Chapter 9
Genomic Designing for Abiotic Stress Resistant Sugarcane

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Abstract Abiotic stress in crops is a relevant and persistent problem in global agriculture scenario. Crop production technologies of the past may not hold good for the future with the climate change challenge looming in the horizon and is happening right away as we read this chapter. Several crops, traditional seeds and knowledge are lost in the battle with nature yet, the ever resilient human spirit brings in new set of tools with the help of scientific interventions to feed the increasing demand from the global population. It is heartening to see that for every challenge we face, there is a bigger network of solutions from different parts of the world. We have learnt and continue to alter our agricultural practices, food habits, and energy consumption and apply sustainable efforts for saving the soil, water and other natural resources. However, there is always little we can do when it comes to nature. With this background, the abiotic stress and its effect on an important commercial, industrial and food crop, sugarcane is discussed in this chapter. Although the modern cultivars are hybrids derived from progenitor species, efforts are underway in broadening the genetic base of sugarcane with different traits obtained from a wide germplasm pool, that includes other genera as well, to meet the current demands like drought tolerance, increased biomass for industrial and pharmacological applications, biofuel and energy related applications and finally as a sugar crop of the tropics. Various abiotic stresses and their effect on the sugarcane growth and development, the status, progress and futuristic aspects of tackling them to design a better sugarcane crop with genomics tool are discussed.

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9.1 Introduction

Sugarcane is an important commercial crop grown primarily for sugar production. Almost 85% of the total global sugar production comes from sugarcane. The bagasse and molasses, byproducts of sugar industry are used in first- and second-generation biofuel production and bioplastic manufacturing worldwide. Globally sugarcane is grown in 2.78 M ha in 92 countries producing 1.9 billion tons of cane (FAOSTAT 2020). The major sugarcane growing countries are Brazil, India, Thailand and China accounting for nearly 70% of acreage and 71% of total production. The total revenue generated and the impact on farmer's livelihood aggregately contribute immensely to India's agricultural economy. It provides employment, and livelihood security to the farmers mostly settled in developing countries. Sugarcane is seen as a potential crop to meet the rising sugar and energy demand. The huge biomass produced provides many opportunities for myriad applications in various industries. As the opportunities are many so are the challenges in production and processing of sugarcane. One of the major production constraints experienced by a long duration crop like sugarcane is abiotic stress, which takes nearly 10 months for harvesting. The changing climatic conditions in the last few decades had been major challenge in agriculture globally. In India sugarcane is planted during March to May which is peak of summer and water scarcity. The heavy rains during the vegetative stage demands waterlogging tolerance in sugarcane. Reduction up to 15–45% of cane yield is observed under waterlogged condition. In the later stages, coinciding with the time of harvesting, the crop experiences severe cold in the winters during December to January. In Brazil sugarcane is cultivated year-round and varieties are developed to mitigate drought, frost and lodging resistance. The degrading soil and water conditions add to the existing stress to the crop. High salt concentration lowers the osmotic potential which causes stunted growth reducing the cane yield up to 50% (Akhtar et al. 2003; Wiedenfeld 2008). The crop is highly prone to reduced fertility in degrading soils causing nutrition deficit, a physiological stress affecting entire crop growth and development.

Mitigating abiotic stress in sugarcane and its management is devised worldwide through systematic research and development. The focus of enhancing sugar yield vis-à-vis imparting inherent resistance to biotic stress and tolerance to abiotic stress requires an integrated approach in sugarcane improvement program. Understanding various mechanisms of sugarcane biotic and abiotic stress tolerance at phenotypic, physiological, biochemical and molecular level and addressing with the conventional and modern biotechnological tools is the right approach for sugarcane improvement. Some of the strategies that are (may be) effective in sugarcane improvement are (i) marker assisted selection (MAS) for traits governed by one or few genes, thereby use of functional genomics to develop DNA based markers for selection. Mapping and tagging of genes/quantitative trait loci (QTLs) had been less effective in improvement of quantitative traits in sugarcane. However, it provided better understanding of the crop's response to various stress. (ii) Structural and functional genomics in sugarcane have led to generation of enormous data on genomic constitution and spatio-temporal expression of gene sets. Sugarcane is a unique crop which has complex genome(s)

and the economic part is the culm that accumulates high sucrose. The complexity in enhancing the economic part is two folds; the crop has to produce high biomass as well as high sucrose. The stress factor adds to the complexity of genetic networking exponentially within the crop system. (iii) Technologies to change the genetic regulation like gene silencing and use of micro-RNA. (iv) Gene editing tools to develop novel phenotype or alter the regulatory mechanism for higher yield and imparting biotic and abiotic stress tolerance. An overview of abiotic stress and the recent developments in sugarcane abiotic stress tolerance is discussed in this chapter.

9.2 Physiology of Abiotic Stresses in Sugarcane

Fertile soil and good quality water makes sugarcane thrive very well, however most of the arable land globally is drought prone, degraded or contaminated with heavy metals. A very low proportion of arable land is irrigated; the water has become hard with high concentration of Ca^{2+} and Mg^{2+} salts. The changing climatic condition has led to increase in global temperature by 1.5 °C. Sugarcane crop is semi-perineal which makes the crop vulnerable to range of abiotic stresses in one crop cycle, starting from drought/low moisture stress, high temperature, waterlogging, salinity, heavy metals and cold. These abiotic stresses are complex and are interrelated at the molecular and gene expression level. Drought and heat stress and salinity are highly interrelated that the condition aggravates causing irreparable damage in sugarcane and crops in general. Sugarcane thrives well under large amount of water however it cannot withstand prolonged exposure to saturated waterlogging stress. Optimal temperature for growth and development is essential in sugarcane, as it succumbs to high temperature, water deficit or soil salinity. Individual or combined effect of abiotic stress factors trigger yield loss, with significant effect on juice quality and sugar recovery. Sugarcane possess certain favorable morphological, physiological and biochemical adaptations to abiotic stress situations which is regulated by intricately woven molecular networks. The physiology of the crop is significantly affected under abiotic stress which can be observed as stunted growth, reduced leaf margins, low chlorophyll content, wilting, chlorosis, necrosis, leaf drying and senescence, with lethal effects under severe stress (Gandonou and Skali-Senhaji 2015; Endres et al. 2016; Phan et al. 2016; Shrivastava et al. 2017; Javed et al. 2020; Wang et al. 2020; Kaur et al. 2022). At molecular level the plant system shows similar abiotic stress responses like inducing reactive oxygen species (ROS), proline and abscisic acid, ethylene responsive factors and gene expression modified by transcription factors like bZIP, WRKY, WUS, LFY and DREB. Identifying sugarcane genotypes tolerant to various abiotic stresses and understanding the component traits imparting tolerance would help in sustaining the production of sugar and bioenergy, as efficient use of the dwindling agricultural inputs is the need of the hour in the scenario of global climate change.

The morphological indicators in sugarcane for abiotic stress are very important for screening large accessions or breeding lines in field evaluation trials. Trait specific

characterization based on morphological indicators like leaf rolling, pigmentation, chlorosis and necrosis under abiotic stress condition will aid in selection and identification of stress tolerant lines. A genotype with higher degree of stress tolerance can be used as a genetic stock in crop improvement. Other than morphological traits a narrow down approach to physiological indicators like relative water content (RWC) in leaf, root elongation, water use efficiency (WUE), photosynthetic rate (Pn), membrane stability, ionic flux, chlorophyll content and levels of proline or abscisic acid help in understanding the underlying mechanism of stress tolerance. Phytohormones have a major role in stress adaptation, wherein abscisic acid (ABA), ethylene, and cytokinins are implicated in perception, integration, and transduction of various environmental cues to alleviate abiotic stresses (Wilkinson et al. 2012). Plants exposed to moisture stress, high temperature, salinity, and cold stress respond with enhanced ABA accumulation, resulting in cellular dehydration. Based on the physiological mechanism of stress mitigation in sugarcane, in-depth studies made through genomics, transcriptomics and proteomics in model plants will unravel the complete networking of genetic regulation underlying the mechanism of stress tolerance.

