

Review

Modern Biotechnologies: Innovative and Sustainable Approaches for the Improvement of Sugarcane Tolerance to Environmental Stresses

Rubab Shabbir ^{1,2,3,4} , Talha Javed ^{2,3,4} , Irfan Afzal ⁴ , Ayman El Sabagh ^{5,6}, Ahmad Ali ^{2,3}, Oscar Vicente ^{7,*}  and Pinghua Chen ^{1,2,*}

- ¹ Key Laboratory of Ministry of Education for Genetics, Breeding and Multiple Utilization of Crops, College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China; rubabshabbir28@gmail.com
 - ² National Engineering Research Center for Sugarcane, Fujian Agriculture and Forestry University, Fuzhou 350002, China; mtahaj@fafu.edu.cn (T.J.); ahmad03348454473@yahoo.com (A.A.)
 - ³ College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, China
 - ⁴ Seed Physiology Lab, Department of Agronomy, University of Agriculture, Faisalabad 38040, Pakistan; iafzal@uaf.edu.pk
 - ⁵ Department of Agronomy, Faculty of Agriculture, University of Kafrelsheikh, Kafrelsheikh 33516, Egypt; ay-man.elsabagh@agr.kfs.edu.eg
 - ⁶ Department of Field Crops, Faculty of Agriculture, Siirt University, Siirt 56100, Turkey
 - ⁷ Institute for the Conservation and Improvement of Valencian Agrodiversity (COMAV), Universitat Politècnica de València, Camino de Vera s/n, 46022 Valencia, Spain
- * Correspondence: ovcicente@upvnet.upv.es (O.V.); 000q010027@fafu.edu.cn (C.P.)



Citation: Shabbir, R.; Javed, T.; Afzal, I.; Sabagh, A.E.; Ali, A.; Vicente, O.; Chen, P. Modern Biotechnologies: Innovative and Sustainable Approaches for the Improvement of Sugarcane Tolerance to Environmental Stresses. *Agronomy* **2021**, *11*, 1042. <https://doi.org/10.3390/agronomy11061042>

Academic Editor: Samir C. Debnath

Received: 27 April 2021

Accepted: 20 May 2021

Published: 22 May 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Abstract: Sugarcane (*Saccharum* spp.) is one of the most important industrial cash crops, contributing to the world sugar industry and biofuel production. It has been cultivated and improved from prehistoric times through natural selection and conventional breeding and, more recently, using the modern tools of genetic engineering and biotechnology. However, the heterogeneity, complex polyan euploid genome and susceptibility of sugarcane to different biotic and abiotic stresses represent impediments that require us to pay greater attention to the improvement of the sugarcane crop. Compared to traditional breeding, recent advances in breeding technologies (molecular marker-assisted breeding, sugarcane transformation, genome-editing and multiple omics technologies) can potentially improve sugarcane, especially against environmental stressors. This article will focus on efficient modern breeding technologies, which provide crucial clues for the engineering of sugarcane cultivars resistant to environmental stresses.

Keywords: *Saccharum* spp.; biotic and abiotic stresses; genetic engineering; stress tolerance

1. Introduction

Sugarcane (*Saccharum* spp.) is an important agricultural crop for various subtropical and tropical countries, such as Brazil, India, Thailand, China, Australia, Pakistan, Philippines, Cuba, Colombia, and the USA [1]. It can produce various industrially valuable products, such as sugar, waxes, biofuels, and bio-fibres [2,3]. Globally, about 75% of sucrose is obtained from sugarcane, whereas the remaining 25% is obtained from *Beta vulgaris* [4]. There are two wild (*S. robustum* and *S. spontaneum*) and four cultivated (*S. edule*, *S. barberi*, *S. sinense*, and *S. officinarum*) main species in the *Saccharum* complex. However, the current commercial sugarcane cultivars, which are allopolyploids of high ploidy level, contain a narrow genetic range due to breeding via popular cultivars of the early 1900s, e.g., NCo310, Co419, and POJ2878 [5], whereas more recent cultivars have been developed via interspecific hybridisation of *S. officinarum* and *S. spontaneum* [6]. These commercial varieties are selected from an intervarietal hybrid population through clonal selection. The mechanism of diploidised meiosis makes them responsive to further breeding through hybridisation, even at the ploidy level of 10× or more. Gene introgression to produce

intergeneric/interspecific hybrids with *Erianthus*, *Miscanthus*, *Narenga*, *Sclerostachya*, and *Sorghum* has been performed through intergeneric hybridisation. These sugarcane hybrids are fertile, vigorous, and could be maintained through vegetative propagation for various years; thus, they are termed perennial hybrids. Allopolyploid hybrids of *Saccharum*, due to their duplicated genomes, proved an ideal material for genetic modification research as compared to other seed-propagated annual plants. Transgenic protocols through *Agrobacterium*-mediated transformation and the biolistic approach have been used in sugarcane in the study of duplicated genes (mechanism of generating new genetic material during molecular evolution) [7]. Sugarcane can act as a model organism for studies on hybridisation, genome restructuring, chromosomal elimination, the effect of gene dosage, chromosomal bivalent pairing of higher polyploids, allelic variation, and so much more [8]. *Erianthus arundinaceus* presents a strong tolerance against abiotic stresses and could be widely used in modern breeding of sugarcane for producing cultivars with better stress tolerance, as well as enhanced sucrose content [9,10].

Considering the interest in rapidly improving sugarcane tolerance to stress under the current climate change scenario, conventional breeding programmes are time-consuming and ineffective [11]. Most importantly, several biotic and abiotic stresses can restrain the performance of sugarcane cultivars from emergence to harvesting [12]. Sugarcane's heterogeneous nature, its vegetative propagation through stem cuttings, and poly-aneuploid genome make the research advances quite complex [13]. High sucrose/cellulose, ethanol-based biofuel production, and resistance to biotic and abiotic stresses are the distinct components considered by breeders for sugarcane improvement [14,15]. Beyond the overall improvement in carbohydrate contents, other traits such as higher emergence potential, plant vigour, and agronomic parameters—tillering, plant height, stem diameter, flowering, and resistance to biotic and abiotic stresses—are of paramount importance for achieving the highest potential of the sugarcane production system [16,17].

In the last decade, sugarcane genetic transformation and multiple omics technologies have received increasing attention, as they can open novel and unique research pathways [18]. The advent of recombinant DNA technology holds great potential, incorporating one or more specific genes via genetic engineering, to control single or multiple traits [19]. More recently, genome-editing techniques have been developed to achieve genetic modifications in which a gene/DNA fragment is replaced, deleted, or inserted into an organism genome using nucleases: meganucleases, zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and, at present, mostly the CRISPR/Cas9 system. During the process, the nucleases cause double-strand breaks (DSBs) at specific DNA regions; finally, non-homologous end joining (NHEJ) or homologous recombination (HR) result in the restoration of the induced DSBs [16]. Molecular markers associated with specific loci of the genome could be used for the analysis and detection of various genotypes in a gene pool of sugarcane [20]. Omics technologies, such as genomics, transcriptomics, proteomics, and metabolomics reveal complex connections between metabolites, proteins, and genes, which could help understand the genetic regulation and molecular mechanisms controlling both yield and stress resistance [21]. However, the limited use of this omics knowledge in sugarcane improvement against environmental stresses is presently frustrating. On the contrary, the development of transgenic sugarcane plants with significantly higher yield potential and better adaptability to fluctuating environmental conditions is moving towards a remarkable success [22]. Nevertheless, a comprehensive statistical genetic analysis is still required to identify genes associated with the increased yield and resistance to biotic and abiotic stresses of sugarcane cultivars.

This review attempts to provide comprehensive information regarding sugarcane genetic improvement for developing cultivars possessing better survival capacity in response to biotic and abiotic stresses, highlighting the different molecular techniques that can be applied to achieve this goal.

2. Molecular Markers-Assisted Breeding (MAB)

2.1. Diverse Molecular Markers

The complex poly-aneuploid genome and narrow genetic base are the main limitations in breeding commercial varieties of sugarcane. Despite sugarcane nobilisation, little improvement has been achieved in enhancing sugar content [23]. Knowledge relevant to genetic diversity among elite cultivars is essential for further crop improvement and can be obtained through investigating morphological traits, pedigree analysis, or molecular markers [24]. Among these approaches, molecular markers have been widely used in the germplasm characterisation of *Saccharum* species. Besides, paternity analysis, phylogenetic relationship analysis, genetic mapping, QTL (quantitative trait loci) mapping, and MAS (molecular-assisted selection) have also received greater attention from researchers. Molecular markers and fragments/small regions/pieces of DNA sequence show polymorphism and reveal variations among different organisms (Figure 1). Different molecular markers, such as RAPDs (random amplified polymorphic DNA), RFLPs (restriction fragment length polymorphisms), AFLPs (amplified fragment length polymorphisms), SNPs (single nucleotide polymorphisms), TRAPs (target region amplification polymorphisms), SSRs (simple sequence repeats), and ISSRs (inter simple sequence repeats) have been widely used in the genetic study of sugarcane [20,25,26].

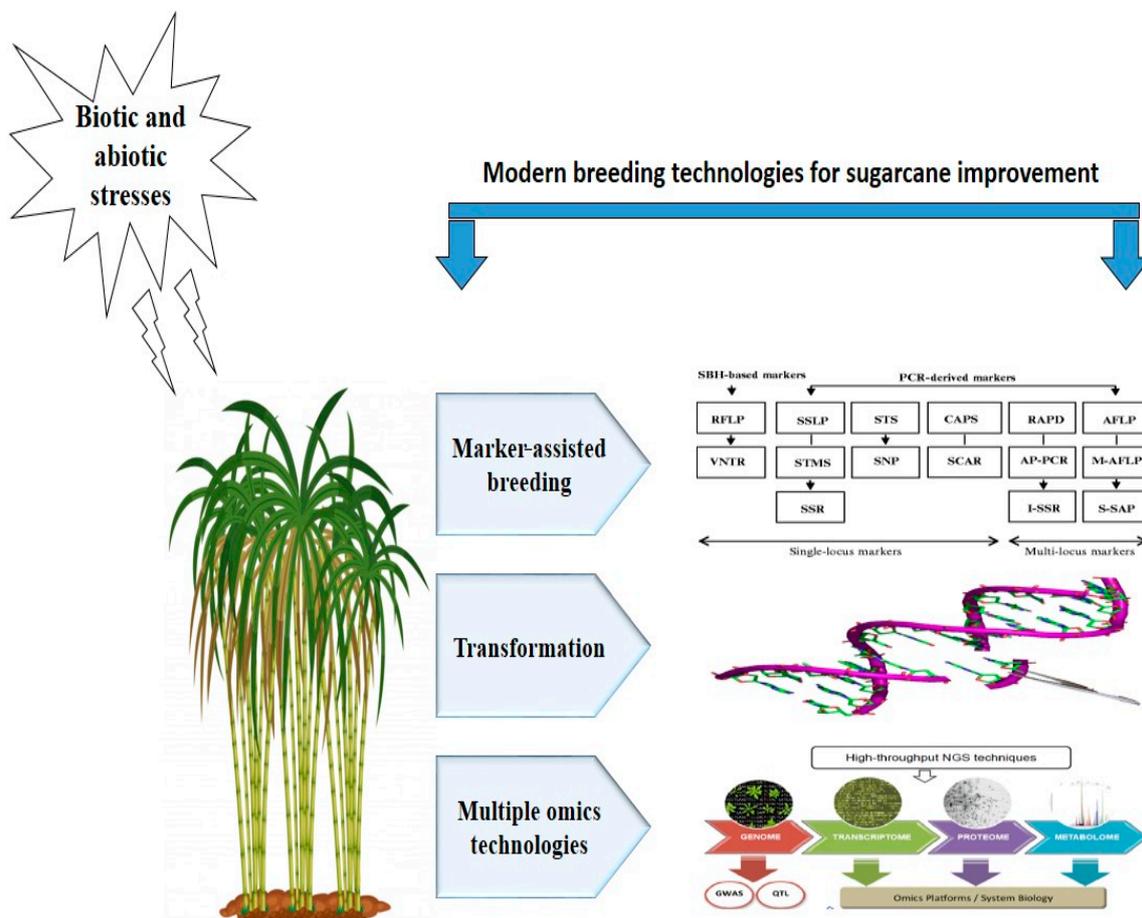


Figure 1. Modern breeding technologies for sugarcane genetic improvement against environmental stresses.

Genetic diversity in sugarcane germplasm was successfully identified using microsatellite DNA markers based on phenotypic traits and pedigree records [27]. Genetic diversity and phylogenetic relationship assessment by DNA markers can be crucial in germplasm classification and selecting breeding methods in sugarcane [28]. Molecular markers such as AFLP, SSR, and TRAPs have been successfully used in sugarcane to evaluate the genetic variations on a molecular level without the influence of environmental factors [29].

