heat stress. Plant Cell Rep. 2017, 36, 1009–1025. [CrossRef]

156. Hu, Z.; Li, J.; Ding, S.; Cheng, F.; Li, X.; Jiang, Y.; Yu, J.; Foyer, C.H.; Shi, K. The protein kinase CPK28 phosphorylates ascorbate peroxidase and enhances thermotolerance in tomato. Plant Physiol. 2021, 186, 1302–1317. [CrossRef]

157. Kagale, S.; Divi, U.K.; Krochko, J.E.; Keller, W.A.; Krishna, P. Brassinosteroid confers tolerance in Arabidopsis thaliana and Brassica napus to a range of abiotic stresses. Plantas 2007, 225, 353–364. [CrossRef] [PubMed]

158. Yin, Y.; Qin, K.; Song, X.; Zhang, Q.; Zhou, Y.; Xia, X.; Yu, J. BZR1 transcription factor regulates heat stress tolerance through FERONIA receptor-like kinase-mediated reactive oxygen species signaling in tomato. Plant Cell Physiol. 2018, 59, 2239–2254. [CrossRef] [PubMed]

159. Wang, B.; Zhong, Z.; Wang, X.; Han, Y.; Yu, D.; Wang, C.; Song, W.; Zheng, X.; Chen, C.; Zhang, Y. Knockout of the OsNAC006 transcription factor causes drought and heat sensitivity in rice. Int. J. Mol. Sci. 2020, 21, 2288. [CrossRef] [PubMed]

160. Legris, M.; Kloes, C.; Burgie, E.S.; Rojas, C.C.R.; Neme, M.; Hilbrunner, A.; Wigge, P.A.; Schäfer, E.; Vierstra, R.D.; Casal, J.J. Phytochrome B integrates light and temperature signals in Arabidopsis. Science 2016, 354, 897–900. [CrossRef] [PubMed]

161. Jung, J.-H.; Domijan, M.; Kloes, C.; Biswas, S.; Ezer, D.; Gao, M.; Khattak, A.K.; Box, M.S.; Charoensawan, V.; Cortijo, S. Phytochromes function as thermosensors in Arabidopsis. Science 2016, 354, 886–889. [CrossRef] [PubMed]

162. Arico, D.; Legris, M.; Castro, L.; Garcia, C.F.; Laino, A.; Casal, J.J.; Mazzella, M.A. Neighbour signals perceived by phytochrome B increase thermotolerance in Arabidopsis. Plant Cell Environ. 2019, 42, 2554–2566. [CrossRef]

163. Abdellatif, I.M.; Yuan, S.; Na, R.; Yoshihara, S.; Hamada, H.; Suzuki, T.; Ezura, H.; Miura, K. Functional characterization of tomato procera mutants. Int. J. Mol. Sci. 2017, 18, 9643–9684. [CrossRef] [PubMed]

164. Arico, D.; Legris, M.; Castro, L.; Garcia, C.F.; Laino, A.; Casal, J.J.; Mazzella, M.A. Neighbour signals perceived by phytochrome B increase thermotolerance in Arabidopsis. Plant Cell Environ. 2019, 42, 2554–2566. [CrossRef]

165. Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int. J. Mol. Sci. 2013, 14, 9643–9684. [CrossRef] [PubMed]

166. Kashojiya, S.; Lu, Y.; Takayama, M.; Komatsu, H.; Minh, L.H.T.; Nishida, K.; Shirasawa, K.; Miura, K.; Nonaka, S.; Masuda, J.-i. Functional characterization of tomato procera mutants. Int. J. Mol. Sci. 2017, 18, 9643–9684. [CrossRef] [PubMed]

167. Hassani, M.U.; Chattha, M.U.; Khan, I.; Chattha, M.B.; Barbanti, L.; Aamer, M.; Iqbal, M.M.; Nawaz, M.; Mahmood, A.; Ali, A. Heat stress in cultivated plants: Nature, impact, mechanisms, and mitigation strategies—A review. Plant Biosyst. 2021, 155, 211–234. [CrossRef]

168. Trivedi, P.; Batista, B.D.; Bazany, K.E.; Singh, B.K. Plant–microbiome interactions under a changing world: Responses, consequences and perspectives. New Phytol. 2022, 234, 1951–1959. [CrossRef]

169. Abdelrahman, M.; El-Sayed, M.; Jogaiah, S.; Burritt, D.J.; Tran, L.-S.P. The “STAY-GREEN” trait and phytohormone signaling networks in plants under heat stress. Plant Cell Rep. 2017, 36, 1009–1025. [CrossRef]

170. Hu, Z.; Li, J.; Ding, S.; Cheng, F.; Li, X.; Jiang, Y.; Yu, J.; Foyer, C.H.; Shi, K. The protein kinase CPK28 phosphorylates ascorbate peroxidase and enhances thermotolerance in tomato. Plant Physiol. 2021, 186, 1302–1317. [CrossRef]