9.2.1 Moisture Stress and Heat Tolerance

Sugarcane is one of the high water demanding crops, requiring 1000 to 2900 mm irrigation water, depending on the agroecological conditions (Robertson and Muchow 1997). WUE measured in Hawaii, Australia, and South Africa ranged from 4.8 to 27.0 tons cane per mega liter of water (Kingston 1994; Robertson and Muchow 1997). Since majority of water absorbed by plants is lost as transpiration, only 1–2% of the water is utilized by plants for photosynthetic and metabolic processes. Moderate moisture stress at actively growing stage of the sugarcane crop with fully developed canopy can lead to as much as 60% reduction in biomass (Robertson et al. 1999) (Fig. 9.1). Planted setts experiencing moisture stress inhibits root meristem which leads to poor establishment of sugarcane crop (Panje and Rao 1964). Soil moisture potential close to zero is the most ideal condition for sprouting of buds and at −2.0 MPa, the germination of buds completely ceases (Inman-Bamber and Smith

Fig. 9.1 Effect of moisture deficit stress on sugarcane at grand growth phase. *Source* Krishnapriya et al. (2020)

2005). Root hydraulic conductance is closely associated with leaf area expansion. Due to the strong correlation between root length and total leaf area, the latter may be used as a surrogate trait to screen for root length density (Van Antwerpen 1999).

Culm elongation is the most sensitive character to moisture stress in sugarcane (Nable et al. 1999; Koonjah et al. 2016), followed by leaf elongation, which in turn reduces photosynthetic area and total canopy Pn (Inman-Bamber and Smith 2005; Koonjah et al. 2016; Singels et al. 2010). Sugarcane in general is relatively tolerant to moisture stress, but even under moderate stress, the crop yield may be drastically reduced (Basnayake et al. 2012). Greater shoot number with stunted growth, larger length of roots with higher rate of root extension per day facilitate mining of water from deeper water tables while drought tolerance was also related to capacity of producing fresh functional roots under very low moisture conditions (Shrivastava et al. 2003). Rate of expansion in young internode declines with moisture stress along with decreasing RWC (Vasantha and Rao 2003). Thin stalked varieties with more number of millable canes, lower shoot: root ratio, deeper and extensive root system aids in maintaining higher leaf sheath moisture at 105–165 days after planting (Shrivastava et al. 2015). Simulation studies using APSIM-Sugarcane model indicated that increasing the rooting depth resulted in 20% increase in biomass accumulation, as deeper rooting was beneficial in the shallow than the deep soil which had a smaller fraction of stored water (Inman-Bamber et al. 2012).

Under water stressed conditions, root hydraulic conductance of drought-tolerant cultivars was twice that of susceptible clones (Saliendra and Meinzer 1992). Clones with higher root hydraulic conductance maintainthe isohydric condition in even under severe moisture deficit in soil. The crop maintains relatively constant leaf water potential by regulating stomatal closure (Saliendra and Meinzer 1989), coordinated by stomatal action, water status of the roots and root-derived signaling metabolites in the xylem sap (Meinzer and Grantz 1990; Meinzer et al. 1991). Leaf water potential and Pn show a strong correlation in sugarcane, wherein for every 0.1 MPa decrease in water potential, Pn decreases by 1.6 μmol m^{-2} s^{-1}. For every 1 °C rise in leaf temperature from 25 to 45 °C, Pn reduced by 0.4 μmol m^{-2} s^{-1}. With decline in the available soil moisture due to stress, water potential, osmotic potential and RWC in sugarcane varieties reduced by 46, 50 and 25%, respectively (Pooja et al. 2018). Leaf anatomical characters associated with drought tolerant sugarcane genotypes are lower frequency of stomata and stomatal index, and smaller cell sizes (Shrivastava et al. 2003). Water stress significantly reduced SPAD chlorophyll index, stomatal conductance (gs), Pn, transpiration rate (Tr) and transpiration use efficiency of photosynthesis, leading to significant reduction in shoot biomass (Zhao et al. 2013; Pooja et al. 2018). These indices may be useful for evaluation of genotypes for stress tolerance. Simulation studies indicated that increased intrinsic WUE and reduced gs leading to increased transpiration efficiency are best traits for selection of sugarcane clones in water-limited environments (Inman-Bamber et al. 2012). Traits such as gs, Tr and SPAD chlorophyll index may be given priority during breeding programs for drought tolerance and also to promote considerable gains in Pn (da Silva et al. 2012). Sugarcane clones with significant reduction in canopy temperature depression (CTD), chlorophyll fluorescence (F_v/F_m) and leaf rolling index under water-limited

condition recorded significant positive correlation with cane yield, indicating their usefulness for selecting tolerant clones (Arunkumar et al. 2020).

Under natural field condition, moisture and heat stress occur simultaneously, nevertheless temperature of 38 °C increased leaf and tiller emergence in sugarcane (Bonnett et al. 2006). In general, sugarcane is tolerant to heat stress, which is evident by some of the practices followed in sugarcane cultivation. In Australia, sugarcane setts are subjected to high temperature (52 °C) for 2 h as a part of phytosanitization. High temperature not exceeding 38 °C with two folds elevated CO_2 level resulted in significantly higher leaf area, dry matter production and juice volume (Vu et al. 2006). These studies indicate that heat tolerance can be used as a proxy trait to select drought tolerant sugarcane genotypes. In some of the studies discussed here, high temperature stress decreased leaf chlorophyll content, chlorophyll stability index (CSI), F_v/F_m, Pn, Tr, RWC and activity of enzymes such as nitrate reductase, sucrose phosphate synthase (SPS), sucrose synthase (SS), acid invertase (AI) and neutral invertase (NI) (Kohila and Gomathi 2018). On the contrary, activity of antioxidant enzymes peroxidase (POX) and superoxide dismutase (SOD) in sugarcane increased up to 15 h of exposure to 40 °C temperature stress and declined afterwards. High temperature tolerant isoforms of POX and SOD protect the cells from oxidative damage under heat stress suggesting that the plants have developed enzymatic control of scavenging the ROS under short term stress (Gomathi et al. 2013). High temperature also induced proline accumulation, total phenolics content, lipid peroxidation and soluble sugar content in all clones irrespective of their tolerance potential (Kohila and Gomathi 2018).

Elevated abscisic acid (ABA), K^+ flux and proline are biochemical indicators of response to moisture and heat stress in sugarcane. These are the major osmotic regulators in sugarcane. ABA is involved in water stress signaling and regulates stomatal conductance for maintaining water balance in sugarcane (Riera et al. 2005; Wilkinson and Davies 2010). ABA and K^+ flux has more important role in maintaining osmotic balance in sugarcane whereas, proline mostly reduces the stress-induced cellular acidification, enabling the synthesis of nucleotides and secondary metabolites to drive growth during the stress or recovery period (Hare and Cress 1997). High osmotic pressure, along with high solute concentration, less chlorophyll and carotene content, (Shrivastava et al. 2003), proline accumulation, high ratio of unsaturated fatty acids with lower membrane permeability (Shrivastava et al. 2015) are important features of drought and thermal (50–57 °C) tolerance. Peroxidase and IAA oxidase activity doubled during moisture stress, while the increase in polyphenol oxidase activity was four fold, which reverted to normal on stress recovery (Vasantha and Rao 2003). Severe water stress increased total soluble carbohydrates, proline and lipid peroxidation in sugarcane varieties (Pooja et al. 2018), whereas high temperature tolerant sugarcane genotypes exhibited higher chlorophyll content, CSI and RWC with significantly low level of lipid peroxidation and membrane injury (Kohila and Gomathi 2018).

9.2.2 Salinity Stress Tolerance

Sugarcane is a glycophyte with low tolerance to considerable high sodium ion concentration in soil. The high salt stress leads to reduction in cane yield and sucrose accumulation (Patade et al. 2011). At salinity levels of ~14 dS/m, about 50% reduction in bud sprouting and plantlet establishment was reported across a range of cultivars (Wahid and Rasul 1997; Akhtar et al. 2003). The salt tolerant genotypes similar to the moisture stress tolerant lines have shown higher Pn, gs, and shoot growth than sensitive ones at 2–12 dS/m salinity levels (Meinzer et al. 1994). Morphological traits such as pink pigmentation and waxiness in leaves, accumulation of nitrogenous solutes, restrained chlorine uptake and/or its accumulation in leaf tissue helps sugarcane to adapt to salinity stress (Shrivastava et al. 2015). Salinity reduces chlorophyll content (Winicov and Button 1991) and an overall reduction in Pn is observed (Burman et al. 2003). Juice yield and sucrose content drastically declined in sugarcane grown in salt affected soils (Lingle and Wiegand 1997).