Genetic diversity analysis of 1002 accessions of sugarcane and related grasses, using 36 SSR markers, revealed the identification of 209 alleles associated with traits of agronomic importance [30]. Furthermore, 25 genotypes of sugarcane were also subjected to genetic diversity analysis through TRAP markers, which resulted in 70% genetic similarity between all accessions [31]. More recently, 150 of the most popular sugarcane accessions of China were successfully subjected to genetic diversity analysis with 21 fluorescence-labelled SSR markers and HPCE (high-performance capillary electrophoresis) for parental germplasm management [32]. Molecular markers help understand the genetic structures by identifying QTLs and developing genomic maps [33]. Incomplete genetic maps and linkages have been obtained using F1 individuals for *Saccharum* species such as *S. officinarum* [34], *S. spontaneum* [35], and a few modern sugarcane cultivars [36]. Genetic mapping of *S. officinarum* with 72 SSR and 40 AFLP primer pairs revealed 28 repulsion linkages [37]. A genetic mapping study of *S. officinarum* and *S. spontaneum* was carried out with AFLP, SRAP, and TRAP markers, which proved that AFLP is a better choice than SRAP and TRAP markers for sugarcane genetic mapping [24]. Whole-genome mapping of 183 sugarcane accessions was genotyped with 3327 DArT (diversity arrays technology), SSR, and AFLP markers to study 13 traits associated with morphology, the residual content of bagasse, resistance against diseases, and yield [38]. Phenotypic characterisation of 32 traits of 92 sugarcane varieties was performed using 174 SSR primers [39]. Linkage map construction and identification of genomic regions associated with traits of interest in the bi-parental population were carried out by QTL mapping [40].

2.2. Molecular Markers Related to Biotic Stresses

Genetic mapping of sugarcane is a challenge due to the presence of multiple alleles for a single gene. However, QTL mapping was proven to effectively discover various trait loci concerning yield [41] and disease resistance [42]. Several QTLs for disease resistance have been successfully identified in sugarcane, including 11 DNA markers for smut disease, five for resistance against fiji leaf gall, four for resistance against leaf scald and pachymetra root-rot [43], *Bru1* for resistance against brown rust [44], and 18 QTLs for resistance against yellow leaf virus [45]. QTL mapping in association with AFLP and SSR markers resulted in the identification of one QTL link for resistance against yellow leaf spot disease [46]. Similarly, another QTL mapping via AFLP and SSR markers identified one major QTL link against the sugarcane yellow leaf virus [47]. *Chilo sacchariphagus*, the spotted-stem borer (SSB), is one of the major sugarcane pests. To understand its genetic basis, QTL mapping was performed with AFLP, RFLP, and SSR markers and resulted in identifying nine QTLs, ranging in phenotypic variation from 6 to 10% [48]. Additionally, genome regions associated with sugarcane resistance against leaf scald disease were successfully identified by QTL mapping with SNPs, SSRs, and ISSRs [49].

2.3. Molecular Markers Related to Abiotic Stresses

Molecular markers have been utilised to select resistant genotypes against abiotic stresses, including drought, salinity, or cold, in major crops such as wheat, rice, sugarcane, maize, sorghum, and barley [50]. For example, AFLP, RAPD, and SSR markers have been widely used for improving the adaptability of sugarcane to abiotic stresses. Thus, RAPD markers analysis resulted in identifying molecular markers associated with resistance to drought and salinity [51,52]. Drought tolerance screening of 23 sugarcane genotypes was carried out using a SCAR marker generated from OPAK-12724 (RAPD DNA-sequence). This study identified 12 drought-tolerant genotypes, indicating that SCAR was 92% specific in the selection and occupied the same locus as OPAK-12724 [53]. Molecular selection in eight sugarcane genotypes (BOT-41, Co-775, Co-997, F-153, F-161, GT54-9, G84-47, and Sp80-32-80) regarding drought tolerance was also performed using RAPD, ISSR, and R-ISSR (combined RAPD and ISSR) markers. Among these, the use of R-ISSR was proved to be more efficient than the independent selection with RAPD or ISSR [54]. A further selection of these genotypes was carried out with AFLP primers by amplifying 886 amplicons with

6.2% polymorphism; this analysis established Co-997 as the most tolerant and Co-775 as the most susceptible to drought of the eight tested genotypes [55].

Sugarcane, being a glycophyte, exhibits growth reduction, nutritional imbalance, low-sprout emergence, low sugar content, and low productivity under salt stress [56]. Molecular markers can be used to track genetic loci related to salt resistance, avoiding time-consuming phenotypic measurements. Indeed, several PCR-based markers have been employed to assess the genetic diversity of sugarcane cultivars regarding their relative salt tolerance. For example, *in vitro* mutagenesis and RAPD markers were used for the selection and molecular characterisation of salt-tolerant lines in *S. officinarum*; RAPD markers indicated the genetic polymorphism in the control and salt-tolerant lines [57]. Screening of salt-tolerant and susceptible lines in sugarcane was also conducted with 15 ISSR markers, based on the similarity index among cultivars. This study proved that the ISSR markers effectively assessed the genetic diversity of sugarcane cultivars [58]. Characterisation between parents and mutant lines (drought and salt-tolerant) was carried out with RAPD markers under tissue culture selection pressure. Sugarcane embryonic calli treated with ethyl-methane sulphonate (EMS) were grown under drought and salt stress conditions, and tolerant lines were separated from the controls, based on the polymorphisms revealed by the RAPD profiles [59]. Similarly, *in vitro* molecular profiling of sugarcane variants was carried out using RAPD markers under water deficit and salt stress treatments [60]. Furthermore, molecular characterisation of 18 sugarcane genotypes under salt stress was undertaken using five TRAP markers. A similarity coefficient of 0.72 was observed, with 25 to 100% polymorphisms, which revealed a limited variation between the tested genotypes under salt stress [61]. Overall, molecular markers are powerful tools for identifying phenotypic and genetic diversity and provide a platform for introducing new desirable traits in sugarcane against abiotic and biotic stresses.

3. Sugarcane Genetic Transformation

3.1. Transformation Approaches

Different physical and biological transformation methods, with varying success rates, have been applied in sugarcane, such as electroporation, particle bombardment, PEG, and *Agrobacterium*-mediated gene transfer. Particle bombardment and *Agrobacterium*-mediated gene transformation are the most common methods applied in sugarcane due to a higher transformation efficiency [62]. Over the past several years, biotechnologists found particle bombardment a more helpful tool for direct gene transfer over a broad range of cells and tissues [63]. In the case of sugarcane, due to its heterozygosity and long generation time during vegetative propagation, bio-ballistics—commonly known as the DNA-gun method—represents a more reliable tool for gene transfer [64]. Sugarcane cultivars amenable to obtain embryonic callus cultures have been transformed by ballistic bombardment. The first transgenic sugarcane plants regenerated from embryogenic calli, transformed with the *npt-II* gene under the control of the Emu promoter, were successfully produced in Australia and required higher-velocity DNA bombardment compared to suspension culture cells [65]. On the contrary, the development of transgenic non-chimeric plants via direct bombardment to meristems or other tissues might be inefficient or non-productive [66].

Agrobacterium tumefaciens is a Gram-negative soil bacterium that can transfer a copy of the T-DNA sequences, present in its tumour-inducing (Ti) plasmid, to plant cells, where they are integrated into the plant genome [67]. The natural DNA transfer system has been adapted to use *A. tumefaciens* as a vector for gene transfer in plants [68]. *Agrobacterium*-mediated gene transfer can be efficiently accomplished in sugarcane using improved vector and promoters, signal molecules, meristematic tissues, and super-virulent strains [69]. In sugarcane, this transformation method benefits from numerous technical advances, such as the availability of a reliable and stable *in vitro* plant regeneration system, high virulent strains, chemicals that activate the *vir* genes, and reduced necrosis resulting from *Agrobacterium* callus browning [70]. In sugarcane, stem cuttings are also used for vege-

tative propagation of crops due to its highly heterogeneous nature [71]. Protocols have been standardised for in vitro production, micro-propagation, and tissue culture of sugarcane through shoot tip, meristem, or callus cultures. A decrease in the dispersal of seed cane-related diseases (e.g., leaf gall, mosaic virus, or leaf scald) has been observed after micro-propagation. Furthermore, seed cane developed by tissue culture performs better in sprouting, growth, and sugar and cane yield, as compared to production through conventional breeding [72,73].

3.2. Genome Editing

Genetic modification through DNA insertion/deletion in an organism is known as genome editing (GE). Four nuclease families can be used for genome editing, namely, meganucleases, transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease (CRISPR/Cas9 technologies) [74]. CRISPR/Cas9 is the adaptive immunity of prokaryotes against invading genomes or viral predators, established by memorising the previous infections and integrating the spacers (short sequences of the invading genomes) within the CRISPR locus. Integrated complexes, termed crRNAs (CRISPR RNAs), are then used by Cas nucleases for targeting invader sequences in case of reinfection. This ability of CRISPR/CAS9 to target the DNA sequences through programmed RNAs opened new approaches in genome editing [75]. Although CRISPR is, by far, the genome-editing method most used at present, TALEN-mediated GE has been proved successful in sugarcane to increase bioethanol production [76].

With a genome size of approximately 10 GB and 8 to 12 homologous gene copies, sugarcane is a classic example of a complex polyploid crop that faces several difficulties regarding genome editing [77]. A drawback in sugarcane molecular improvement is transgene-silencing at both transcription and post-transcriptional levels [78]. Efficient promoters can regulate specific GE tools, e.g., Cas9. Not unlike other GE nucleases, Cas9 may also have off-target effects, resulting in unwanted mutations. The gRNA–Cas9 complexes, in addition to cutting target DNA, can cleave off-target DNA sequences [79]. However, selecting specific promoters with truncated gRNA–Cas9 complexes can provide high mutation rates with minimum off-target effects [80]. GE can be further improved using modified variants of Cas9 to solve off-target cleaving issues [81]. Overall, although several challenges still need to be faced, the use of different Cas9 variants and other CRISPR-associated nucleases can be soon a powerful tool to enable successful GE in sugarcane and other polyploid crops.

3.3. Transformation Approaches against Biotic Stresses

Plant genetic engineering can enhance crop productivity and resistance to various biotic and abiotic stresses and, therefore, can be utilised for genetic improvement of sugarcane [82,83]. Over the last decades, rapid developments in genetic modification methods allowed the direct manipulation of the genetic makeup of an organism for the sake of introducing desirable characteristics [84]. Fundamental advances have been made in plant molecular biology and genetic engineering, bringing scientists to the efficient dissection and cloning of genes [85]. Cry genes such as *cry1A* [86], *cry1Ab* [87], *cry1Ac* [88], and *cry1Aa3* [89] from *Bacillus thuringiensis* have been used for the genetic modification of sugarcane to enhance its resistance to borers.

Herbicide resistance is known as a prominent example of a trait introduced by genetic transformation. Sugarcane plants transformed with the Bt insecticidal gene *cry1Ab*, the glyphosate-tolerant gene *EPSPS*, and the selection marker gene *PMI* showed strong herbicide tolerance under both laboratory and field conditions [90]. Independent transgenic lines of herbicide-resistant, fertile transgenic sugarcane plants, with high transformation efficiency and up-regulated *bar* transgene expression were successfully developed using the DNA-gun [91]. Herbicide-resistant sugarcane plants with normal physiological and morphological functions have also been obtained successfully using *Agrobacterium*-mediated

transformation. In fact, *Agrobacterium* transformation was first employed in sugarcane to develop herbicide-resistant sugarcane plants, in which the *Pga492* binary vector contained the *neomycin phosphotransferase-II*, *bar*, and β -glucuronidase (GUS) genes inserted between the T-DNA borders [92]. Several similar experiments have been reported, also describing the *Agrobacterium*-mediated transformation of sugarcane cultivars to obtain herbicide-resistant plants [93,94].

3.4. Transformation Approaches against Abiotic Stresses

Abiotic stresses can disturb the growth, development, and metabolism of a plant [82]. There are various abiotic stress conditions that plants must face in a field, but low temperature, drought, and salinity are the main environmental stressful factors negatively influencing crop performance [95]. Molecular studies have revealed that, to withstand these stresses, plants trigger various defence mechanisms, mediated by the activation through different pathways of the synthesis of specific proteins, such as protease inhibitors, antioxidant enzymes, late-embryogenesis abundant (LEA) proteins, and transcription factors (WRKY, MYB, AP2/ERF, and NAC) [96,97].

Drought is one of the main concerns of sugarcane growers, as it constrains the growth and productivity of the crop. Sugarcane, being a delta crop, needs heavy irrigation; therefore, only drought-tolerant varieties can grow in areas with limited water supply [98]. Numerous studies have been conducted to increase the resistance of sugarcane to drought stress, for example, by the transfer of the Trehalose synthase (*TSase*) and the vacuolar-pyrophosphatase (*AVP1*) genes to sugarcane apical meristem tissues [99]. In sugarcane, genes expressed under abiotic stresses were identified through expression profiling. A wild-type cultivar (Q117) was grown under drought, cold, and salinity conditions, and changes in gene expression were evaluated. Significant variation was observed in four genes encoding stress-related proteins, namely, late-embryogenesis abundant protein-3 (LEA3), early responsive dehydration protein-4 (ERD4), pyrroline-5-carboxylase synthase (P5CS), and galactinol synthase (GolS). These stress-induced genes showed up-regulation in response to specific stresses: ERD4 under drought and LEA4 under cold, whereas P5CS and GolS were strongly induced by salt stress. Further up-regulation of P5CS and ERD4 was achieved by transforming Q117 with the *Arabidopsis CBF4* gene; however, no such results were reported in transgenic plants in the case of GolS and LEA3 [100]. An R2R3-MYB sugarcane transcription factor (TF) gene '*ScMYB2*' and its alternatively spliced forms (*ScMYB2S1* and *ScMYB2S2*) were observed to play a positive role in drought tolerance mechanisms through the ABA signalling pathway [101]. Over-expression of *EaDREB2* (*DREB2* gene from *E. arundinaceus*) and pyramiding with *PDH45* (a pea DNA helicase gene) can enhance the salinity and drought tolerance in transgenic sugarcane cultivars [102]. Similarly, the transformation of sugarcane plants with the *PDH45* gene under the control of the *PortUbi2.3* promoter was also shown to be effective against drought and salinity stresses [103].