171. Kagale, S.; Divi, U.K.; Krochko, J.E.; Keller, W.A.; Krishna, P. Brassinosteroid confers tolerance in Arabidopsis thaliana and Brassica napus to a range of abiotic stresses. Plantas 2007, 225, 353–364. [CrossRef] [PubMed]

172. Yin, Y.; Qin, K.; Song, X.; Zhang, Q.; Zhou, Y.; Xia, X.; Yu, J. BZR1 transcription factor regulates heat stress tolerance through FERONIA receptor-like kinase-mediated reactive oxygen species signaling in tomato. Plant Cell Physiol. 2018, 59, 2239–2254. [CrossRef] [PubMed]

173. Wang, B.; Zhong, Z.; Wang, X.; Han, Y.; Yu, D.; Wang, C.; Song, W.; Zheng, X.; Chen, C.; Zhang, Y. Knockout of the OsNAC006 transcription factor causes drought and heat sensitivity in rice. Int. J. Mol. Sci. 2020, 21, 2288. [CrossRef] [PubMed]

174. Arico, D.; Legris, M.; Castro, L.; Garcia, C.F.; Laino, A.; Casal, J.J.; Mazzella, M.A. Neighbour signals perceived by phytochrome B increase thermotolerance in Arabidopsis. Plant Cell Environ. 2019, 42, 2554–2566. [CrossRef]

175. Abdellatif, I.M.; Yuan, S.; Na, R.; Yoshihara, S.; Hamada, H.; Suzuki, T.; Ezura, H.; Miura, K. Functional characterization of tomato procera mutants. Int. J. Mol. Sci. 2017, 18, 9643–9684. [CrossRef] [PubMed]
175. Wang, T.-Y.; Wu, J.-R.; Duong, N.K.T.; Lu, C.-A.; Yeh, C.-H.; Wu, S.-J. HSP70-4 and farnesylated Atf3 constitute a specific HSP70/HSP40-based chaperone machinery essential for prolonged heat stress tolerance in Arabidopsis. *J. Plant Physiol.* 2021, 261, 153430. [CrossRef]

176. FAO. *Responding to the Impact of the COVID-19 Outbreak on Food Value Chains through Efficient Logistics*; FAO: Rome, Italy, 2020.

177. Carol, K. Reconstructing patterns of temperature, phenology, and frost damage over 124 years. *Ecology* 2013, 94, 41–50. [CrossRef]

178. Shi, Y.; Ding, Y.; Yang, S. Molecular regulation of CBF signaling in cold acclimation. *Trends Plant Sci.* 2018, 23, 623–637. [CrossRef] [PubMed]

179. Liu, Y.; Zhou, J. MAPping kinase regulation of ICE1 in freezing tolerance. *Trends Plant Sci.* 2018, 23, 91–93. [CrossRef] [PubMed]

180. Guo, X.; Liu, D.; Chong, K. Cold signaling in plants: Insights into mechanisms and regulation. *J. Integr. Plant Biol.* 2018, 60, 745–756. [CrossRef] [PubMed]

181. Yadav, S. Cold stress tolerance mechanisms in plants. A review. *Agron. Sustain. Dev.* 2010, 30, 515–527. [CrossRef]

182. Adams, W.W., III; Stewart, J.J.; Cohu, C.M.; Muller, O.; Demmig-Adams, B. Habitat temperature and precipitation of Arabidopsis thaliana ecotypes determine the response of foliar vasculature, photosynthesis, and transpiration to growth temperature. *Front. Plant Sci.* 2016, 7, 1026. [CrossRef]

183. Muller, O.; Stewart, J.J.; Cohu, C.M.; Polutchno, S.K.; Demmig-Adams, B.; Adams, W.W., III. Leaf architectural, vascular and photosynthetic acclimation to temperature in two biennials. *Physiol. Plant* 2014, 152, 763–772. [CrossRef]

184. Hassan, M.A.; Xiang, C.; Farooq, M.; Muhammad, N.; Yan, Z.; Hui, X.; Yuanyuan, K.; Bravo, A.K.; Lele, Z.; Jincal, L. Cold stress in wheat: Plant acclimation responses and management strategies. *Front. Plant Sci.* 2016, 171, 2744–2759. [CrossRef] [PubMed]

185. Venegas-Rioseco, J.; Ginocchio, R.; Ortiz-Calderón, C. Increase in Phytoextraction Potential by Genome Editing and Transformation: A Review. *Plants* 2021, 10, 86. [CrossRef]

186. Basharat, Z.; Novo, L.A.; Yasmin, A. Genome editing wedrs CRISPR: What is in it for phytoremediation? *Plants* 2018, 7, 51. [CrossRef]

187. Auguy, F.; Fahr, M.; Moulin, P.; El Mzibri, M.; Smouni, A.; Filali-Maltouf, A.; Béna, G.; Doumas, P. Transcriptome changes in *Hirschfeldia incana* in response to lead exposure. *Front. Plant Sci.* 2016, 6, 1231. [CrossRef]