Salt stress induces osmotic stress caused externally on plant roots and disturbs the ionic flux inside of the cell (Munns and Termaat 1986). There is surge in Na^+ ions in the cell which causes reduction in K^+/Na^+ balance in cell. Significant decrease in sugarcane biomass accumulation is observed in genotypes grown in salinity of 10 dS/m (Rao et al. 2021). High K^+ content in the juice indicates the salt-tolerant behavior of sugarcane cultivars (Lingle et al. 2000). It is observed that the K^+ ion is closely associated with moisture stress tolerance which is involved in osmoregulation of leaf water potential in sugarcane. The effect of ion toxicity is pronounced under salinity stress, as enhanced levels of leaf proline content was observed when the sugarcane plantlets were exposed to iso-osmotic stress imposed by NaCl as against mannitol (Cha-um and Kirdamanee 2009). Tolerant genotypes showed enhanced proline, polyols, and total free amino acid content than the salinity sensitive ones (Wahid 2004; Gomathi et al. 2010).

Sugarcane genotypes exhibit variation for various levels of salinity (Wahid and Rasul 1997; Vasantha et al. 2010). Progressive stress responses suggested that tolerant varieties showed stable pigment (chlorophyll and carotenoids) levels in plastids and high proline accumulation along with increased activity of oxidative enzymes. Lipid peroxidation was higher in sensitive variety, and the difference between genotypes became significant from fourth day of stress imposition (Vasantha and Rajalakshmi 2009). Gas exchange parameters were not much affected due to salinity during the formative stage, while Pn, Tr and water potential decreased significantly during grand growth of the crop. Significant reduction in sink size including cane length and stem biomass corresponded to the tolerance potential of clones (Vasantha et al. 2010). Salinity in general affected transport of sucrose from the source leaf, wherein the tolerant genotypes exhibited better sucrose biosynthesis as well as efficient partitioning towards sink tissues, reiterating that under stress, carbon allocation and partitioning was more important than the carbohydrate availability per se (Gomathi and Thandapani 2004). Salinity stress imposed at formative phase of the crop led to drastic reduction in SPAD chlorophyll index, F_v/F_m, RWC, stalk height and weight

and other yield attributes (Brindha et al. 2019). The SOS pathway genes including *SOS1* (Na^+/H^+ antiporter), *SOS2* (CIPK), and *SOS3* (CBL) associated with ion homeostasis were reported to play a major role in imparting salinity tolerance in the sugarcane variety Co 85,019. The differential accumulation of Na^+ and K^+ ions in the contrasting genotypes (Co 85,019 and Co 97,010) confirmed the role of SOS pathway in sugarcane (Brindha et al. 2021). Traits such as SPAD chlorophyll index, F_v/F_m, leaf area index and biomass production were drastically reduced under combined stresses of drought and salinity, with significant impact on the juice quality and cane yield (Vasantha et al. 2017). Some sugarcane genotypes screened in controlled salinity conditions exhibited tolerance irrespective of the phenophases and were able to maintain the leaf area at salinity level as high as 21 dS/m. It is suggested to conduct salinity screening in controlled salinity in hydroponics rather in field, owing to the high inter plot variation in soil properties in the latter (Ashraf et al. 2010). High concentration of Na^+ in soils with exchangeable sodium percent (ESP) between 3 and15 are considered sodic (Sumner and Naidu 1997). The sodicity causes poor drainage along with Ca^{2+} deficiency induced by the presence of excess Na^+ ions. Salinity also reduces the activity of nitrate reductase which hampers the uptake of nitrogen assimilation and metabolism (Mahajan et al. 2013; Medeiros et al. 2014).

9.2.3 Waterlogging and Flooding Tolerance

Intermittent flooding and waterlogging in well drained soils is not a major problem in sugarcane. However, regions with high water table up to 15 cm below the soil surface induce waterlogging stress in sugarcane. Sugarcane grown in water table ranging from 30 to 76 cm below soil surface for the entire crop cycle showed no reduction in yield up to the third ratoon (Carter and Floyd 1975). Low-lying areas with high rainfall in USA, Bangladesh, Indonesia and India experience recurring flooding and waterlogging which are one of the major production constraints to sugarcane (Bailey-Serres and Voe-senek 2008). The excess water in soil fills the voids and airspaces causing a significant reduction in O_2, CO_2, and ethylene exchange between the plant and its environment. Plants deprived of oxygen exhibit low levels of aerobic respiration and ATP production, resulting in reduced Pn and consequently plant growth. Under such hypoxic conditions, high concentration of ethylene triggers signal transduction pathways regulating various adaptive and survival responses (Bailey-Serres and Voesenek 2008). Presence of organic compounds in waterlogged soils accelerate the production of free radicals to toxic levels. Leaching and run-off of essential nutrients and secondary metabolites is common during flooding. Although sugarcane is only moderately tolerant to waterlogging stress, the water uptake patterns revealed that transpiration proceeds without much change even under flooding conditions (Chabot et al. 2002).

Flooding resulted in altered morphology including the formation of more number of adventitious roots with large aerenchyma cells Presence of aerenchymatous cells in aerial roots of tolerant cane may be a useful screening tool to identify sugarcane

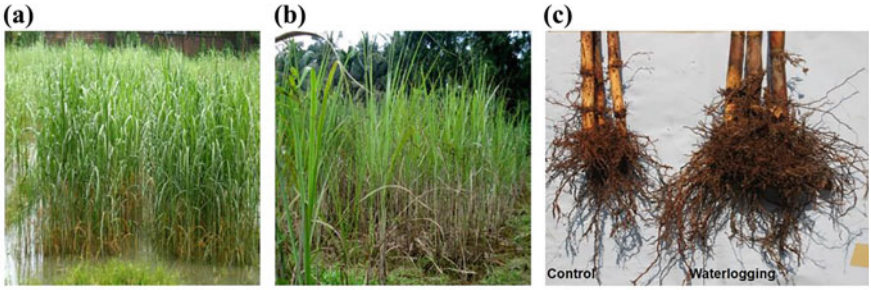

Fig. 9.2 Effect of waterlogging stress on sugarcane growth at **a** formative phase when the stress was predominant, **b** maturity phase, and **c** variation in root morphology due to waterlogging stress. Source for Fig. 9.2a, b: Gomathi et al. (2014); C: unpublished data

cultivars adapted to waterlogging stress (Gilbert et al. 2007). Flooding increased total dry weight of sugarcane, with concomitant increase in leaf, stalk and root biomass (Fig. 9.2). Sugarcane plants exposed to prolonged flooding developed three distinct roots such as reddish-black aerial roots above the water surface, whitish underwater roots from pre-existing root primordia, and thin and pinkish colored negatively geotropic roots. Root growth showed an allometric relationship, increasing along with shoot growth. Pn decreased under waterlogging, but gs and intercellular CO_2 concentration increased, indicating a non-stomatal limitation to Pn. Basal and middle internodes of the sugarcane showed higher concentration of total soluble solids measured as Brix in the flood affected plants (Tetsushi and Karim 2007).

Waterlogging stress significantly increased RWC, proline accumulation and content and activity of antioxidant enzymes SOD and POX, with considerable reduction in chlorophyll and carotenoid content (Bajpai and Chandra 2015). Traits favorable under stress included bobbin-shaped internodes, enhanced activity of polyphenol oxidase, relatively less increase in activity of alcohol dehydrogenase (ADH), aiding the quick restitution of growth upon cessation of flooding (Shrivastava et al. 2015). Leaf and stem expansion id inhibited by waterlogging stress, along with reduced tiller production and altered orientation of shoot extension. Aerial roots are important for continued supply of oxygen to the plant under flooding, in turn contributing to high dry matter production. Ethylene is also implicated in aerenchyma formation in adventitious roots under flooding stress. Genes ADH, ACC oxidase, submergence induced proteins and G-box binding factor-1 were up regulated in tolerant sugarcane varieties (Gomathi et al. 2014).