Enhanced proline accumulation was reported under drought [104] and salt stresses [105] in sugarcane plants, in agreement with the positive role of proline accumulation in the mechanisms of abiotic stress tolerance established in many other species, e.g., soybean, onion, or rice [106,107]. Transformation of sugarcane plants with the *P5C5* gene from *Vigna aconitifolia* L. showed a 25% increase in proline content and better tolerance to salt stress than non-transgenic control plants [108]. Similarly, sugarcane transformation with the *PDH45* gene, under the control of the *Port Ubi2.3* promoter, was effective against water deficit and salinity stresses [92]. Additionally, an increase in water stress tolerance was observed in sugarcane plants transformed with the *Arabidopsis Bax inhibitor-1* (*AtBI-1*) gene [109]. Under cold stress, several differentially expressed genes were observed in a clone of *S. spontaneum* (IND00-1037), such as the cold-responsive gene *CBF6*, associated with cold sensing, genes of phytochrome signalling, MAP kinases, lignin and pectin biosynthesis, soluble sugar, lipid, and calcium signalling pathways [110]. Metabolic (ethylene and Ca^+) pathways were investigated, searching for the activation of genes increasing the tolerance of sugarcane to potassium deficiency [111]. Overall, one of the most relevant

goals of sugarcane transformation is enhancing crop fitness against biotic (insect pests, viruses, and bacteria) and abiotic (temperature, salinity, metal toxicity, and drought) stress, which is challenging to accomplish without the use of modern breeding technologies.

4. Multiple Omics Technologies

4.1. Sugarcane Genomics

Genome sequencing of modern sugarcane cultivars is a far cry from completion due to its heterozygosity, high polyploidy level, and repetitive content of the homologous/homeologous chromosomes [112]. Modern sugarcane (*Saccharum* spp.) cultivars originated from two progenitors of different genome size, *S. officinarum* (930 Mb) and *S. spontaneum* (750 Mb) [113]. Progress on sugarcane genome sequencing has been achieved through de novo assembly using both short- and long-read techniques [37], BAC (bacterial artificial chromosome) sequencing [114], studies on gene remodelling [115], the functional transcriptomic approach [21], and genome-wide association studies (GWAS) [116].

The autopolyploid genome of *S. spontaneum* has been assembled using AP85-441 (haploid), which revealed that fission and translocation of two ancestral chromosomes resulted in four rearranged chromosomes, thereby resulting in a reduction in the number of chromosomes from 10 to 8. Surprisingly, in four rearranged chromosomes, almost 80% of the nucleotide-binding site-encoding genes were associated with disease resistance [117]. Genome sequencing of sugarcane based on BACs was carried out to exploit the co-linearity of sugarcane and sorghum. By referring to the sorghum genome, it was revealed that *S. spontaneum* and *S. officinarum* differ by their chromosomal rearrangements and transposable elements, which explains their distinct number of chromosomes, genome size, and their role in high polyploidisation and divergence. Based on whole-genome profiling, a minimal tilling path of the 4660 sugarcane-BAC was selected that covered the gene-rich part in the genome of sorghum. The selected BAC was sequenced and assembled in a higher quality single tilling path (382 Mb). As a result, almost 25,318 gene models (protein-coding) were predicted, out of which 17% showed no collinearity with the respective sorghum orthologs [112]. GWAS was used to identify 23 MTAs (marker-trait associations) associated with quantitative traits such as stalk number, stalk height, stalk weight, and cane yield and sugar contents, which were adequate for the identification of superior sugarcane cultivars [116]. The same approach also allowed identifying sugarcane cultivars resistant to yellow leaf virus and orange rust [118]. *Xanthomonas albilineans* is the causative agent of leaf scald bacterial disease of sugarcane in most countries. Genomic analysis can aid in understanding the genetic diversity of pathogens. Genome sequencing of Xa-FJ1 (a strain of *X. albilineans*) and a comparative genomic analysis of GPE-PC73 (another strain of *X. albilineans*) and Xa-FJ1 were carried out. The results revealed that 82 genes in GPE-PC73 and ten genes in Xa-FJ1 were associated with DNA methyltransferases, *Zonula occludens* toxin, and phage-related proteins, as well as homologous recombination, transposable elements, and prophage integration. CRISPR systems linked with 16 in/dels (insertions/deletions) were revealed between both strains under comparative genomic analysis [119]. The whole reference genome of sugarcane is not available for public use due to challenges of genome association. However, allelic variation and expression patterns of target regions can be carried out by analysing the deep-sequence data of target-enriched regions and RNA-seq data, respectively [120].

4.2. Sugarcane Transcriptomics

The transcriptomic analysis provides data on gene expression profiles, employing various techniques, such as expressed sequence tags (ESTs), probe hybridisation arrays, or known genes of allied crops. The Brazilian SUCEST (sugarcane EST) project by the ONSA Virtual Genomics Institute [121] represents a major achievement in the EST collection, with 237,965 ESTs collected from 37 cDNA libraries [122]. A total of 43,141 SASs (sugarcane-assembled sequences), encoding the putative transcripts, were organised from these ESTs through cluster analysis [123]. The Gene Index (3.0 version) of sugarcane

consists of 282,683 ESTs, which comprises 499 cDNA-sequences along with 121,342 uni-genes. However, there are approximately 10,000 coding genes of sugarcane that are still unidentified [124]. In addition to the ESTs, transcriptomics approaches have also been used to define and validate the expression patterns of sugarcane genes [125], e.g., identification of genes for developmental responses, quality and quantitative traits, associated with photosynthesis [126], leaf abscission [127], ripening [128], or lignin and cellulose biosynthesis [129].

Red rot caused by *Colletotrichum falcatum* is notorious for affecting sugarcane development and yield. Many studies have been conducted to understand the mechanism of *C. falcatum* invasion and the development of resistant sugarcane cultivars. These studies provided some essential information, such as the early induction of transcription factors (NAC, WRKY, MYB, TLP, and bZIP) that could be actively involved or coordinate the defence reactions against pathogen attack [130]. *Acidovorax avenae* subsp. *avenae* (*Aaa*) is the causative agent of red stripe disease in sugarcane. Under *Aaa* invasion, sugarcane transcriptomic analysis identified various differentially expressed genes (DEGs) involved in oxidative bursts, ethylene biosynthesis, and cell wall fortification [131]. Furthermore, transcriptome analysis of resistant (ROC22) and susceptible (MT11-610) sugarcane cultivars revealed the positive role of eight DEGs of sugarcane resistance to *Aaa* [132]. Previous literature extensively reported the participation of different genes in biotic stress resistance in plants; some examples are mentioned in Table 1.

Table 1. Biotic stresses and potential control in sugarcane.

Causal Agent	Disease	Main Findings	Reference
<i>Colletotrichum falcatum</i>	Red rot	Early induction of TFs (NAC, WRKY, MYB, TLP, and bZIP) can be actively involved or coordinate the defence against pathogen attack	[133]
<i>Colletotrichum falcatum</i>	Red rot	Clusters of ESTs expressed in resistant cultivars of sugarcane against red rot	[134]
<i>Fusarium verticillioides</i>	Pokkah boeng	A total of 1779 differentially expressed transcripts were identified under <i>F. verticillioides</i> stress. Among these, several DEGs were associated with pathogenicity	[135]
<i>Colletotrichum falcatum</i>	Red rot	Expression of genes to control pathogenesis in sugarcane	[136]
<i>Sporisorium scitamineum</i>	Smut	Activation of <i>MAPK</i> and <i>NBS-LRR</i> genes and signalling pathways of various hormones, e.g., auxins, ethylene, abscisic acid, and salicylic acid, in response to sugarcane smut	[137]
<i>Sporisorium scitamineum</i>	Smut	Differentially expressed genes (DEGs) involved in the biosynthesis of the cell wall, disease resistance, signal transduction, and phenylpropanoid pathway under disease stress	[138]
<i>Puccinia melanocephala</i>	Brown rust	Brown rust-responsive genes identification in Louisiana clone.	[139]
<i>Leifsonia xyli</i> subsp. <i>xyli</i>	Ratoon stunt	Identification of <i>NBS-LRR</i> , <i>ZFP</i> , and <i>PAL</i> genes responsive to <i>Lxx</i> infection.	[140]
<i>Leifsonia xyli</i> subsp. <i>xyli</i>	Ratoon stunt	Identification of 267 DEGs and 150 differentially expressed proteins (DEPs) involved in plant growth, signal transduction, hormone metabolism, and defence mechanisms.	[141]
<i>Fusarium verticillioides</i>	Pokkah boeng	DEGs were involved in phenylpropanoid biosynthesis, nitrogenous metabolism, and wax and cutin biosynthesis under disease stress.	[142]
<i>Xanthomonas albilineans</i> (<i>Xa</i>) <i>Acidovorax avenae</i> subsp. <i>avenae</i> (<i>Aaa</i>)	Leaf scald Red stripe	Induction of the stress-responsive genes <i>ShMAPK07</i> and <i>ShMAPKKK02</i> against <i>Xa</i> and <i>Aaa</i> stimuli	[143]

Environmental abiotic stresses also induce significant changes in gene expression as a plant activates certain transcription factors (WRKY, MYB, AP2/ERF, and NAC), through different transduction pathways, to trigger specific defence mechanisms [144].

Among these stressful conditions, drought is one of the major limitations for the growth, development, and yield of sugarcane throughout the world [82,145]. It has been observed that, under drought conditions, various genes encoding aquaporin, coenzyme A-ligase, ascorbate peroxidase, E3 enzyme for SUMO-protein-ligase SIZ2, and MYB are activated in the drought-tolerant sugarcane cultivar SP81-3250 but not in the drought-sensitive cultivar RB855453 [146]. Stress-induced genes such as *RLK* (receptor-like protein-kinase), *ACC* (1-aminocyclopropane-1-carboxylate) oxidase, and *bHLH* (basic helix-loop-helix) transcription factors were also shown to be effective against drought and other abiotic stresses [146]. Sugarcane cultivar Mex 69-290 responded to stress via up-regulation of genes related to transcriptional regulation, antioxidant activities, flavonoid biosynthesis, carbohydrate catabolism, ABA signalling, and biosynthesis of other important secondary metabolites [147]. The HT-Super SAGE technique was used to evaluate four drought-sensitive and -resistant cultivars, which resulted in the identification of 9831 unitags induced in resistant cultivars under drought stress. Various genes play an essential role in sugarcane metabolic processes, e.g., thioredoxin-like 1-2 in oxidative detoxification and protein degradation, β -expensin 8 as a precursor in root growth, acetyl-CoA carboxylase in fatty acids biosynthesis, 1-aminocyclopropane-1-carboxylate deaminase in ethylene-stress attenuation, transketolase in the pentose-phosphate pathway, and fructose biphosphate aldolase/triosephosphate isomerase/6-phosphofructo-2-kinase in glycolysis of carbohydrate metabolism [148]. Similarly, another study was conducted under drought stress on *S. narenga*, which led to the identification of 3389 differentially expressed genes (DEGs) involved in signal transduction via hormones, blue light response, and other metabolic pathways [149]. Overall, thousands of TFs have been identified in different plants, many involved in the responses to abiotic stress. However, there is a need in the future for more extensive studies to identify and test the applications of these TF genes for producing stress-resistant sugarcane cultivars.

4.3. Sugarcane Proteomics

Proteomic analysis through protein quantification and post-translational derivatives aids in providing insight into biological systems. An individual proteome shows variable expression and post-translational modifications (acetylation, methylation, phosphorylation, glycosylation) under different environmental stimuli. Various protein isolation and quantification tools, such as 2D-electrophoresis (2-DE), mass spectrometry (MS), and matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF-MS), are used for the determination of comparative and differential expression of sugarcane proteins under biotic and abiotic stress conditions [150]. iTRAQ (isobaric tag for relative and absolute quantitation) is another major proteome quantification tool [151].

Proteomic analysis of host-pathogen interaction aids in a better understanding of the virulence of pathogens and disease mechanisms; it should also help develop tolerant/resistant cultivars. The identification of 20 DEPs (differentially expressed proteins) related to photosynthesis, signal transduction, and defence responses was achieved through 2-DE/MALDI-TOF-MS techniques in sugarcane infected with *Sporisorium scitamineum* [152]. Similar experiments led to the identification of 53 proteins after the emergence stage; those proteins were related to cell division, protein folding, metabolism, energy, stress, and defence mechanisms [153]. Comparative proteomic analysis using the iTRAQ technique in sugarcane cultivars susceptible and resistant to *S. scitamineum* identified 341 and 273 DEPs, respectively. Most of these proteins, including endo-1,4- β -xylanase, β -1,3-glucanase, peroxidase, heat-shock proteins, lectins, and PR1, were involved in resistance against smut disease, whereas in the resistant cultivar, gibberellic acid and ethylene pathways, PRs, and phenylpropanoid metabolism were particularly active [154]. The up-regulation of proteins related to the ABA, ROS, calcium, and photosynthesis pathways was detected in both smut-susceptible and -resistant sugarcane cultivars [155]. Proteomic analysis of sugarcane (stalk tissues) under *Colletotrichum falcatum* infection was performed by 2-DE and resulted in developing a reference map and establishing an extraction map [156]. On the other hand, proteomic analysis by 2-DE/MALDI-TOF-MS led to the identification of

the *C. falcatum* EPL1 protein as a potential PAMP (pathogen-associated molecular pattern) for the induction of systemic resistance in sugarcane [157]. Additionally, iTRAQ-based proteomic analysis revealed differential expression of proteins in response to infection stress caused by *X. albilineans* [158].