188. Biskr, M.; Béa, B.; Masson, P.; Jolivet, C.; Faure, J.; Dumas, P. Overexpression of the copper tolerance protein Ccbf1p in tobacco plants enhances copper tolerance and accumulation in root cytoplasm and decreases hydrogen peroxide production. *J. Hazard. Mater.* 2012, 189, 65–71. [CrossRef] [PubMed]

189. LeDuc, D.L.; AbdelSamie, M.; Môntes-Bayon, M.; Wu, C.P.; Reisinger, S.J.; Terry, N. Overexpressing both ATP sulfurylase and selenocysteine methyltransferase enhances selenium phytoremediation traits in Indian mustard. *Environ. Pollut.* 2006, 144, 70–76. [CrossRef] [PubMed]
202. Lv, Y.; Deng, X.; Quan, L.; Xia, Y.; Shen, Z. Metallothioneins BcMT1 and BcMT2 from *Brassica campestris* enhance tolerance to cadmium and copper and decrease production of reactive oxygen species in *Arabidopsis thaliana*. *Plant Soil* 2013, 367, 507–519. [CrossRef]

203. Shahid, M.; Khalid, S.; Abbas, G.; Shahid, N.; Nadeem, M.; Sabir, M.; Aslam, M.; Durnat, C. Heavy metal stress and crop productivity. In *Crop Production and Global Environmental Issues*; Springer: Berlin/Heidelberg, Germany, 2015; pp. 1–25.

204. Hossain, M.A.; Piyatida, P.; da Silva, J.A.T.; Fujita, M. Molecular mechanism of heavy metal toxicity and tolerance in plants: Central role of glutathione in detoxification of reactive oxygen species and methylglyoxal and in heavy metal chelation. *J. Bot.* 2012, 2012, 872875. [CrossRef]

205. Ghori, N.-H.; Ghori, T.; Hayat, M.; Imadi, S.; Gul, A.; Altay, V.; Ozturk, M. Heavy metal stress and responses in plants. *Int. J. Environ. Sci. Technol. (Tehran)* 2019, 16, 1807–1828. [CrossRef]

206. Gage, K.L.; Krausz, R.F.; Walters, S.A. Emerging challenges for weed management in herbicide-resistant crops. *Agriculture* 2019, 9, 180. [CrossRef]

207. Green, J.M. Current state of herbicides in herbicide-resistant crops. *Pest Manag. Sci.* 2014, 70, 1351–1357. [CrossRef]

208. Hasanuzzaman, M.; Hakeem, K.R.; Nahar, K.; Alharby, H.F. *Plant Abiotic Stress Tolerance: Agronomic, Molecular and Biotechnological Approaches*; Springer: Berlin/Heidelberg, Germany, 2019.

209. Baeg, G.-J.; Kim, S.-H.; Choi, D.-M.; Tripathi, S.; Han, Y.-J.; Kim, J.-I. CRISPR/Cas9-mediated mutation of 5-oxoprolinase gene confers resistance to sulfonamide compounds in *Arabidopsis*. *Plant Biotechnol. Rep.* 2021, 15, 753–764. [CrossRef]

210. Chen, J.; Zou, W.; Meng, L.; Fan, X.; Xu, G.; Ye, G. Advances in the uptake and transport mechanisms and QTLs mapping of cadmium in rice. *Int. J. Mol. Sci.* 2019, 20, 3417. [CrossRef]

211. Mao, Y.; Botella, J.R.; Liu, Y.; Zhu, J.-K. Gene editing in plants: Progress and challenges. *Nat. Sci. Rev.* 2019, 6, 421–437. [CrossRef] [PubMed]

212. Dong, H.; Huang, Y.; Wang, K. The development of herbicide resistance crop plants using CRISPR/Cas9-mediated gene editing. *Genes* 2021, 12, 912. [CrossRef] [PubMed]

213. Toda, E.; Okamoto, T. CRISPR/Cas9-Based Genome Editing Using Rice Zygotes. *Curr. Protoc. Plant Biol.* 2020, 5, e20111. [CrossRef] [PubMed]

214. Yang, S.H.; Kim, E.; Park, H.; Koo, Y. Selection of the high efficient sgRNA for CRISPR-Cas9 to edit herbicide related genes, PDS, ALS, and EPSPS in tomato. *Appl. Biol. Chem.* 2022, 65, 13. [CrossRef]

215. Jogam, P.; Sandhya, D.; Alok, A.; Peddaboina, V.; Allini, V.R.; Zhang, B. A review on CRISPR/Cas-based epigenetic regulation in plants. *Int. J. Biol. Macromol.* 2022, 219, 1261–1271. [CrossRef]

216. Li, C.; Brant, E.; Budak, H.; Zhang, B. CRISPR/Cas: A Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement. *J. Zhejiang Univ. Sci. B* 2021, 22, 253–284. [CrossRef]