9.2.4 Cold Stress Tolerance

Sugarcane is generally cold sensitive, although the magnitude of damage depends on the severity and duration of stress, inherent resistance of cultivar, and time lapse and/or temperature fluctuations between cold stress and harvest (Tai and Lentini

1998). The wide variation in response to cold stress among sugarcane varieties was evident from field observations. wherein, sub-tropical clones were more tolerant to stress compared to their tropical counterparts (Du et al. 1999). Using the available germplasm to identify cold-responsive genes in sugarcane would greatly advance conventional breeding programs as well as development of transgenic plants with improved tolerance to cold stress. Requirement of an optimum growing temperature is one of the common factors limiting the geographical distribution and growing season of many plant species. Sugarcane cultivation, mostly restricted to tropical and sub-tropical regions does not experience drastic reduction in temperature during its crop season, although significant reduction in yield and juice quality has been observed in the event of cold stress. Cold acclimation is regulated at the post-transcriptional level in sensitive crops such as rice, corn, cotton and tomato, wherein ice formation occurs during cold stress. Several genes induced by drought stress are also expressed during cold stress, as they are commonly regulated by the phytohormone ABA. When the gene encoding the enzyme isopentenyltransferase (ipt) was overexpressed in sugarcane cv. RB855536 along with a cold inducible promoter *AtCOR15a*, the senescence rate of excised leaves subjected to low temperature reduced considerably as compared to control plants (Belintani et al. 2012). Overexpression of *ipt* gene enhanced cold tolerance of non-acclimated whole plants, with 31% higher total chlorophyll content compared to control plants, reduced malondialdehyde content, and electrolyte leakage indicating less damage due to stress. Expression of *ipt* driven by the stress inducible *COR15a* promoter enhanced tolerance to cold stress in sugarcane without negative effect on plant growth. In a similar study, the gene encoding α-tubulin from tolerant sugarcane variety GT28 (*SoTUA*)) was overexpressed in cold susceptible variety ROC22 (Chen et al. 2020). Increased expression of α-tubulin in the transgenic lines improved the soluble protein, sugars and peroxidase activity, while malondialdehyde content reduced considerably under chilling treatment as compared to control plants. Likewise, cold-defense related genes showed higher expression in the transgenic lines overexpressing *SoTUA*, indicating its protective role during chilling stress (Chen et al. 2021).

9.3 Sugarcane Genetic Resources for Abiotic Stress Tolerance

Sugarcane belongs to *Andropogoneae* tribe in the family *Poaceae*. The subtribe *Saccharine* includes the genus *Saccharum* and other related genera such as *Erianthus* (Fig. 9.3a), *Miscanthus*, *Narenga* and *Sclerostachya*. Genus *Saccharum* comprises three cultivated species *Saccharum officinarum*, *S. barberi* and *S. sinense*, and three wild species *S. robustum*, *S. spontaneum* (Fig. 9.3b) and *S. edule*. These six species of *Saccharum* along with related genera form the basic genetic resources of sugarcane, together termed as the 'Saccharum Complex' (Mukherjee 1957; Daniels et al. 1975). The entire gamut of sugarcane genetic resources is conserved in two world

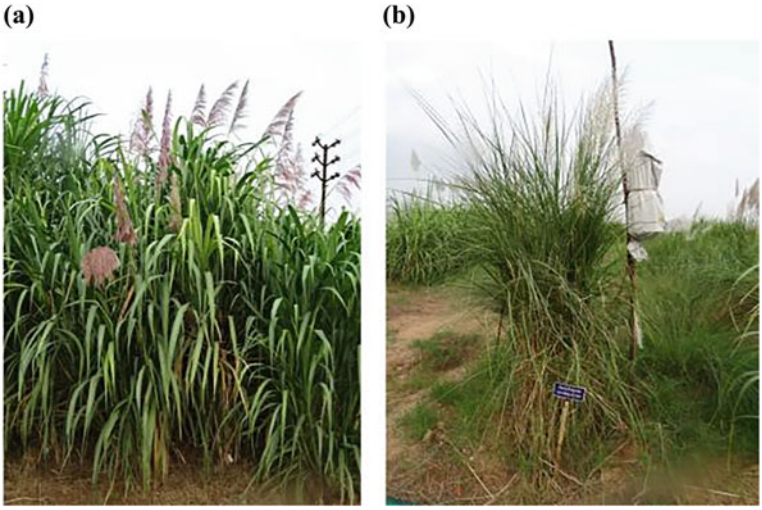

Fig. 9.3 Valuable genetic resources for abiotic stress tolerance in sugarcane (A) *Erianthus* spp. and (B) *Saccharum spontaneum* (b) clones

repositories; one in India at the ICAR-Sugarcane Breeding Institute (SBI) Regional Centre, Kannur, Kerala and the other in USA at the World Collection of Sugarcane and Related Grasses (WCSRG), Miami, Florida.

The ICAR-SBIRC repository is the world's largest in situ germplasm collection, with 2397 accessions of different *Saccharum* sp., allied genera and man-made historical and commercial hybrids (Table 9.1). Another set of germplasm consisting of 1709 *S. spontaneum*, 406 *Erianthus* sp. and 63 allied genera collected across India and exotic clones from different parts of the world are conserved in the field gene bank of ICAR-SBI Coimbatore (Table 9.1). In addition to this, 2013 clones including Co and

Table 9.1 World collection of sugarcane germplasm available at ICAR-Sugarcane Breeding Institute, India

Location	Species	Accessions
Kannur (Kerala)	*S. officinarum*	757
	S. robustum	129
	S. barberi	42
	S. sinense	30
	S. edule	16
	S. spontaneum	387
Coimbatore (Tamil Nadu)	*S. spontaneum*	1709
	Erianthus arundinaceous	230
	Erianthus sp.	176
	Allied genera	63

Co allied hybrids, interspecific and inter generic hybrids, foreign hybrids and other genetic stocks like CYM, CD clones etc. are maintained at ICAR-SBI, Coimbatore. Few *S. spontaneum* clones collected from Arunachal Pradesh, and *Erianthus* sp. and *Mischanthus sp.* clones collected from Meghalaya are maintained at Indian Agricultural Research Institute (IARI) Regional Station, Wellington, Tamil Nadu. Apart from this, a total of 1380 genotypes including Co canes, Co allied hybrids, exotic clones, inter-specific and inter-generic hybrids, core collection of *S. officinarum*, species clones of *S. barberi*, *S. sinense*, *S. robustum*, *Erianthus* sp., S*clerostachya* sp. and *Narenga* sp. are maintained at ICAR-SBI Research Centre, Agali, Kerala.

One of the important strategies for crop improvement is through wide hybridization, which is possible due to the vast germplasm collection available in sugarcane. Wild germplasm sources impart genes for high biomass producing ability, resistance to pests and diseases and adaptability for growth under different stress conditions in the modern cultivars. Nobilization of sugarcane is one of the pioneering works in the modern history, which involves planned introgression of wild forms of *Saccharum* sp. and related genera into noble canes, to improve yield and ancillary characteristics. The first interspecific hybrid released as a variety of sugarcane (Co 205) was derived from hybridization of *S. officinarum* clone Vellai and *S. spontaneum* clone Coimbatore local, leading the future course of sugarcane breeding across the globe. Modern sugarcane cultivars are complex aneupolyploids derived from the crosses involving *S. officinarum*, *S. spontaneum*, *S. barberi*, *S. sinense* and *S. robustum*. The identified sources of abiotic stress tolerance are given in Table 9.2.

9.3.1 Primary Gene Pool

The primary gene pool comprises commercial sugarcane hybrids, derived from *S. officinarum*, *S. spontaneum*, *S. barberi* and *S. sinense*, because potential of cultivars may be fixed in the first sexual generation itself.

9.3.2 Secondary Gene Pool

The cultivated species *S. officinarum*, *S. barberi* and *S. sinense* form the secondary gene pool, wherein their involvement in crop improvement programs persists for a few generations of breeding.