The extent of drought tolerance is affected by the synthesis, degradation, and modification of proteins. Therefore, various proteomic studies have been conducted to address this situation in sugarcane. The enhanced expression of *SoDip22*, a drought-inducible gene regulated by ABA, was observed under drought stress in the bundle sheath cells of sugarcane leaves [159]. Using the 2-DE method, an accumulated protein (18 KDa) was detected under the increased expression of *SoDip22*, suggesting a possible role in drought stress tolerance [160]. Another comparative proteomic study, based on 2-DE and MALDI-TOF-MS, indicated that proteins associated with signal transduction, regulatory processes, and photosynthesis were up-regulated in a drought-tolerant sugarcane cultivar and down-regulated in a drought-susceptible one [161]. A quantitative proteomic analysis of sugarcane stems under drought stress revealed that most affected proteins (74) were associated with cell wall metabolism. Besides, 37 transcription factors, including the NAC TF family, C2H2 (Cys2-His2) TF family, C3H TF family, myeloblastosis (MYB) TF family, ARF (auxin response factors), or HSF (heat shock factors), belonging to the group of low-abundance nuclear proteins, were also identified [162].

Differentially delayed responses of sugarcane root proteomes under salt stress were studied through physiological and proteomic analyses. The results revealed that the differentially expressed proteins were associated with growth, energy metabolism, carbohydrate metabolism, development, ROS metabolism, membrane stabilisation, and protein protection [163]. Comparative proteomic analysis of two sugarcane cultivars, RB855536 and RB867515, revealed that four proteins related to defence responses and energy metabolism, i.e., germin-like protein, HSP70, fructose-1,6-bisphosphate aldolase (FBA), and glyceraldehyde-3-phosphate dehydrogenase (G3PDH), were differentially expressed in stressed and control conditions, suggesting a role in salt stress responses [164]. Overall, these research findings highlight the importance of diverse proteins for maintaining homeostasis in sugarcane under salt stress.

4.4. Sugarcane Metabolomics

A study that connects physiological and phenotypic alterations to external stimuli, based on changes in the levels of low-molecular weight compounds, is known as metabolomics [165]. Sugarcane metabolomics is still at an early stage due to various challenges and difficulties; however, determining metabolites levels, mainly carbohydrates (glucose, fructose, sucrose, raffinose, and inositol), does aid in understanding sugarcane biology [166]. Metabolomics analysis is carried out using various techniques, such as mass spectrometry (MS) [167], nuclear magnetic resonance (NMR) [168], and gas chromatography (GC) [169]. Furthermore, a metabolomics study is also necessary to understand the interaction of sugar-related transcriptomes and their proteomes [170]. The metabolomics approach has recently been used to understand host–pathogen interactions and defence mechanisms in sugarcane, to help develop resistant cultivars. With advances in genetic transformation and molecular genetics, transgenic sugarcane cultivars with desirable traits, e.g., insect resistance, have been developed by manipulating different molecules, such as proteinase inhibitors, secondary metabolites lectins, insecticidal proteins, delta endotoxins, or ribosome-inactivating proteins [171]. Metabolomics analysis of sugarcane plants infected by sugarcane yellow leaf virus (SCYLV) revealed high levels of carbohydrates, hexose, sucrose, and starch, the latter particularly observed in infected leaves. This high content of carbohydrates in leaves indicated a reduced assimilates' export [172]. Under yellow canopy syndrome (YCS), high sucrose content and lower stomatal conductivity and photosynthetic rate were observed in infected leaves in a metabolomics study, with another increment in sugar levels as a secondary effect [173]. In an independent study, a metabolomics analysis using liquid chromatography coupled with electrospray ionisation tandem MS

(LC-ESI-MS) was performed on smut-susceptible sugarcane genotypes inoculated with SSC39 teliospores of *S. scitamineum* to identify marker molecules. Biosynthesis of Apigenin 7-O-(600-O-acetylglucoside) was hindered due to infection; however, after the whip development, an increasing pattern was also observed, making it a marker metabolite to identify healthy plants [174].

Elongated roots and a high rate of photosynthesis were recorded under drought stress, associated with high malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents [175]. H_2O_2 acted as a secondary signalling metabolite and oxidant due to its high penetration ability across membranes and relatively long half-life [176]. Heat stress is another major constraint in sugarcane production, and metabolomics studies have also been conducted regarding this stress factor. It was observed that an exogenous proline (20 mM) treatment of sugarcane buds alleviated heat stress by decreasing H_2O_2 and increasing osmolytes and soluble sucrose contents. Similarly, exogenous proline application was also shown to improve Ca^{2+} and K^+ levels in crop plants [177]. Increased proline accumulation was reported in stressed sugarcane plants, which pointed to a possible positive role of proline in the mechanisms of tolerance to salinity and drought [178]. Similarly, high proline content and lower concentration of Na^+ were observed in leaves of sugarcane plants subjected to salt stress [179]. A research study on K92-80 (salt-sensitive) and K88-92 (salt-tolerant) sugarcane cultivars revealed ~ 2-fold increased proline content in salt-stressed K88-92 plants, as compared to the control, whereas no change was observed in K-92-80 [180]. Thus, the above-mentioned and similar studies indicate the importance of metabolomics for identifying stress-tolerant sugarcane genotypes.

5. Conclusions

Sugarcane, one of the most important industrial cash crops worldwide, has been improved for agronomic traits and resistance against biotic and abiotic stresses from prehistoric times. However, conventional breeding is time-consuming and not efficient enough to deliver the improved varieties needed under the current climate change scenario. Successful developments in the sphere of the genetic manipulation of plants using molecular tools, as compared to classical breeding, have been proven beneficial for obtaining sustainable and higher yields with lower susceptibility towards biotic and abiotic stresses. Besides, advances in breeding technologies, such as molecular marker-assisted breeding, genetic transformations, genome-editing, and multiple omics technologies, have a great potential for sugarcane genetic improvement.

Molecular markers-assisted breeding strategies have been undertaken for pyramiding multiple stress-tolerant genes/QTLs to develop robust tolerant sugarcane varieties to ensure food security. Previously, several QTLs for disease resistance have been successfully identified in sugarcane, including 11 DNA markers for smut disease, five for resistance against fiji leaf gall, four for resistance against leaf scald and pachymetra root-rot, *Bru1* for resistance against brown rust, and 18 QTLs for resistance against yellow leaf virus. In addition, AFLP, RAPD, and SSR markers have been widely used for improving the adaptability of sugarcane to abiotic stressors such as drought, extreme temperature, and salinity, among other stressful conditions. Multiple omics technologies, such as sugarcane genomics (genome sequencing of Xa-FJ1, a strain of *X. albilineans*), transcriptomics (transcriptomic identification of several DEGs for sugarcane resistance to *Aaa*), proteomics (quantitative proteomic analysis of the sugarcane defence responses incited by *Acidovorax avenae* subsp. *avenae*, causing red stripe), and metabolomics (smut-susceptible sugarcane liquid chromatography coupled with electrospray ionisation tandem MS) have been successfully employed to ensure sustainable sugarcane production under a changing climate.

Overall, modern biotechnologies can lead to the release of genetically improved cultivars with better physiological and morphological traits and improved field performance under varying environmental conditions and in a shorter time than conventional breeding.

6. Future Prospects

Future trends and expectations should be aimed at cracking the current main hurdles (biotic and abiotic stressors) to plants. Modern genome-editing tools (CRISPR/Cas9, base editing) can provide a permanent solution by developing stress tolerant/resistant varieties, although further studies are needed to reach these goals. The integration of all these approaches will lead to the sustainable production of the sugarcane crop, through the effective management of environmental stresses under the present scenario of changing climate. In addition, plant epigenetics, which is a conserved gene expression regulatory mechanism including histone modification, DNA methylation, noncoding RNA, and chromatin remodelling, represents an emerging and efficient tool to better understand biological processes in sugarcane. Overall, there is an urgent need for employing molecular breeding tools to improve sugarcane cultivars, as this is a vegetatively propagated crop, with narrow genetic pools and a complicated genome. Finally, an important step towards crop improvement would be to promote a transparent dialogue between molecular biologists and plant physiologists, on the one hand, and between farmers, breeding companies, and the public, on the other, to jointly resolve the economic, sociological, legal, and ethical hurdles.

Author Contributions: Conceptualisation, P.C. and O.V.; resources, P.C.; writing—original draft preparation, R.S. and T.J.; writing—review and editing, T.J., I.A., A.E.S., A.A., O.V., and P.C.; supervision, P.C.; project administration, P.C.; funding acquisition, P.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Natural Science Foundation, PR China (grant numbers: KF2015080, KF2015118, KFA17263A, KJG16005R).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analysed in this study. Data sharing is not applicable to this article.

Acknowledgments: The authors thank Gao San-Ji (National Engineering Research Centre for Sugarcane, China) for critically reviewing the manuscript.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Sucden. Available online: <http://www.sucden.com/> (accessed on 13 January 2021).
2. Singh, A.; Lal, U.R.; Mukhtar, H.M.; Singh, P.S.; Shah, G.; Dhawan, R.K. Phytochemical profile of sugarcane and its potential health aspects. *Phcog. Rev.* **2015**, *9*, 45. [[CrossRef](#)]
3. Iryani, D.A.; Kumagai, S.; Nonaka, M.; Sasaki, K.; Hirajima, T. Characterization and production of solid biofuel from sugarcane bagasse by hydrothermal carbonization. *Waste Biomass Valori.* **2017**, *8*, 1941–1951. [[CrossRef](#)]
4. Kroger, M.; Meister, K.; Kava, R. Low—Calorie sweeteners and other sugar substitutes: A review of the safety issues. *Food Sci.* **2006**, *5*, 35–47. [[CrossRef](#)]
5. Hunsigi, G. *Production of Sugarcane Theory and Practice*; Springer: Berlin, Germany; Heidelberg, Germany, 1993; pp. 12–28.
6. Fukuhara, S.; Terajima, Y.; Irei, S.; Sakaigaichi, T.; Ujihara, K.; Sugimoto, A.; Matsuoka, M. Identification and characterization of intergeneric hybrid of commercial sugarcane (*Saccharum* spp. hybrid) and *Erianthus arundinaceus* (Retz.) Jeswiet. *Euphytica* **2013**, *189*, 321–327. [[CrossRef](#)]
7. Arvinth, S.; Arun, S.; Selvakesavan, R.K.; Srikanth, J.; Mukunthan, N.; Kumar, P.A.; Premachandran, M.N.; Subramonian, N. Genetic transformation and pyramiding of aprotinin-expressing sugarcane with cry1Ab for shoot borer (*Chilo infuscatellus*) resistance. *Plant Cell Rep.* **2010**, *29*, 383–395. [[CrossRef](#)] [[PubMed](#)]
8. Premachandran, M.N.; Prathima, P.T.; Lekshmi, M. Sugarcane and polyploidy: A review. *J. Sugarcane Res.* **2011**, *1*, 1–15.
9. Yan, J.; Zhang, J.; Sun, K.; Chang, D.; Bai, S.; Shen, Y.; Huang, L.; Zhang, J.; Zhang, Y.; Dong, Y. Ploidy level and DNA content of *Erianthus arundinaceus* as determined by flow cytometry and the association with biological characteristics. *PLoS ONE* **2016**, *11*, e0151948. [[CrossRef](#)]
10. Cai, Q.; Aitken, K.S.; Fan, Y.H.; Piperidis, G.; Liu, X.L.; McIntyre, C.L.; Huang, X.Q.; Jackson, P. Assessment of the genetic diversity in a collection of *Erianthus arundinaceus*. *Genet. Resour. Crop Evol.* **2012**, *59*, 1483–1491. [[CrossRef](#)]

11. Scortecci, K.C.; Creste, S.; Calsa, J.T.; Xavier, M.A.; Landell, M.G.; Figueira, A.; Benedito, V.A. Challenges, opportunities and recent advances in sugarcane breeding. *Plant Breed* **2012**, *1*, 267–296.
12. Meena, M.R.; Kumar, R.; Chinnaswamy, A.; Karuppaiyan, R.; Kulshreshtha, N.; Ram, B. Current breeding and genomic approaches to enhance the cane and sugar productivity under abiotic stress conditions. *3 Biotech* **2020**, *10*, 440. [[CrossRef](#)]
13. Tew, T.L.; Cobill, R.M. Genetic improvement of sugarcane (*Saccharum* spp.) as an energy crop. In *Genetic Improvement of Bioenergy Crops*; Springer: New York, NY, USA, 2008; pp. 273–294.
14. Tahir, M.; Rahman, H.; Gul, R.; Ali, A.; Khalid, M. Genetic divergence in sugarcane genotypes. *J. Exp. Agric. Int.* **2013**, *3*, 102–109. [[CrossRef](#)]
15. Singh, S.P.; Singh, P.; Sharma, B.L. Methods to improve germination in sugarcane. In *Emerging Trends of Plant Physiology for Sustainable Crop Production*; CRC Press: Washington, DC, USA, 2018; pp. 331–344.
16. Güell, M.; Yang, L.; Church, G.M. Genome editing assessment using CRISPR Genome Analyzer (CRISPR-GA). *Bioinform* **2014**, *30*, 2968–2970. [[CrossRef](#)] [[PubMed](#)]
17. Sanghera, G.S.; Malhotra, P.K.; Singh, H.; Bhatt, R. Climate change impact in sugarcane agriculture and mitigation strategies. In *Harnessing Plant Biotechnology and Physiology to Stimulate Agricultural Growth*; The Journal of Plant Science and Research: New Delhi, India, 2019; pp. 99–115.
18. Khan, M.T.; Khan, I.A.; Yasmeen, S. Genetically modified sugarcane for biofuels production: Status and perspectives of conventional transgenic approaches, RNA interference, and genome editing for improving sugarcane for biofuels. In *Sugarcane Biofuels*; Springer: Cham, Switzerland, 2019; pp. 67–96.
19. Wright, S. Recombinant DNA technology and its social transformation. *Osiris* **1986**, *1*, 303–360. [[CrossRef](#)] [[PubMed](#)]
20. Ali, A.; Pan, Y.B.; Wang, Q.N.; Wang, J.D.; Chen, J.L.; Gao, S.J. Genetic diversity and population structure analysis of *Saccharum* and *Erianthus* genera using microsatellite (SSR) markers. *Sci. Rep.* **2019**, *9*, 1–10. [[CrossRef](#)]
21. Devarumath, R.M.; Kalwade, S.B.; Kulkarni, P.A.; Sheelavanthmath, S.S.; Suprasanna, P. *Integrating OMICS Approaches in Sugarcane Improvement. OMICS Applications in Crop Science*; CRC Press: New York, NY, USA, 2013; pp. 191–250.
22. Mirajkar, S.J.; Devarumath, R.M.; Nikam, A.A.; Sushir, K.V.; Babu, H.; Suprasanna, P. Sugarcane (*Saccharum* spp.): Breeding and genomics. In *Advances in Plant Breeding Strategies: Industrial and Food Crops*; Springer: Cham, Switzerland, 2019; pp. 363–406.
23. Liu, P.; Chandra, A.; Que, Y.; Chen, P.H.; Grisham, M.P.; White, W.H.; Dalley, C.D.; Tew, T.L.; Pan, Y.-B. Identification of quantitative trait loci controlling sucrose content based on an enriched genetic linkage map of sugarcane (*Saccharum* spp. hybrids) cultivar ‘LCP 85–384’. *Euphytica* **2016**, *207*, 527–549. [[CrossRef](#)]
24. Alwala, S.; Kimbeng, C.A.; Veremis, J.C.; Gravois, K.A. Linkage mapping and genome analysis in *Saccharum* interspecific cross using AFLP, SRAP and TRAP markers. *Euphytica* **2008**, *164*, 37–51. [[CrossRef](#)]
25. Palhares, A.C.; Rodrigues-Morais, T.B.; Van Sluys, M.A.; Domingues, D.S.; Maccheroni, W.; Jordão, H.; Souza, A.P.; Marconi, T.G.; Mollinari, M.; Gazaffi, R.; et al. A novel linkage map of sugarcane with evidence for clustering of retrotransposon-based markers. *BMC Genet.* **2012**, *13*, 51. [[CrossRef](#)]
26. Manimekalai, R.; Suresh, G.; Govinda Kurup, H.; Athiappan, S.; Kandalam, M. Role of NGS and SNP genotyping methods in sugarcane improvement programs. *Critic. Rev. Biotechnol.* **2020**, *40*, 1–16. [[CrossRef](#)] [[PubMed](#)]
27. Cordeiro, G.M.; Pan, Y.B.; Henry, R.J. Sugarcane microsatellites for the assessment of genetic diversity in sugarcane germplasm. *Plant Sci.* **2003**, *165*, 181–189. [[CrossRef](#)]
28. You, Q.; Pan, Y.B.; Xu, L.P.; Gao, S.W.; Wang, Q.N.; Su, Y.C.; Yang, Y.Q.; Wu, Q.B.; Zhou, D.G.; Que, Y.X. Genetic diversity analysis of sugarcane germplasm based on fluorescence—Labeled simple sequence repeat markers and a capillary electrophoresis—Based genotyping platform. *Sugar Tech.* **2016**, *18*, 380–390. [[CrossRef](#)]
29. Creste, S.; Sansoli, D.M.; Tardiani, A.C.S.; Silva, D.N.; Goncalves, F.K.; Favero, T.M.; Medeiros, C.N.F.; Festucci, C.S.; Carlini-Garcia, L.A.; Landell, M.G.; et al. Comparison of AFLP, TRAP and SSRs in the estimation of genetic relationships in sugarcane. *Sugar Tech.* **2010**, *12*, 150–154. [[CrossRef](#)]
30. Nayak, S.N.; Song, J.; Villa, A.; Pathak, B.; Ayala-Silva, T.; Yang, X.; Todd, J.; Glynn, N.C.; Kuhn, D.N.; Glaz, B.; et al. Promoting utilization of *Saccharum* spp. genetic resources through genetic diversity analysis and core collection construction. *PLoS ONE* **2014**, *9*, e110856. [[CrossRef](#)] [[PubMed](#)]
31. Singh, R.B.; Singh, B.; Singh, R.K. Study of genetic diversity of sugarcane (*Saccharum*) species and commercial varieties through TRAP molecular markers. *Indian J. Plant Physiol.* **2017**, *22*, 332–338. [[CrossRef](#)]
32. Wu, J.; Wang, Q.; Xie, J.; Pan, Y.B.; Zhou, F.; Guo, Y.; Chang, H.; Xu, H.; Zhang, W.; Zhang, C.; et al. SSR marker-assisted management of parental germplasm in sugarcane (*Saccharum* spp. hybrids) breeding programs. *Agron* **2019**, *9*, 449. [[CrossRef](#)]
33. Pinto, L.R.; Garcia, A.A.F.; Pastina, M.M.; Teixeira, L.H.M.; Bressiani, J.A.; Ulian, E.C.; Bidoia, M.A.P.; Souza, A.P. Analysis of genomic and functional RFLP derived markers associated with sucrose content, fiber and yield QTLs in a sugarcane (*Saccharum* spp.) commercial cross. *Euphytica* **2010**, *172*, 313–327. [[CrossRef](#)]
34. Guimaraes, C.T.; Sills, G.R.; Sobral, B.W.S. Comparative mapping of Andropogoneae: *Saccharum* L. (sugarcane) and its relation to sorghum and maize. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 14262–14266. [[CrossRef](#)]
35. Silva, J.A.G.; Sorrells, M.E.; Burnquist, W.; Tanksley, S.D. RFLP linkage map and genome analysis of *Saccharum spontaneum*. *Genome* **1993**, *36*, 782–791. [[CrossRef](#)]
36. Zhou, J.R.; Sun, H.D.; Ali, A.; Rott, P.C.; Javed, T.; Fu, H.Y.; Gao, S.J. Quantitative proteomic analysis of the sugarcane defense responses incited by *Acidovorax avenae* subsp. *avenae* causing red stripe. *Ind. Crops Prod.* **2021**, *162*, 113275. [[CrossRef](#)]

37. Aitken, K.S.; Jackson, P.A.; McIntyre, C.L. QTL identified for sugar related traits in a sugarcane (*Saccharum* spp.) cultivar 9 *S. officinarum* population. *Theor. Appl. Genet.* **2007**, *112*, 1306–1317. [[CrossRef](#)]
38. Gouy, M.; Rousselle, Y.; Chane, A.T.; Anglade, A.; Royaert, S.; Nibouche, S.; Costet, L. Genome wide association mapping of agro—Morphological and disease resistance traits in sugarcane. *Euphytica* **2015**, *202*, 269–284. [[CrossRef](#)]
39. Siraree, A.; Banerjee, N.; Kumar, S.; Khan, M.S.; Singh, P.K.; Sharma, S.; Singh, R.K.; Singh, J. Identification of marker—Trait associations for morphological descriptors and yield component traits in sugarcane. *Physiol. Mol. Biol. Plants* **2017**, *23*, 185–196. [[CrossRef](#)]
40. Collard, B.C.; Jahufer, M.Z.; Brouwer, J.B.; Pang, E.C. An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: The basic concepts. *Euphytica* **2005**, *142*, 169–196. [[CrossRef](#)]
41. Singh, R.K.; Singh, S.P.; Tiwari, D.K.; Srivastava, S.; Singh, S.B.; Sharma, M.L.; Singh, R.; Mohapatra, T.; Singh, N.K. Genetic mapping and QTL analysis for sugar yield-related traits in sugarcane. *Euphytica* **2013**, *191*, 333–353. [[CrossRef](#)]
42. Aitken, K.S. Introgression of a large effect QTL for smut resistance inherited from *S. Spontaneum*. In Proceedings of the Plant and Animal Genome XXIV Conference, San Diego, CA, USA, 8–13 January 2016.
43. Wei, X.; Jackson, P.A.; McIntyre, C.L.; Aitken, K.S.; Croft, B. Associations between DNA markers and resistance to diseases in sugarcane and effects of population substructure. *Theor. Appl. Genet.* **2006**, *114*, 155–164. [[CrossRef](#)] [[PubMed](#)]
44. Daugrois, J.H.; Grivet, L.; Roques, D.; Hoarau, J.Y.; Lombard, H.; Glaszmann, J.C.; D’Hont, A.A. A putative major gene for rust resistance linked with a RFLP marker in sugarcane cultivar ‘R570’. *Theor. Appl. Genet.* **1996**, *92*, 1059–1064. [[CrossRef](#)] [[PubMed](#)]
45. You, Q.; Yang, X.; Peng, Z.; Islam, M.S.; Sood, S.; Luo, Z.; Comstock, J.; Xu, L.; Wang, J. Development of an Axiom Sugarcane100K SNP array for genetic map construction and QTL identification. *Theor. Appl. Genet.* **2019**, *132*, 2829–2845.
46. Aljanabi, S.M.; Parmessur, Y.; Kross, H.; Dhayan, S.; Sauntally, S.; Ramdoyal, K.; Autrey, L.J.; Dookun-Sauntally, A. Identification of a major quantitative trait locus (QTL) for yellow spot (*Mycovellosiella koepkei*) disease resistance in sugarcane. *Mol. Breed.* **2007**, *19*, 1–14. [[CrossRef](#)]
47. Costet, L.; Raboin, L.; Payet, M.; D’Hont, A.; Nibouche, S. A major quantitative trait allele for resistance to the *Sugarcane yellow leaf virus* (Luteoviridae). *Plant Breed.* **2012**, *131*, 637–640. [[CrossRef](#)]
48. Nibouche, S.; Raboin, L.M.; Hoarau, J.Y.; D’Hont, A.; Costet, L. Quantitative trait loci for sugarcane resistance to the spotted stem borer *Chilo sacchariphagus*. *Mol. Breed.* **2012**, *29*, 129–135. [[CrossRef](#)]
49. Gutierrez, A.; Hoy, J.; Kimbeng, C.; Baisakh, N. Identification of genomic regions controlling leaf scald resistance in sugarcane using a bi—Parental mapping population and selective genotyping by sequencing. *Front. Plant Sci.* **2018**, *9*, 877. [[CrossRef](#)]
50. Winicov, I. Molecular markers and abiotic stresses. In *Molecular Techniques in Crop Improvement*; Springer: New York, NY, USA, 2010.
51. Abdel-Tawab, F.M.; Fahmy, E.M.; Allam, A.I.; El-Rashidy, H.A.; Shoaib, R.M. Development of RAPD and SSR marker associated with stress tolerance and some technological traits and transient transformation of sugarcane (*Saccharum* spp.). In Proceedings of the International Conference. The Arab Region and Africa in the World Sugar Context, Aswan, Egypt, 9–12 March 2003; pp. 10–12.
52. Abdel-Tawab, F.M.; Fahmy, E.M.; Allam, A.I.; El-Rashidy, H.A.; Shoaib, R.M. Marker assisted selection for abiotic stress tolerance in sugarcane (*Saccharum* spp.). *Egypt. J. Agric. Res.* **2003**, *81*, 635–646.
53. Srivastava, M.K.; Li, C.N.; Li, Y.R. Development of sequence characterized amplified region (SCAR) marker for identifying drought tolerant sugarcane genotypes. *Aus. J. Crop Sci.* **2012**, *6*, 763.
54. Khaled, K.; El-Sherbeny, S.; Abdelhadi, A. R-ISSR as marker assisted selection for drought tolerance in sugarcane. *J. Agric. Chem. Biotechnol.* **2017**, *8*, 91–97. [[CrossRef](#)]
55. Khaled, K.A.; El-Arabi, N.I.; Sabry, N.M.; El-Sherbiny, S. Sugarcane genotypes assessment under drought condition using amplified fragment length polymorphism. *Biotechnology* **2018**, *17*, 120–127. [[CrossRef](#)]
56. Chaum, S.; Chuencharoenm, S.; Mongkolsiriwatanam, C.; Ashrafm, M.; Kirdmanee, C. Screening sugarcane (*Saccharum* sp.) genotypes for salt tolerance using multivariate cluster analysis. *Plant Cell Tiss. Org. Cult.* **2012**, *110*, 23–33. [[CrossRef](#)]
57. Suprasanna, P.; Patade, V.Y.; Vaidya, E.R.; Patil, V.D. Radiation induced in vitro mutagenesis, selection for salt tolerance and characterization in sugarcane. In *Induced Plant Mutations in the Genomics Era: Proceedings of an International Joint FAO/IAEA Symposium*; International Atomic Energy Agency: Vienna, Austria, 2009; pp. 145–147.
58. Markad, N.R.; Kale, A.A.; Pawar, B.D.; Jadhav, A.S.; Patil, S.C. Molecular characterization of sugarcane (*Saccharum officinarum* L.) genotypes in relation to salt tolerance. *Bioscan* **2014**, *9*, 1785–1788.
59. Gadakh, S.; Patel, D.; Singh, D. Use of RAPD markers to characterize salt and drought lines of sugarcane. *Int. J. Adv. Res. Biol. Sci.* **2017**, *4*, 50–57. [[CrossRef](#)]
60. Yadav, P.V.; Suprasanna, P.; Gopalrao, K.U.; Anant, B.V. Molecular profiling using RAPD technique of salt and drought tolerant regenerants of sugarcane. *Sugar Tech.* **2006**, *8*, 63–68. [[CrossRef](#)]
61. Farsangi, F.J.; Thorat, A.S.; Devarumath, R.M. Molecular characterization of sugarcane genotypes for their salinity and susceptibility using TRAP markers. *Int. J. Curr. Res.* **2018**, *10*, 68947–68951.
62. Suprasanna, P. Biotechnological interventions in sugarcane improvement: Strategies, methods and progress. *BARC Newsl.* **2010**, *45*, 47–53.
63. Feng, D.; Wang, Y.; Wu, J.; Lu, T.; Zhang, Z. Development and drought tolerance assay of marker-free transgenic rice with OsAPX2 using biolistic particle-mediated co-transformation. *Crop J.* **2017**, *5*, 271–281.