S. officinarum

Six drought responsive candidate genes viz., *DREB1A*, *NAC2*, *Snac1*, *SHN*1, *SIZ*1 and *PIN3* involved in ABA independent pathway of drought stress response was identified in *S. officinarum* clones. *DREB1A* gene present in Fiji B and Fiji 30 clones, induced other abiotic stress responsive genes in order to maintain the water balance during stress. *NAC2* gene which was reported in rice for cold and salt tolerance was

Table 9.2 Sugarcane genotypes identified as potential sources of different abiotic stress tolerance

Species	Genotypes	References
Moisture stress tolerant genotypes		
S. officinarum	Gungera, 57 NG 73, IJ 76–412, IJ 76–564, Caledonia Ribbon	Vasantha et al. (2017)
S. robustum	NG 77–79, 57 NG-19, NG 77–146, NG 77–23, 57 NG-27, NG 77–38, NG 77–59, NG 77–122, IJ 76–336, IJ 76–337	Vasantha et al. (2017), Priji and Hemaprabha (2014)
S. barberi	Nargori, Lalri, Manga sic, MatnaShaj, Pararia Shaj, Saretha, Pathri, Kewali, Khatuia	Vasantha et al. (2017), Priji and Hemaprabha (2014)
S. spontaneum	TS 76–216, US 56–20-1, Taiwan 96, Pamba, Ponape-1, SES 32A, IND 90–805, IND 90–796, IND 85–503, Tabongo, IND 84–351, Iritty 2, SES 168, SES 600, SES 106B, SES 515/7, SES 561, IND 90–813 and *S. spontaneum* Coimbatore	Vasantha et al. (2017), Priji and Hemaprabha (2014)
S. sinense	Mcilkrum, Reha, Lalkhadi, Kalkya, Kheli, Chukche, Uba white, Ikhri	Vasantha et al. (2017), Priji and Hemaprabha (2014)
ISH clones	ISH 9, ISH 23, ISH 41, ISH 58, ISH 100, ISH 110, ISH 118, ISH 175	Vasantha et al. (2017)
Erianthus sp.	IK76-81, IK 76–48, IK 76–62, IK 76–91, IK 76–99, IND 84–863	Augustine et al. (2015), Priji and Hemaprabha (2014)
Saccharum sp. clones	Co 997, Co 86011, Co 1148, Co89003, Co 720, Co 86032, CoLk 8102, Co 86010, ISH 100, Co 85019, Co 86002, Co 85004, Co 87023, CoC 671, Co 97008, BO 91, Co 88025, Co 2000–10, Co 99006, Co 94008, Co 98008, Co 99008, NS 83/247, Co 740, Co 419	Hemaprabha and Simon (2012)
Salinity stress tolerant genotypes		

(continued)

Table 9.2 (continued)

Species	Genotypes	References
S. officinarum	Blanche reunion, Chapina, Fiji 28, Tjiing Bali, Green german, Horne, Hawaii original 24, Hina Hina 18, IJ 76- 315, IJ 76–316, IJ 76–422, IJ 76–470, Keong, Koelz 1132, Kaludaiboothan, Luzon white, Manteiga 1295, Manteiga 1585, Manjiri red, Maxwell, Mogali, Miavoi, Mikokio- 44, Maur-55 str, Mongetgayam, NC-15, NC-33, NG 21–12, Local red, Waxy red, NG 77–67, NG 77–70, NG 77–92, NG 21–42, 21 NG-2, 21 NG-5, 21 NG-6, 21 NG-21, 28 NG 12, 28 NG 13, 28 NG 21, 28 NG 32, 28 NG 54, 28 NG 68, 28 NG 72, 28 NG 80, 28 NG 87, 28 NG 110, 28 NG 206, 28 NG 210, 28 NG 211, 28 NG 273, 28 NG 287, 51 NG 11, 51 NG 12, 51 NG 14, 51 NG 53, 51 NG 59, 51 NG 77, 51 NG 147, 51 NG 159, 51 NG 287, 57 NG 26, 57 NG 57, 57 NG 67,57 NG 68, 57 NG 100, 57 NG 126, 57 NG 71, 57 NG 159, 57 NG 166, 57 NG 172, 57 NG 184, 57 NG 191, 57 NG 196, 57 NG 198, 57 NG 199, 57 NG 203, 57 NG 237, 57 NG 241, 57 NG 272, 77 NG 15, 77 NG 18, 77 NG 31, 77 NG 32, 77 NG 65, 77 NG 66, 77 NG 117, Old Jamaica, Ogle's selection, Oramboo, Otaheite, Pynmana ribbon, Pattacheruku, Pakaweli -2, Patta Patti, Pohina -51, Selemibali, Shamsara, Sinense, Sarawak unknown, Tahiti -3, Tibbomird, UB-1, UB-14, White transparent, Zwart manila, Tjerpering, Koelz 11,132, 57 NG 78, 57 NG 215, 57 NG 50, 57 NG 212, 57 NG 110, Poona, Caledonia, Fiji 10, IM 76 - 360, IM 76- 252, Katha, Uba white, Ansali, 77 NG 242, Rayada, IJ 76 543, IJ 76 522, IJ 76 556, IJ 76 470, IJ 76 418, IJ 76 316, IJ 76 315, IM 76–507, IM 76–253, IM 76–232, Black Fiji, IK 76–31, 77 NG 1, 77 NG 221, Zwart Cheribon	Vasantha et al. (2017)
S. robustum	28 NG 219, 28 NG 251, 57 NG 6, 57 NG 201, 77 NG-10, 77 NG-26, 77 NG-34,77 NG -55, 77 NG-136, 77 NG-160, 77 NG-167, 77 NG-170, 77 NG-221, 77 NG-237, 57 NG 231	Vasantha et al. (2017)
S. barberi	Khakai, Khatuia-124, Kewali 14G, Kuswarottur, Lalri, Nargori, Pansahi, Pathri, Uba seedling, Reha	Vasantha et al. (2017)

identified from *officinarum* clones viz., 28 NG 224, Keong, 21 NG 2, Fiji B and Fiji 30. The three genes *Snac1*, *SHN1* and *SIZ1* which were reported to be involved in drought tolerance mechanism in other crops. Among these three, *Snac1* was reported to be present in 28 NG 210, SHN 1 in Penang and SIZ 1in Keong. *PIN3* gene a regulator of auxin efflux was found to be present in seven clones viz. 57 NG 136, 28 NG 224, Keong, Mia Moi, 28 NG 210, Fiji B and Fiji 30 (Priji and Hemaprabha 2014).

S. barberi

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were found to be present in *S. barberi* clones Kewali and Khatuia. Saretha clone harboured nine drought responsive genes without *DREB1A* and *Hep2*. Pathri contained all the ten genes excluding *HRD* gene (Priji and Hemaprabha 2014).

S. sinense

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were reported to be present in *S. sinense* clone Ikhri. Chuckche harbored seven drought responsive genes apart from *DREB1A*, *DRF1*, *HRD* and *SHN1*. Uba White clone was with eight drought tolerant genes without *DRF1*, *SHN1* and *Wrky 38* (Priji and Hemaprabha 2014).

9.3.3 Tertiary Gene Pool

S. spontaneum and *S. robustum* constitute the tertiary gene pool, contributing to abiotic stress tolerant traits and improvement in fiber content of the progenies. These are considered tertiary as it takes a number of generations to eliminate their undesirable effects during varietal development.

S. spontaneum

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were reported to be present in *S. spontaneum*. All these 11 genes were found to be present in three *S. spontaneum* clones viz., Iritty 2, SES 600 and *S. spontaneum* Coimbatore. Four drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2* and *Wrky 38 factor* were identified in eight clones of *S. spontaneum* viz., Iritty 2, SES 168, SES 600, SES 106B, SES 515/7, SES 561, IND 90–813 and *S. spontaneum* Coimbatore. All these eight genotypes except SES 168 harbored *Snac1* and *Hep2* genes. Similarly, *HRD*, *SHN1* and *PIN3* were found to be present in all these eight genotypes except SES 106B. SES 515/7 and SES 561 were bestowed with nine drought responsive genes excluding *PIN3* and *SHN1*. IND 90–813 harbored eight

drought responsive genes excluding *DREB1A*, *SIZ1* and *PIN3* (Priji and Hemaprabha 2014).