64. Arencibia, A.; Molina, P.R.; DelaRiva, G.; Selman-Housein, G. Production of transgenic sugarcane (*Saccharum officinarum* L.) plants by intact cell electroporation. *Plant Cell Rep.* **1995**, *14*, 305–309. [[CrossRef](#)] [[PubMed](#)]
65. Bower, R.; Birch, R.G. Transgenic sugarcane plants via micro-projectile bombardment. *Plant J.* **1992**, *2*, 409–416. [[CrossRef](#)]
66. Snyman, S.J.; Meyer, G.M.; Richards, J.M.; Haricharan, N.; Ramgareeb, S.; Hockett, B.I. Refining the application of direct embryogenesis in sugarcane: Effect of the developmental phase of leaf disc explants and the timing of DNA transfer on transformation efficiency. *Plant Cell Rep.* **2006**, *25*, 1016–1023. [[CrossRef](#)]
67. Guo, M.; Ye, J.; Gao, D.; Xu, N.; Yang, J. Agrobacterium-mediated horizontal gene transfer: Mechanism, biotechnological application, potential risk and forestalling strategy. *Biotechnol. Adv.* **2018**, *37*, 259–270.
68. Herrera-Estrella, L.; Depicker, A.; Van Montagu, M.; Schell, J. Expression of chimaeric genes transferred into plant cells using a Ti-plasmid-derived vector. *Nature* **1983**, *303*, 209–213. [[CrossRef](#)]
69. Anderson, D.J.; Birch, R.G. Minimal handling and super-binary vectors facilitate efficient, *Agrobacterium*-mediated, transformation of sugarcane (*Saccharum* spp. hybrid). *Trop. Plant Biol.* **2012**, *5*, 183–192. [[CrossRef](#)]
70. Wu, H.; Awan, F.S.; Vilarinho, A.; Zeng, Q.; Kannan, B.; Phipps, T.; McCuiston, J.; Wang, W.; Caffall, K.; Altpeter, F. Transgene integration complexity and expression stability following biolistic or *Agrobacterium*-mediated transformation of sugarcane. *In Vitro. Cell. Dev. Biol. Plant.* **2015**, *51*, 603–611.
71. Behera, K.K.; Sahoo, S. Rapid in vitro micropropagation of sugarcane (*Saccharum officinarum* L. cv-Nayana) through callus culture. *Nat. Sci.* **2009**, *7*, 1–10.
72. Tolera, B.; Diro, M.; Belew, D. Response of sugarcane (*Saccharum officinarum* L.) varieties to BAP and IAA on in vitro shoot multiplication. *Adv. Crop Sci. Technol.* **2014**, *2*, 1–5.
73. Tolera, B.; Shimelis, D. Comparison of micropropagated and conventional raised sugarcane planting materials as initial source of seed cane at Metahara Sugar Estate, Ethiopia. *Adv. Tissue Eng. Regen. Med.* **2016**, *1*, 1–3. [[CrossRef](#)]
74. Kathiria, P.; Eudes, F. Nucleases for genome editing in crops. *Biocatal. Agric. Biotechnol.* **2014**, *3*, 14–19. [[CrossRef](#)]
75. Hille, F.; Charpentier, E. CRISPR-Cas: Biology, mechanisms and relevance. *Philos. Trans. R. Soc. B Biol. Sci.* **2016**, *5*, 371. [[CrossRef](#)] [[PubMed](#)]
76. Nekrasov, V.; Staskawicz, B.; Weigel, D.; Jones, J.D.; Kamoun, S. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nat. Biotechnol.* **2013**, *31*, 691. [[CrossRef](#)] [[PubMed](#)]
77. Shan, Q.; Wang, Y.; Li, J.; Zhang, Y.; Chen, K.; Liang, Z.; Zhang, K.; Liu, J.; Xi, J.J.; Qiu, J. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.* **2013**, *31*, 686. [[CrossRef](#)]
78. Birch, R.G.; Bower, R.S.; Elliott, A.R. Highly efficient, 5'-sequencespecific transgene silencing in a complex polyploid. *Trop. Plant Biol.* **2010**, *3*, 88–97. [[CrossRef](#)]
79. 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](#)] [[PubMed](#)]
80. Kleinstiver, B.P.; Pattanayak, V.; Prew, M.S.; Tsai, S.Q.; Nguyen, N.T.; Zheng, Z.; Joung, J.K. High-fidelity CRISPR-Cas9 nucleases with no detectable genome-wide off-target effects. *Nature* **2016**, *529*, 490–495. [[CrossRef](#)]
81. Joyce, P.; Kuwahata, M.; Turner, N.; Lakshmanan, P. Selection system and co-cultivation medium are important determinants of *Agrobacterium* mediated transformation of sugarcane. *Plant Cell Rep.* **2010**, *29*, 173–183. [[CrossRef](#)]
82. Javed, T.; Shabbir, R.; Ali, A.; Afzal, I.; Zaheer, U.; Gao, S.J. Transcription factors in plant stress responses: Challenges and potential for sugarcane improvement. *Plants* **2020**, *9*, 491. [[CrossRef](#)]
83. Cui, D.; Huang, M.T.; Hu, C.Y.; Su, J.B.; Lin, L.H.; Javed, T.; Deng, Z.H.; Gao, S.J. First Report of *Pantoea stewartii* subsp. *stewartii* Causing Bacterial Leaf Wilt of Sugarcane in China. *Plant Dis.* **2021**, *101*, 1190.
84. Wang, L.; Zheng, Y.; Ding, S.; Zhang, Q.; Chen, Y.; Zhang, J. Molecular cloning, structure, phylogeny and expression analysis of the invertase gene family in sugarcane. *BMC Plant Biol.* **2017**, *17*, 109.
85. Torres, J.B.; Ruberson, J.R. Interactions of *Bacillus thuringiensis* Cry1Ac toxin in genetically engineered cotton with predatory heteropterans. *Transgenic Res.* **2008**, *17*, 345. [[CrossRef](#)] [[PubMed](#)]
86. Arencibia, A.D.; Carmona, E.R.; Cornide, M.T.; Castiglione, S.; O'Reilly, J.; Chinea, A.; Oramas, P.; Sala, F. Somaclonal variation in insect-resistant transgenic sugarcane (*Saccharum* hybrid) plants produced by cell electroporation. *Transgenic Res.* **1999**, *8*, 349–360. [[CrossRef](#)]
87. Braga, D.P.V.; Arrigoni, E.D.B.; Silva-Filho, M.C.; Ulian, E.C. Expression of the Cry1Ab protein in genetically modified sugarcane for the control of *Diatraea saccharalis* (Lepidoptera: Cram-bidae). *J. New Seeds* **2003**, *5*, 209–222. [[CrossRef](#)]
88. Jing-Sheng, X.; Shiwu, G.; Liping, X.; Rukai, C. Construction of expression vector of CryIA(c) gene and its transformation in sugarcane. *Sugar Tech.* **2008**, *10*, 269–273.
89. Kalunke, R.M.; Kolge, A.M.; Babu, K.H.; Prasad, D.T. Agrobacterium-mediated transformation of sugarcane for borer resistance using cry1Aa3 gene and one-step regeneration of transgenic plants. *Sugar Tech.* **2009**, *11*, 355–359. [[CrossRef](#)]
90. Wang, W.Z.; Yang, B.P.; Feng, X.Y.; Cao, Z.Y.; Feng, C.L.; Wang, J.G.; Xiong, G.R.; Shen, L.B.; Zeng, J.; Zhao, T.T.; et al. Development and characterization of transgenic sugarcane with insect resistance and herbicide tolerance. *Front. Plant Sci.* **2017**, *8*, 1535. [[CrossRef](#)] [[PubMed](#)]
91. Gallo-Meagher, M.; Irvine, J.E. Herbicide resistant transgenic sugarcane plants containing the bar gene. *Crop Sci.* **1996**, *36*, 1367–1374. [[CrossRef](#)]

92. Manickavasagam, M.; Ganapathi, A.; Anbazhagan, V.R.; Sudhakar, B.; Selvaraj, N.; Vasudevan, A.; Kasthuriengan, S. Agrobacterium-mediated genetic transformation and development of herbicide-resistant sugarcane (*Saccharum* spp. hybrids) using axillary buds. *Plant Cell Rep.* **2004**, *23*, 134–143. [[CrossRef](#)]
93. Enriquez-Obregon, G.A.; Vazquez-Padron, R.I.; Pioto-Samsonov, D.L.; De la Riva, G.A.; Selman-Housein, G. Herbicide-resistant sugarcane (*Saccharum officinarum* L.) plants by *Agrobacterium*-mediated transformation. *Planta* **1998**, *206*, 20–27. [[CrossRef](#)]
94. Leibbrandt, N.B.; Snyman, S.J. Stability of gene expression and agronomic performance of a transgenic herbicide-resistant sugarcane line in South Africa. *Crop Sci.* **2003**, *43*, 671–677. [[CrossRef](#)]
95. Ahmad, B.; Raina, A.; Khan, S. Impact of biotic and abiotic stresses on plants, and their responses. In *Disease Resistance in Crop Plants*; Springer: Cham, Switzerland, 2019; pp. 1–19.
96. Somerville, C.; Youngs, H.; Taylor, C.; Davis, S.C.; Long, S.P. Feed stocks for lignocellulosic biofuels. *Science* **2010**, *329*, 790–792. [[CrossRef](#)] [[PubMed](#)]
97. Aydin, S.; Buyuk, I.; Aras, E.S. Expression of SOD gene and evaluating its role in stress tolerance in NaCl and PEG stressed *Lycopersicon esculentum*. *Turk J. Bot.* **2014**, *38*, 89–98. [[CrossRef](#)]
98. Selvi, A.; Devi, K.; Manimekalai, R.; Prathima, P.T. Comparative analysis of drought-responsive transcriptomes of sugarcane genotypes with differential tolerance to drought. *Biotech* **2020**, *10*, 236.
99. Wang, Z.Z.; Zhang, S.Z.; Yang, B.P.; Li, Y.R. Trehalose synthase gene transfer mediated by *Agrobacterium tumefaciens* enhances resistance to osmotic stress in sugarcane. *Sugar Tech.* **2005**, *7*, 49–54. [[CrossRef](#)]
100. McQualter, R.B.; Dookun-Saumtally, A. Expression profiling of abiotic-stress inducible genes in sugarcane. In Proceedings of the XXVI Congress, International Society of Sugar Cane Technologists. ICC, Durban, South Africa, 29 July–2 August 2007; pp. 878–888.
101. Guo, J.; Ling, H.; Ma, J.; Chen, Y.; Su, Y.; Lin, Q.; Gao, S.; Wang, H.; Que, Y.; Xu, L. A sugarcane R2R3-MYB transcription factor gene is alternatively spliced during drought stress. *Sci. Rep.* **2017**, *7*, 41922.
102. Augustine, S.M.; Narayan, J.A.; Syamaladevi, D.P.; Appunu, C.; Chakravarthi, M.; Ravichandran, V.; Tuteja, N.; Subramonian, N. Over-expression of EaDREB2 and pyramiding of EaDREB2 with the pea DNA helicase gene (PDH45) enhance drought and salinity tolerance in sugarcane (*Saccharum* spp. hybrid). *Plant Cell Rep.* **2015**, *34*, 247–263. [[CrossRef](#)]
103. Augustine, S.M.; Ashwin, N.J.; Syamaladevi, D.P.; Appunu, C.; Chakravarthi, M.; Ravichandran, V.; Tuteja, N.; Subramonian, N. Introduction of pea DNA helicase 45 into sugarcane (*Saccharum* spp. hybrid) enhances cell membrane thermostability and upregulation of stress-responsive genes leads to abiotic stress tolerance. *Mol. Biotechnol.* **2015**, *57*, 475–488. [[CrossRef](#)]
104. Errabii, T.; Gandonou, C.; Essalmani, H.; Abrini, J.; Idaomar, M.; Senhaji, N. Growth, proline and ion accumulation in sugarcane callus cultures under drought-induced osmotic stress and its subsequent relief. *Afr. J. Biotechnol.* **2006**, *4*, 1250–1255.
105. Patade, V.Y.; Suprasanna, P.; Bapat, V.A. Gamma irradiation of embryogenic callus cultures and in vitro selection for salt tolerance in sugarcane (*Saccharum officinarum* L.). *Agric. Sci. China* **2008**, *7*, 101–105. [[CrossRef](#)]
106. Strizhov, N.; Ábrahám, E.; Ökrész, L.; Blicling, S.; Zilberstein, A.; Schell, J.; Koncz, C.; Szabados, L. Differential expression of two P5CS genes controlling proline accumulation during salt—Stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in *Arabidopsis*. *Plant J.* **1997**, *12*, 557–569. [[PubMed](#)]
107. Ashraf, M.; Fooladm, M.R. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.* **2007**, *59*, 206–216. [[CrossRef](#)]
108. Guerzoni, J.T.S.; Belintani, N.G.; Moreira, R.M.P.; Hoshino, A.A.; Domingues, D.S.; Filho, J.C.B.; Vieira, L.G.E. Stress-induced D1-pyrroline-5-carboxylate synthetase (P5CS) gene confers tolerance to salt stress in transgenic sugarcane. *Acta Physiol. Plant* **2014**, *36*, 2309–2319. [[CrossRef](#)]
109. Ramiro, D.A.; Melotto, P.D.M.; Barbosa, M.D.A.; Santos, F.D.; Gomez, S.G.P.; Junior, N.S.M.; Lam, E.; Carrer, H. Expression of *Arabidopsis* bax inhibitor-1 in transgenic sugarcane confers drought tolerance. *Plant Biotechnol. J.* **2016**, *14*, 1826–1837. [[CrossRef](#)] [[PubMed](#)]
110. Selvarajan, D.; Mohan, C.; Dhandapani, V.; Nerkar, G.; Jayanarayanan, A.N.; Vadakkancherry, M.M.; Murugan, N.; Kaur, L.; Chennappa, M.; Kumar, R.; et al. Differential gene expression profiling through transcriptome approach of (*Saccharum spontaneum* L.) under low temperature stress reveals genes potentially involved in cold acclimation. *Biotech* **2018**, *8*, 195. [[CrossRef](#)]
111. Zeng, Q.; Ling, Q.; Fan, L.; Li, Y.; Hu, F.; Chen, J.; Huang, Z.; Deng, H.; Li, Q.; Qi, Y. Transcriptome profiling of sugarcane roots in response to low potassium stress. *PLoS ONE* **2015**, *10*, e0126306. [[CrossRef](#)]
112. Garsmeur, O.; Droc, G.; Antonise, R.; Grimwood, J.; Potier, B.; Aitken, K.; Jenkins, J.; Martin, G.; Charron, C.; Hervouet, C. A mosaic monoploid reference sequence for the highly complex genome of sugarcane. *Nat. Commun.* **2018**, *9*, 2638. [[CrossRef](#)]
113. Mustafa, G.; Joyia, F.A.; Anwar, S.; Parvaiz, A.; Khan, M.S. Biotechnological interventions for the improvement of sugarcane crop and sugar production. In *Sugarcane-Technology and Research*; In Tech Open: London, UK, 2018; pp. 113–138.
114. Garsmeur, O.; Aitken, K.S.; Potier, B.; Grimwood, J.; Charron, C.; Droc, G. A reference sequence of the monoploid genome of sugarcane [W889]. In Proceedings of the Plant and Animal Genome Conference, San Diego, CA, USA, 14–18 January 2017.
115. Chuong, E.B.; Elde, N.C.; Feschotte, C. Regulatory activities of transposable elements: From conflicts to benefits. *Nat. Rev. Genet.* **2017**, *18*, 71. [[CrossRef](#)]
116. Yang, X.; Sood, S.; Luo, Z.; Todd, J.; Wang, J. Genome-wide association studies identified resistance loci to orange rust and yellow leaf virus diseases in sugarcane (*Saccharum* spp.). *Phytopathology* **2019**, *109*, 623–631. [[CrossRef](#)]

117. Zhang, J.; Zhang, X.; Tang, H.; Zhang, Q.; Hua, X.; Ma, X.; Zhu, F.; Jones, T.; Zhu, X.; Bowers, J. Allele-defined genome of the autopolyploid sugarcane *Saccharum spontaneum* L. *Nat. Genet.* **2018**, *50*, 1565. [CrossRef]
118. Barreto, F.Z.; Rosa, J.R.B.F.; Balsalobre, T.W.A.; Pastina, M.M.; Silva, R.R.; Hoffmann, H.P.; de Souza, A.P.; Garcia, A.A.F.; Carneiro, M.S. A genome-wide association study identified loci for yield component traits in sugarcane (*Saccharum* spp.). *PLoS ONE* **2019**, *14*, e0219843. [CrossRef] [PubMed]
119. Zhang, H.L.; Ntambo, M.S.; Rott, P.C.; Chen, G.; Chen, L.L.; Huang, M.T.; Gao, S.J. Complete genome sequence reveals evolutionary and comparative genomic features of *Xanthomonas albilineans* causing sugarcane leaf scald. *Microorganisms* **2020**, *8*, 182. [CrossRef] [PubMed]
120. Santiago, T.R.; Pereira, V.M.; de Souza, W.R.; Steindorff, A.S.; Cunha, B.A.; Gaspar, M.; Favaro, L.C.; Formighieri, E.F.; Kobayashi, A.K.; Molinari, H.B. Genome-wide identification, characterization and expression profile analysis of expansins gene family in sugarcane (*Saccharum* spp.). *PLoS ONE* **2018**, *13*, e0191081.
121. Simpson, A.J.; Perez, J.F. Latin America: ONSA, the São Paulo Virtual Genomics Institute. *Nat. Biotechnol.* **1998**, *16*, 795–796. [CrossRef]
122. SUCEST. Available online: <http://sucest.lad.ic.unicamp.br/en/> (accessed on 24 January 2021).
123. Vettore, A.L.; da Silva, F.R.; Kemper, E.L.; Souza, G.M.; da Silva, A.M.; Ferro, M.I.T.; Henrique-Silva, F.; Gigliotti, É.A.; Lemos, M.V.; Coutinho, L.L. Analysis and functional annotation of an expressed sequence tag collection for tropical crop sugarcane. *Genome Res.* **2003**, *13*, 2725–2735. [CrossRef]
124. Xu, S.; Wang, J.; Shang, H.; Huang, Y.; Yao, W.; Chen, B.; Zhang, M. Transcriptomic characterization and potential marker development of contrasting sugarcane cultivars. *Sci. Rep.* **2018**, *8*, 1683. [CrossRef]
125. Manners, J.M.; Casu, R.E. Transcriptome analysis and functional genomics of sugarcane. *Trop. Plant. Biol.* **2011**, *4*, 9–21. [CrossRef]
126. Mattiello, L.; Riaño-Pachón, D.M.; Martins, M.C.M.; da Cruz, L.P.; Bassi, D.; Marchiori, P.E.R.; Ribeiro, R.V.; Labate, M.T.V.; Labate, C.A.; Menossi, M. Physiological and transcriptional analyses of developmental stages along sugarcane leaf. *BMC Plant. Biol.* **2015**, *15*, 300. [CrossRef]
127. Li, M.; Liang, Z.; Zeng, Y.; Jing, Y.; Wu, K.; Liang, J.; He, S.; Wang, G.; Mo, Z.; Tan, F. De novo analysis of transcriptome reveals genes associated with leaf abscission in sugarcane (*Saccharum officinarum* L.). *BMC Genom.* **2016**, *17*, 195. [CrossRef]
128. Cunha, C.P.; Roberto, G.G.; Vicentini, R.; Lembke, C.G.; Souza, G.M.; Ribeiro, R.V.; Machado, E.C.; Lagôa, A.M.; Menossi, M. Ethylene-induced transcriptional and hormonal responses at the onset of sugarcane ripening. *Sci. Rep.* **2017**, *7*, 43364. [CrossRef]
129. Kasirajan, L.; Hoang, N.V.; Furtado, A.; Botha, F.C.; Henry, R.J. Transcriptome analysis highlights key differentially expressed genes involved in cellulose and lignin biosynthesis of sugarcane genotypes varying in fiber content. *Sci. Rep.* **2018**, *8*, 11612. [CrossRef] [PubMed]
130. Nishiyama, M.Y., Jr.; Ferreira, S.S.; Tang, P.-Z.; Becker, S.; Poertner-Taliana, A.; Souza, G.M. Full-length enriched cDNA libraries and ORFeome analysis of sugarcane hybrid and ancestor genotypes. *PLoS ONE* **2014**, *9*, e107351. [CrossRef] [PubMed]
131. Santa, B.A.B.; Rojas, C.A.; Grativol, C.; de Armas, E.M.; Entenza, J.O.; Thiebaut, F.; Lima, M.F.; Farrinelli, L.; Hemerly, A.S.; Lifschitz, S.; et al. Sugarcane transcriptome analysis in response to infection caused by *Acidovorax avenae* subsp. *avenae*. *PLoS ONE* **2016**, *11*, e0166473.
132. Chu, N.; Zhou, J.R.; Fu, H.Y.; Huang, M.T.; Zhang, H.L.; Gao, S.J. Global gene responses of resistant and susceptible sugarcane cultivars to *Acidovorax avenae* subsp. *avenae* identified using comparative transcriptome analysis. *Microorganisms* **2020**, *8*, 10. [CrossRef]
133. Muthiah, M.; Ramadass, A.; Amalraj, R.S.; Palaniyandi, M.; Rasappa, V. Expression profiling of transcription factors (TFs) in sugarcane X *Colletotrichum falcatum* interaction. *J. Plant. Biochem. Biotechnol.* **2013**, *22*, 286–294. [CrossRef]
134. Sathyabhama, M.; Viswanathan, R.; Malathi, P.; Sundar, A.R. Identification of differentially expressed genes in sugarcane during pathogenesis of *Colletotrichum falcatum* by suppression subtractive hybridization (SSH). *Sugar Tech.* **2016**, *18*, 176–183. [CrossRef]
135. Lin, Z.; Wang, J.; Bao, Y.; Guo, Q.; Powell, C.A.; Xu, S.; Chen, B.; Zhang, M. Deciphering the transcriptomic response of *Fusarium verticillioides* in relation to nitrogen availability and the development of sugarcane pokkah boeng disease. *Sci. Rep.* **2016**, *6*, 29692. [CrossRef]
136. Prasanth, C.N.; Viswanathan, R.; Krishna, N.; Malathi, P.; Sundar, A.R.; Tiwari, T. Unraveling the genetic complexities in gene set of sugarcane red rot pathogen *Colletotrichum falcatum* through transcriptomic approach. *Sugar Tech.* **2017**, *19*, 604–615. [CrossRef]
137. Huang, N.; Ling, H.; Su, Y.; Liu, F.; Xu, L.; Su, W.; Wu, Q.; Guo, J.; Gao, S.; Que, Y. Transcriptional analysis identifies major pathways as response components to *Sporisorium scitamineum* stress in sugarcane. *Gene* **2018**, *678*, 207–218. [CrossRef]
138. McNeil, M.D.; Bhuiyan, S.A.; Berkman, P.J.; Croft, B.J.; Aitken, K.S. Analysis of the resistance mechanisms in sugarcane during *Sporisorium scitamineum* infection using RNA-seq and microscopy. *PLoS ONE* **2018**, *13*, e0197840. [CrossRef] [PubMed]
139. Avellaneda, M.C.; Parco, A.P.; Hoy, J.W.; Baisakh, N. Putative resistance-associated genes induced in sugarcane in response to the brown rust fungus, *Puccinia melanocephala* and their use in genetic diversity analysis of Louisiana sugarcane clones. *Plant. Gene* **2018**, *14*, 20–28. [CrossRef]
140. Zhu, K.; Yuan, D.; Zhang, X.Q.; Yang, L.T.; Li, Y.R. The physiological characteristics and associated gene expression of sugar cane inoculated with *Leifsonia xyli* subsp. *xyli*. *J. Phytopathol.* **2018**, *166*, 44–52.
141. Cia, M.C.; de Carvalho, G.; Azevedo, R.A.; Monteiro-Vitorello, C.B.; Souza, G.M.; Nishiyama-Junior, M.Y.; Lembke, C.G.; Antunes de Faria, R.S.D.C.; Marques, J.P.R.; Melotto, M.; et al. Novel Insights Into the Early Stages of Ratoon Stunting Disease of Sugarcane Inferred from Transcript and Protein Analysis. *Phytopathology* **2018**, *108*, 1455–1466. [CrossRef] [PubMed]