S. robustum

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A*, and *SIZ1* of ABA independent pathway were found to be present in *S. robustum* clone NG 77–59. The clone IJ 76 33 contained nine drought responsive candidate genes without *DREB1A* and *DRF1* genes. Similarly, nine drought responsive candidate genes excluding *SIZ1* and *Wrky 38* were present in the clones IJ 76 336 and NG 77–122 (Priji and Hemaprabha 2014).

9.3.4 Distant Gene Pool

The allied genera including *Erianthus, Mischanthus, Narenga and Sclerostachya* can be termed as distant gene pool. In this group only *E. arundinaceous* has been utilized for its high biomass and abiotic stress tolerance.

***Erianthus* species**

Eleven drought responsive candidate genes viz., *DRF1*, *NIT1*, *NAC2*, *Wrky 38 factor*, *Snac1*, *Hep2*, *HRD*, *SHN1*, *PIN3*, *DREB1A* and *SIZ1* of ABA independent pathway were reported to be present in five *Erianthus* sp clones (IK 76–48, IK 76–62, IK 76–91, IK 76–99 and IND 84–863) except *Snac1* in IK 76–91, IND 84–363 and *Wrky 38* in IK 76–99, respectively, making it an important source of drought tolerance (Priji and Hemaprabha 2014). In *E. arundinaceous* the expression of *HSP70* was found to be enhanced under moisture stress. The transgenic sugarcane overexpressing *EaHSP70* exhibited enhanced cell membrane thermostability, RWC, gas exchange parameters, chlorophyll content and photosynthetic efficiency under moisture stress. The chlorophyll retention capacity increased in these plants, with higher germination and establishment under salinity stress as compared to control plants. This demonstrates the potential of *EaHSP70* gene for genetic manipulation to induce drought and salt tolerance in sugarcane (Augustine et al. 2015). The *E. arundinaceus* clone IK 76–81 was found to be drought tolerant with increased expression of *DREB2* and expansin genes with increase in soil moisture stress (Augustine et al. 2015).

9.4 Designing Sugarcane for Abiotic Stress Tolerance

Genome organization of sugarcane reveals a complex structure. The sugarcane cultivars are in general interspecific hybrid clones of *Saccharum officinarum* ($2n = 80$ and $X = 10$) and *Saccharum spontaneum* ($2n = 40$ to 128; and also the basic chromosome number variying from $X = 4$ to 8) (D'Hont et al. 1998; Grivet and Arruda 2002). This sugarcane complex (Mukherjee 1957), show a complex ancestry resulted from

interbreeding among the six species of *Saccharum* (i.e. *S. officinarum*, *S. robustum*, *S. spontaneum*, *S. barbari*, *S. sinense*, *S. edule*) and allied genera (i.e. *Erianthus*, *Sclerostachya*, *Miscanthus*, and *Narenga*). In sugarcane breeding, modern cultivars are derivatives of *Saccharum officinarum* and *Saccharum spontaneum* interspecific crosses. *S. officinarum* is contributes thick stalk (biomass) and high sucrose whereas, biotic and abitotic stress tolerance is imparted by *S. spontaneum.* However, *S. spontaneum* is one of the progenitor of *S. officinarum* (Babu et al. 2010)*;* and the ancestry of *S. barbari* and *S. sinense* is traced back to *S. officinarum* and *S. spontaneum* (Amalraj and Balasundaram 2006). The other *Sacchrum* spp and related genera are used in the pre-pre breeding program for introgression of genes for higher biomass, resistance to pest and diseases and tolerance to abiotic stress or enhanced fitness, *Saccharum* species like *S. barberi* and *S. sinense, S. edule* and allied genera like *Erianthus*, *Sclerostachya*, *Miscanthus*, and *Narenga.* The interspecific crosses of promising sugarcane parents are planted to generate a series of hybrid clone which are subsequently selected for yield and sugar related parameters. Multi-location trials are conducted to test stability and suitability of hybrid clones in target environment. The genomic structure or organization is completely ignored, as the clones are selected by planting setts. The unavailability of chromosomal organization and the information on contribution of parents in manifestation of traits does not hamper selection or release of cultivars in sugarcane. Based on pedigree and lineage of commercial cultivars the sugarcane genome can be characterized as complex of multiple genome fragments (homoeologus), polyploid and multiple gene copies. With the available technology it is extremely difficult to ascertain which allele from which genome is expressing and also the inter- and intra – allelic interaction in the genome(s). A complete account of complexity of sugarcane genome and the challenges in genome analysis is passably reviewed by Thirugnanasambandam et al. (2018). The sugarcane improvement program relies more on chromosomal organization rather than recombination in cross derivatives. The cross derivatives show high rate of aneuploids, which masks the effects of recombination occurring in one or two generations. Under this scenario determination the breeding value of the clone is currently not achievable. The expression profiling and localization of large effect QTLs for complex traits in sugarcane are mere dissection of already formed variety. The tremendous diversity of sugarcane transcripts reveal complexity of gene expression networking in sugarcane (Thirugnanasambandam et al. 2017, 2018, 2019, 2020).

9.4.1 Conventional Breeding and Selection Procedure

Sugarcane improvement for abiotic stress tolerance is challenging. Series of intra and inter specific crosses are made to shuffle genome(s) for achieving higher yield and high sucrose content. The most important part of sugarcane breeding is the phenotyping for evaluating yield levels and stability of expression over locations and years. Multi-location evaluation of sugarcane hybrids and selection of clones for target environment is one of strategies which is practiced and followed till date.

Creating facilities for screening of sugarcane for artificial abiotic stress tolerance is highly challenging. Most of the parameters that can play significant role under natural conditions cannot be imitated in artificial conditions. The abiotic stress in sugarcane is mostly location specific and must be addressed by continuous screening in the target locations. All the scientific reports or research publications indicate that the sugarcane genotypes which performed better under near ideal condition have performed better under abiotic stress too.

9.4.2 Genomics Aided Selection in Sugarcane

In sugarcane the nature of hybrids and its progenies generated after every generation of sexual reproduction are unpredictable and hypervariable. Under such situation the prediction of performance of selfed progeny or the hybrid is very low. The low breeding value in the progenies of a hybrid discourages the use of advanced molecular tools devised for enhancing genetic gains in other crops where the chromosomal inheritance is stable, either diploid or polyploid. Some of the major challenges in practicing genomic selection are broadly classified as (i) sequencing and generation of single dose markers (ii) simplification of complexity of genome(s) in sugarcane (iii) discerning the genetic relatedness and estimating the breeding values based on the clones and parents (iv) improving the accuracy of prediction models (v) extension of one genomic selection over locations and over diverse crosses globally. Among the challenges listed above the generation of single dose marker.

9.4.3 Mi-RNA Based Selection

MicroRNAs (miRNAs) are endogenous, evolutionarily conserved RNAs which are between 19 and 24 nucleotides in length. miRNAs are master regulators of post-transcriptional phases of gene expression. They are known to interfere in translational machinery either to prevent or alter protein synthesis. When miRNA are bound to the target mRNAs, the ensuing association of decay factors lead to destabilization of mRNA (Bhaskaran and Mohan 2014). After the discovery of additional gene regulation mechanism by miRNA, researchers devised experiments to alter gene regulation in various organisms. In sugarcane and many other plant species, several studies indicated a strong role of miRNA association with abiotic stress. Involvement of plant miRNAs under stress conditions have been reported by several workers as most of their target genes are induced by stress (Jones-Rhoades and Bartel 2004; Phillips et al. 2007). Regulation of miRNA expression under stress alters the abundance of their target genes (Jagadeeswaran et al. 2009; Lv et al. 2010). Likewise, repression of miRNA causes accumulation of its target, thereby eliciting stress tolerance responses (Sunkar and Zhu 2004). Role of set of differentially expressed miRNA in sugarcane was studied with two cultivars RB867515 (drought tolerant) and