142. Wang, Z.; Li, Y.; Li, C.; Song, X.; Lei, J.; Gao, Y.; Liang, Q. Comparative transcriptome profiling of resistant and susceptible sugarcane genotypes in response to the airborne pathogen *Fusarium verticillioides*. *Mol. Biol. Rep.* **2019**, *46*, 3777–3789. [[CrossRef](#)] [[PubMed](#)]
143. Ali, A.; Na, C.; Ma, P.; Javed, T.; Zaheer, U.; Huang, M.T.; Fu, H.Y.; Gao, S.J. Genome—Wide analysis of mitogen—Activated protein (MAP) kinase gene family expression in response to biotic and abiotic stresses in sugarcane. *Physiol. Plant.* **2021**, *171*, 86–107.
144. Song, S.; Qi, T.; Huang, H.; Ren, Q.; Wu, D.; Chang, C.; Peng, W.; Liu, Y.; Peng, J.; Xie, D. The jasmonate-ZIM domain proteins interact with the R2R3-MYB transcription factors MYB21 and MYB24 to affect jasmonate-regulated stamen development in *Arabidopsis*. *Plant. Cell* **2011**, *23*, 1000–1013. [[CrossRef](#)]
145. Nawae, W.; Shearman, J.R.; Tangphatsornruang, S.; Punpee, P.; Yoocha, T.; Sangsrakru, D.; Naktang, C.; Sonthirod, C.; Wirojsirasak, W.; Ukoskit, K.; et al. Differential expression between drought-tolerant and drought-sensitive sugarcane under mild and moderate water stress as revealed by a comparative analysis of leaf transcriptome. *PeerJ* **2020**, *8*, p–e9608. [[CrossRef](#)]
146. Belesini, A.; Carvalho, F.; Telles, B.; De Castro, G.; Giachetto, P.; Vantini, J.; Carlin, S.; Cazetta, J.; Pinheiro, D.; Ferro, M. De novo transcriptome assembly of sugarcane leaves submitted to prolonged water-deficit stress. *Genet. Mol. Res.* **2017**, *16*, gmr16028845. [[CrossRef](#)]
147. Pereira-Santana, A.; Alvarado-Robledo, E.J.; Zamora-Briseño, J.A.; Ayala-Sumuano, J.T.; Gonzalez-Mendoza, V.M.; Espadas-Gil, F.; Alcaraz, L.D.; Castaño, E.; Keb-Llanes, M.A.; Sanchez-Teyer, F. Transcriptional profiling of sugarcane leaves and roots under progressive osmotic stress reveals a regulated coordination of gene expression in a spatiotemporal manner. *PLoS ONE* **2017**, *12*, e0189271. [[CrossRef](#)]
148. Da Silva, M.D.; de Oliveira Silva, R.L.; Neto, J.R.C.F.; Benko-Iseppon, A.M.; Kido, E.A. Genotype-dependent regulation of drought-responsive genes in tolerant and sensitive sugarcane cultivars. *Gene* **2017**, *633*, 17–27. [[CrossRef](#)] [[PubMed](#)]
149. Liu, X.; Zhang, R.; Ou, H.; Gui, Y.; Wei, J.; Zhou, H.; Tan, H.; Li, Y. Comprehensive transcriptome analysis reveals genes in response to water deficit in the leaves of *Saccharum narenga* (Nees ex Steud.) hack. *BMC Plant Biol.* **2018**, *18*, 250. [[CrossRef](#)]
150. Barnabas, L.; Ramadass, A.; Amalraj, R.S.; Palaniyandi, M.; Rasappa, V. Sugarcane proteomics: An update on current status, challenges, and future prospects. *Proteome* **2015**, *15*, 1658–1670. [[CrossRef](#)] [[PubMed](#)]
151. Vélez-Bermúdez, I.C.; Wen, T.-N.; Lan, P.; Schmidt, W. Isobaric tag for relative and absolute quantitation (iTRAQ)-based protein profiling in plants. In *Plant Proteostasis*; Springer: New York, NY, USA, 2016; pp. 213–221.
152. Que, Y.; Xu, L.; Lin, J.; Ruan, M.; Zhang, M.; Chen, R. Differential protein expression in sugarcane during sugarcane-*Sporisorium scitamineum* interaction revealed by 2DE and MALDI-TOF/MS. *Comp. Funct. Genom.* **2011**, *989016*, 1–10. [[CrossRef](#)] [[PubMed](#)]
153. Barnabas, L.; Ashwin, N.M.R.; Kaverinathan, K.; Trentin, A.R.; Pivato, M.; Sundar, A.R.; Malathi, P.; Viswanathan, R.; Rosana, O.B.; Neethukrishna, K.; et al. Proteomic analysis of a compatible interaction between sugarcane and *Sporisorium scitamineum*. *Proteome* **2016**, *16*, 1111–1122. [[CrossRef](#)] [[PubMed](#)]
154. Su, Y.; Xu, L.; Wang, Z.; Peng, Q.; Yang, Y.; Chen, Y.; Que, Y. Comparative proteomics reveals that central metabolism changes are associated with resistance against *Sporisorium scitamineum* in sugarcane. *BMC Genom.* **2016**, *17*, 800. [[CrossRef](#)]
155. Song, Q.Q.; Singh, P.; Singh, R.K.; Song, X.P.; Li, H.P.; Nong, Y.Y.; Yang, L.T.; Li, Y.R. Proteomic analysis of sugarcane-*Sporisorium scitamineum* interaction based on iTRAQ technique. *Acta Agron. Sin.* **2019**, *45*, 55–69. [[CrossRef](#)]
156. Amalraj, R.S.; Selvaraj, N.; Veluswamy, G.K.; Ramanujan, R.P.; Muthurajan, R.; Palaniyandi, M.; Agrawal, G.K.; Rakwal, R.; Viswanathan, R. Sugarcane proteomics: Establishment of a protein extraction method for 2-DE in stalk tissues and initiation of sugarcane proteome reference map. *Electrophoresis* **2010**, *31*, 1959–1974. [[CrossRef](#)]
157. Ashwin, N.M.R.; Barnabas, L.; Sundar, A.R.; Malathi, P.; Viswanathan, R.; Masi, A.; Kumar, G.A.; Rakwal, R. Comparative secretome analysis of *Colletotrichum falcatum* identifies a cerato-platanin protein (EPL1) as a potential pathogen-associated molecular pattern (PAMP) inducing systemic resistance in sugarcane. *J. Proteom.* **2017**, *169*, 2–20. [[CrossRef](#)]
158. Meng, J.Y.; Ntambo, M.S.; Rott, P.C.; Fu, H.Y.; Huang, M.T.; Zhang, H.L.; Gao, S.J. Identification of differentially expressed proteins in sugarcane in response to infection by *Xanthomonas albilineans* using iTRAQ quantitative proteomics. *Microorganisms* **2020**, *8*, 76. [[CrossRef](#)]
159. Sugiharto, B.; Ermawati, N.; Mori, H.; Aoki, K.; Yonekura-Sakakibara, K.; Yamaya, T.; Sugiyama, T.; Sakakibara, H. Identification and characterization of a gene encoding drought-inducible protein localizing in the bundle sheath cell of sugarcane. *Plant. Cell Physiol.* **2002**, *43*, 350–354. [[CrossRef](#)] [[PubMed](#)]
160. Jangpromma, N.; Kitthaisong, S.; Daduang, S.; Jaisil, P.; Thammisirak, S. 18KDa protein accumulation in sugarcane leaves under drought stress conditions. *Kmitl Sci. Technol. J.* **2007**, *7*, 44–54.
161. De Almeida, C.M.; Silva, T.D.; Malafaia, C.B.; do Amaral, D.O.; Arruda, I.D.; de Brito, G.G.; Donato, V.; da Silva, M.V.; Correia, M.D. Proteomic and physiological analysis of response to water deficit in sugarcane. *Wudpecker J. Agric. Res.* **2014**, *2*, 1–7.
162. Salvato, F.; Loziuk, P.; Kiyota, E.; Daneluzzi, G.S.; Araújo, P.; Muddiman, D.C.; Mazzafera, P. Label—free quantitative proteomics of enriched nuclei from sugarcane (*Saccharum* ssp) stems in response to drought stress. *Proteome* **2019**, *19*, 1900004. [[CrossRef](#)] [[PubMed](#)]
163. Pacheco, C.M.; Pestana-Calsa, M.C.; Gozzo, F.C.; Mansur Custodio Nogueira, R.J.; Menossi, M.; Calsa Junior, T. Differentially delayed root proteome responses to salt stress in sugar cane varieties. *J. Proteome Res.* **2013**, *12*, 5681–5695. [[CrossRef](#)] [[PubMed](#)]

164. Murad, A.M.; Molinari, H.B.C.; Magalhães, B.S.; Franco, A.C.; Takahashi, F.S.C.; Franco, O.L.; Quirino, B.F. Physiological and proteomic analyses of *Saccharum* spp. grown under salt stress. *PLoS ONE* **2014**, *9*, e98463.
165. Passamani, L.Z.; Barbosa, R.R.; Reis, R.S.; Heringer, A.S.; Rangel, P.L.; Santa-Catarina, C.; Grativol, C.; Veiga, C.F.; Souza-Filho, G.A.; Silveira, V. Salt stress induces changes in the proteomic profile of micropropagated sugarcane shoots. *PLoS ONE* **2017**, *12*, e0176076. [[CrossRef](#)]
166. Sumner, L.W.; Mendes, P.; Dixon, R.A. Plant metabolomics: Large-scale phytochemistry in the functional genomics era. *Phytochem* **2003**, *62*, 817–836. [[CrossRef](#)]
167. Ferreira, D.A.; Martins, M.; Cheavegatti-Gianotto, A.; Carneiro, M.S.; Amadeu, R.R.; Aricetti, J.A.; Wolf, L.D.; Hoffmann, H.P.; de Abreu, L.G.; Caldana, C. Metabolite profiles of sugarcane culm reveal the relationship among metabolism and axillary bud outgrowth in genetically related sugarcane commercial cultivars. *Front. Plant. Sci.* **2018**, *9*, 857. [[CrossRef](#)]
168. Jayaraman, K.; Gokalp, I.; Petrus, S.; Belandria, V.; Bostyn, S. Energy recovery analysis from sugar cane bagasse pyrolysis and gasification using thermogravimetry, mass spectrometry and kinetic models. *J. Anal. Appl. Pyrolysis* **2018**, *132*, 225–236. [[CrossRef](#)]
169. Hao, N.; Lu, K.; Ben, H.; Adhikari, S.; Lacerda, T.B.; Ragauskas, A.J. Effect of autohydrolysis pretreatment conditions on sugarcane bagasse structures and product distribution resulting from pyrolysis. *Energy Technol.* **2018**, *6*, 640–648. [[CrossRef](#)]
170. Naron, D.; Collard, F.-X.; Tyhoda, L.; Görgens, J. Production of phenols from pyrolysis of sugarcane bagasse lignin: Catalyst screening using thermogravimetric analysis–Thermal desorption–Gas chromatography–Mass spectroscopy. *J. Anal. Appl. Pyrolysis* **2019**, *138*, 120–131. [[CrossRef](#)]
171. Mahlanza, T.; Rutherford, R.S.; Snyman, S.J.; Watt, M.P. Methylglyoxal-induced enhancement of somatic embryogenesis and associated metabolic changes in sugarcane (*Saccharum* spp. hybrids). *Plant. Cell Tissue Organ. Cult.* **2019**, *136*, 279–287. [[CrossRef](#)]
172. Lehrer, A.T.; Moore, P.H.; Komor, E. Impact of sugarcane yellow leaf virus (SCYLV) on the carbohydrate status of sugarcane: Comparison of virus-free plants with symptomatic and asymptomatic virus-infected plants. *Physiol. Mol. Plant. Pathol.* **2007**, *70*, 180–188. [[CrossRef](#)]
173. Marquardt, A.; Scalia, G.; Joyce, P.; Basnayake, J.; Botha, F.C. Changes in photosynthesis and carbohydrate metabolism in sugarcane during the development of Yellow Canopy Syndrome. *Funct. Plant. Biol.* **2016**, *43*, 523–533. [[CrossRef](#)] [[PubMed](#)]
174. Schaker, P.D.; Peters, L.P.; Cataldi, T.R.; Labate, C.A.; Caldana, C.; Monteiro-Vitorello, C.B. Metabolome dynamics of smutted sugarcane reveals mechanisms involved in disease progression and whip emission. *Front. Plant. Sci.* **2017**, *8*, 882. [[CrossRef](#)]
175. Marcos, F.C.; Silveira, N.M.; Mokochinski, J.B.; Sawaya, A.C.; Marchiori, P.E.; Machado, E.C.; Souza, G.M.; Landell, M.G.; Ribeiro, R.V. Drought tolerance of sugarcane is improved by previous exposure to water deficit. *J. Plant. Physiol.* **2018**, *223*, 9–18. [[CrossRef](#)]
176. Silva, K.I.; Sales, C.R.; Marchiori, P.E.; Silveira, N.M.; Machado, E.C.; Ribeiro, R.V. Short-term physiological changes in roots and leaves of sugarcane varieties exposed to H₂O₂ in root medium. *J. Plant. Physiol.* **2015**, *177*, 93–99. [[CrossRef](#)]
177. Rasheed, R.; Wahid, A.; Farooq, M.; Hussain, I.; Basra, S.M. Role of proline and glycinebetaine pretreatments in improving heat tolerance of sprouting sugarcane (*Saccharum* sp.) buds. *Plant. Growth Regul.* **2011**, *65*, 35–45. [[CrossRef](#)]
178. Molinari, H.B.C.; Marur, C.J.; Daros, E.; De Campos, M.K.F.; De Carvalho, J.F.R.P.; Filho, J.C.B.; Pereira, L.F.P.; Vieira, L.G.E. Evaluation of the stress-susceptible production of proline in transgenic sugarcane (*Saccharum* spp.): Osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiol. Plant.* **2007**, *130*, 218–229. [[CrossRef](#)]
179. Chiconato, D.A.; Junior, G.d.S.S.; dos Santos, D.M.M.; Munns, R. Adaptation of sugarcane plants to saline soil. *Environ. Exp. Bot.* **2019**, *162*, 201–211. [[CrossRef](#)]
180. Theerawitaya, C.; Tisarum, R.; Samphumphuang, T.; Singh, H.P.; Takabe, T.; Cha-um, S. Expression levels of vacuolar ion homeostasis-related genes, Na⁺ enrichment, and their physiological responses to salt stress in sugarcane genotypes. *Protoplasma* **2020**, *257*, 525–536. [[CrossRef](#)] [[PubMed](#)]