RB855536 (drought sensitive). The miRNA sp-miR394 was down-regulated under drought stress in both tolerant and sensitive sugarcane cultivars, reinstating its role in abiotic stress response. The ssp-miR394 targets the gene encoding a glyceraldehyde-3-phosphate dehydrogenase (GAPDH), while ssp-miR1432 targets bZIP in sugarcane; both these miRNAs were down-regulated under drought in both cultivars. It implies that bZIP is associated with drought response but, activation of transcription factor alone may not be responsible for differential tolerance levels in sugarcane. Under cold stress, 62 of the 412 miRNAs identified in sugarcane showed a significant differential expression (Yang et al. 2017). The cold stress induced upregulation of miR319 in roots and buds was demonstrated by subjecting sugarcane to 4 °C for 24 h (Thiebaut et al. 2012). ABA treatment is also found to trigger the miR319 production in sugarcane, with TCP-PCF5, TCP-PCF6, GAMyb, a protein kinase, and a fasciclin-like glycoprotein, a subclass of arabinogalactan proteins as potential targets. Varying periods of cold stress treatment (0–48 h at 4 °C) induced miR319, with spatial and temporal difference in expression levels in root and shoot tissues (Thiebaut et al. 2012) The up-regulation of miR319 coupled with down-regulation of its targets, a Myb transcription factor (GAMyB) and a TCP transcription factor (PCF5), were observed in cold-tolerant and -sensitive sugarcane varieties exposed to 4 °C. To narrow down the miRNAs implicated in cold tolerance, a tolerant (FN39) and sensitive (ROC22) cultivar of sugarcane was used to generate small RNA libraries, followed by validation through RT-qPCR. The miRNAs involved in targeting of auxin response factors (*ARF*) and transport inhibitor response (*TIR*) genes, miR167 and miR393 showed significant up-regulation under cold stress in both the cultivars. Differential expression of miR160 and miR156 was observed in the cultivars with contrasting cold tolerance nature in sugarcane These findings provide the valuable information for further functional characterization of miRNAs in sugarcane under cold stress. A number of environmental cues which are perceived by plants are transmitted to trigger a cascade of gene expression in response. The variation or the trigger for differential gene expression levels could be due to a number of factors starting from bio-physio-chemical mechanism like osmosis and Na^+ and Ca^{2+} flux signaling (receptors and transporters).

9.4.4 Transcriptomics of Sugarcane Abiotic Stress

Advancement in next-generation sequencing technologies have led to the development of methods for analyzing transcript abundance under various biotic and abiotic stress conditions. Nevertheless, expressed sequence tags (EST) libraries are among the earliest resources for gene discovery in several organisms including agricultural crops. EST databases facilitate large-scale mining of data mining to identify genes involved in specific pathways and traits, and hence prove to be invaluable in analyzing the global response of tissues or whole organisms under stress. A putative

model for global gene expression under cold stress was constructed using ESTs available in the Sugarcane EST Genome Project (SUCEST; http://sucest.lad.ic.unicamp.br) employing high-density filter arrays and extensive data mining (Nogueira et al. 2003). Similarly, ESTs encoding proteins directly involved in chilling tolerance identified till date include WCOR410b (Danyluk et al. 1998), WCOR413 (Allard et al. 1998), dehydrin 2 (DHN2; Zhu et al. 2000), barley ABA-inducible protein (HVA22; Shen et al. 2001), thaumatin-like protein, glucanase-like protein, and chitinase-like protein (Yu and Griffith 1999). In silico analyses confimed the presence of two putative dehydrin like proteins (WCOR410b and DHN2) in sugarcane, which aid in stabilizing macromolecules to protect cellular membranes against chilling injury (Pearce 1999). Thaumatin-like protein, glucanase-like protein (1,3-glucanase), and chitinase like protein are also implicated in pathogenesis-related response in plants, stabilizing the cellular membranes owing to their antifreeze activity, inhibiting leakage across membranes during chilling (Hincha et al. 1997; Pearce 1999; Tomczak et al. 2002). Comparative transcriptomic analysis of cold susceptible sugarcane hybrid (CP72-1210) *versus* cold tolerant *S. spontaneum* (TUS05-05) revealed more than 600 differentially expressed genes in response to cold stress (Park et al. 2015). Expression analysis of one of the differentially expressed genes, encoding a *S. spontaneum* homolog of a NOD26-like major intrinsic protein gene (SspNIP2) showed that cold treatment for 30 min was sufficient to induce SspNIP2 by ~ 2.5 fold, which persisted even up to 24 h of stress exposure. Similarly, transcriptome profiling of low temperature tolerant *S. spontaneum* clone IND 00–1037 collected from high altitude regions of Arunachal Pradesh, India, revealed that about 2583 and 3302 genes were up- and down-regulated due to stress, respectively (Dharshini et al. 2016). Cold-responsive genes such as cold-regulated (COR), dehydrins, LEA proteins, heat shock proteins (HSP), aquaporins and osmolytes play a significant role in cold acclimatization during 24 h exposure to 10 °C stress (Dharshini et al. 2016; Selvarajan et al. 2018). Root transcriptome analyses at different time intervals after stress imposition led to the detection of a total of 4425 differentially expressed transcripts (2715 upregulated and 1710 downregulated). Major genes conferring tolerance to low temperature included COR protein, osmotin, dehydrin, HAL1, chilling tolerant divergence 1 (COLD1) and HSP90, in agreement to previous studies. Further, metabolic sensors such as proline, MDA, calcium-dependent kinase, G-coupled proteins, and histidine kinase were triggered under low temperature stress in *S. spontaneum* roots, activating the signal transduction through MYB, ERF, ARF2, DREB, CAMTA, and C2H2. This resulted in the biosynthesis of annexin, which mediated the plasma membrane calcium permeability and production of cold-responsive genes. Metabolic pathways such as phenylpropanoid which stimulates flavonoid biosynthesis along with synthesis of sucrose, galactose, raffinose, and fructose are involved in triggering cold-responsive TFs.

A de novo assembly of the leaf transcriptome of two sugarcane cultivars (tolerant, SP81-3250 and susceptible, RB855453) were evaluated by the RNA-Seq method. Water deficit stress on the 90th day of stress imposition showed altered gene expression in the tolerant cultivar, while the sensitive cultivar showed differently expressed genes as early as on the 30th day of stress (Belesini et al. 2017). Several important gene families, including aquaporins, late embryogenesis abundant proteins,

auxin related proteins, transcription factors, HSPs, light harvesting chlorophyll a-b binding proteins, disease resistance proteins, and ribosomal proteins were induced in a wild sugarcane type, *S. narenga* exposed to drought stress for 22 days (Liu et al. 2018). Likewise, transcriptomic changes under varying levels of water deficit stress in tolerant hybrid (Co 06022) was compared to susceptible hybrid (Co 8021), revealing a progressive decrease in the expressed genes as the stress period increased from 6 to 10 days (Selvi et al. 2020). The *S. spontaneum* clone GXS87-16 ws considered to be a valuable resistance source to various biotic and abiotic stresses, as it was also profiled for drought responsive genes using RNA-Seq at three water-deficit levels (mild, moderate, and severe) and upon recovery during the elongation stage (Li et al. 2021).

9.4.5 Proteomics

Differentially expressed proteins under moisture and salinity stress were identified in sugarcane genotypes (Sugiharto et al. 2002; Jangpromma et al. 2010; Pacheco et al. 2013; Passamani et al. 2017). Among the many differentially expressing transcripts, ScDR1 and ScDR2 were found to play a predominant role in imparting stress tolerance to sugarcane (Begcy et al. 2012, 2019). With the advancement in screening techniques and use of recombinant DNA technology researchers could put forth the results more convincingly in sugarcane. Chen et al. (2017) reported a novel gene in sugarcane encoding a 10.66 kDa Non-specific Lipid Transfer Protein (*ScNsLTPs*), with 671 bp long cDNA, a 312 bp open reading frame (ORF). Results from RT-qPCR results showed that the overexpression of *ScNsLTPs* under stress was exogenously induced by salicylic acid, PEG and cold. However, treatment with methyl jasmonate downregulated the expression of ScNsLTPs. The genes *ScCBL2-1*, *ScCBL3-1*, and *ScCBL4* in sugarcane possessed ORF in the range of 642 to 678 bp, and encoded polypeptides containing 213 to 225 amino acids. The ScCBL protein expression in transgenic sugarcane was localized in the plasma membrane and cytoplasm. Expression of the CBL genes in *Escherichia coli* cells confirmed their role in enhancing tolerance to salinity (NaCl) and heavy metal ($CuCl_2$) stress. Resistance to invasion by *Ralstonia solanacearum* was observed in *ScCBLs* overexpressed *Nicotiana benthamiana* leaves (Su et al. 2020).

The G-protein-coupled receptors (GPCRs) were implicated in conferring tolerance to multiple abiotic stresses. The GPCRs regulate the G-protein-mediated signaling thereby influencing plant growth, development, and stress responses. The sugarcane ShGPCR1 protein sequence contained nine predicted transmembrane (TM) domains connected by four extracellular and four intracellular loops, which could interact with various ligands and heterotrimeric G proteins in the cells. Abiotic stresses including moisture deficit, salinity and low temperature unregulated the expression of *ShGPCR1*, predominantly localized to the plasma membrane. The protein ShGPCR1 helps in maintaining cell membrane integrity under stress by enhancing intracellular Ca^{2+} levels in response to GTP. Constitutive expression of

ShGPCR1 in transgenic sugarcane led to enhanced expression of genes encoding late embryogenesis abundant protein, dehydrin drought responsive 4, and galactinol synthase under moisture stress; ethylene responsive factor 3, salt overly sensitive 1, and vacuolar Na+/H+ antiporter 1 under salinity stress; and nam/ataf1/2/cuc2, cold responsive factor 2, and alcohol dehydrogenase 3 under cold stress. Transgenic events with overexpression of ShGPCR1 conferred tolerance to drought, salinity and cold stress, confirmed by estimation of RWC in the transgenic stressed plants (Ramasamy et al. 2021). Such stress tolerant transgenic lines may enhance sugarcane production in marginal environments with limited resources. The sugarcane catalase 1 (ScCAT1) protein localized in plasma membrane and cytoplasm was upregulated by smut infection as well as treatments induced by salicylic acid, methyl jasmonate, ABA, H_2O_2, heavy metal ($CuCl_2$), hyper-osmotic (PEG) and salt (NaCl) stresses (Su et al. 2014).

9.4.6 Transgenics for Abtioic Stress Tolerance

Transgenic technology in crops is a powerful tool to introduce novel traits, altering gene expression and silencing. The first transgenic sugarcane plant was developed by Bower and Birch (1992), employing an efficient microprojectile bombardment of the embryogenic callus. The methodology was further optimized for development of herbicide resistant transgenic sugarcane plants (Gallo-Meagher and Irvine 1996). Budeguer et al. (2021) published a comprehensive reviewed of genetic transformation in sugarcane. The list of drought tolerant sugarcane varieties developed though transformation is presented in Table 9.3.

Apart from the structural genes, transcription factors (TFs) may be promising candidates to develop transgenic plants with enhanced tolerance to moisture deficit, salinity and cold stress. The COR/DREB family of TFs were the first to be associated with gene regulation under abiotic stress situation (Moran et al. 1994). Enhanced drought by overexpression of DREB(s) was demonstrated by recording physiological traits like RWC, Pn, sucrose content and bud sprouting in transgenic sugarcane plants exposed to drought stress (Reis et al. 2014; Augustine et al. 2015). Constitutive expression of DEAD-box helicase gene from pea (*PDH45*) improved the salinity tolerance of sugarcane variety Co 86032. Presence of *PDH45* significantly improved cell membrane thermostability, RWC, gas exchange parameters, chlorophyll content, and photosynthetic efficiency of transgenic events of Co 86,032 under moisture stress compared to WT. Overexpression of *PDH45* also led to the upregulation of DREB2-induced downstream stress-related genes in sugarcane, resulting in higher germination ability and better chlorophyll retention compared to WT under salinity stress (Augustine et al. 2015). The role of membrane-bound receptor proteins, such as GPCRs is demonstrated to improved abiotic stress tolerance in sugarcane. GPCRs

Table 9.3 Transgenic sugarcane varieties with improved abiotic stress tolerance

Abiotic stress	Promoter	Candidate gene	Gene function	Variety used for transformation
Drought	P35S enhanced	*Tsase*	Biomolecules stabilization	ROC10
Cold	pCOR15a	*ipt*	Cytoquinin synthesis	RB855536
Drought	P35S	*AVP1*	Osmotic regulation	CP-77–400
Drought	pRab17	*DREB2A CA*	Gene regulation	RB855156
Salinity	pAIPC inducible	*P5CS*	Proline synthesis	RB855156
Drought/Salinity	pUBI	*PDH45/DREB2*	Nucleic acids metabolism, gene regulation	Co 86032
Drought/Salinity	pUBI	*HSP70*	Cellular components stabilization	Co 86032
Drought	pUBI	*BI-1*	Programmed cell death regulation	RB835089
Drought	P35S enchanced	*AVP1*	Osmotic regulation	CSSG-668
Drought	pUBI	*SoP5CS*	Proline synthesis	Guitang 21
Salinity	pUBI	*EaGly III*	Reduced oxidative damage	Co 86032
Drought	pUBI	*AtBBX29*	Gene regulation	NCo310
Drought	P35S	*TERF1*	Gene regulation	XintaitangR22
Cold	pUBI	*SoTUA*	A-tubulin synthesis	ROC22

Source Budeguer et al. (2021) and references thereof

are associated with signal perception with a major control over plant growth, development, and response to stresses. Upregulation of *ShGPCR1* through constitute overexpression enhanced tolerance to drought, salinity and cold stress (Ramasamy et al. 2021).

9.5 Genome Editing Tools and Future Prospects in Sugarcane

Genome editing (GE) is a tool for *in-vivo* modification of DNA in the genome by creating insertion, deletion or substitution within a specific sequence. It uses engineered nucleases for creating double stranded breaks in the genome. These breaks are repaired by non-homologous end joining (NHEJ) or homology recombination (HR) for creation of site directed mutations in vivo. Meganucleases (MN), Zinc-finger nucleases (ZFNs), Transcription Activator-like Effector Nucleases (TALENs) and the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Associated Nuclease 9 are the four families of engineered nucleases available at present (Mohan 2016). GE has enabled creation of novel variant alleles or altogether new phenotype. GE is a powerful tool which has many advantages over, site directed mutagenesis and transgenics. GE in agricultural crops has multiple application including but not limited to increasing yield, nutritional quality, weed protection and tolerance/resistance to biotic and abiotic stress (Ahmad et al. 2019). The first GE milestone achieved in sugarcane was using TALEN to reduce the lignin content in cell wall to improve saccharification efficiency, facilitating higher production of lingocellulosic bioethanol (Jung and Altpeter 2016). The TALEN-mediated mutants of gene encoding O-methyltransferase showed a significant reduction in total lignin and altered lignin composition, along with 43.8% improved saccharification efficiency (Kannan et al. 2018). The complex sugarcane genome which is large, highly polyploid and aneuploid in nature poses many challenges. Targeting and localizing specific sequence in multiple genomes of sugarcane with multiple alleles and high copy are the obvious impediments in the use of genome editing tools in sugarcane. Gene silencing of the non-targeted alleles or copies in the genome can be attempted to achieve expression of single copy.

As the chapter discusses the various stresses and sugarcane's response to them, it is clearly evident that the abiotic stress tolerance forms a very large, complex, over-lapping network of several genes and transcription factors with many layers of regulation at protein, mRNA, miRNA, transcripts/alternative transcripts/transcript variants and finally the genes and genomes. In addition to these, there are various retrotransposons, transposable elements and several uncharacterized genes involved in the regulatory network of abiotic stress response and tolerance in sugarcane. The complex sugarcane genome with 12–15 copies of a gene in its large, mixed genomic composition of 2–3 progenitor genomes offers a real challenge to the present day biotechnological tools. Every genotype/variety differs in the chromosome number and composition although the abiotic stress response seemingly involves a definitive pattern as seen from the recent genomics studies. Genomic designing of sugarcane combining the best of traits, and biotic and abiotic resistance sourcing genes from different germplasms is currently a dream to plant breeders and molecular biologists with more emphasis on ever- changing, hostile environs of the global climate scenario. With more advances in genomics and computing facilities, the dream must be realized in the near future.

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