Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7473

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (3):** 484-502 (2019)

International Journal of Pure & Applied Bioscience

Review Article

Perspective for Genetic Amelioration of Sugarcane towards Water Logged Conditions

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Received: 1.05.2019 | Revised: 29.05.2019 | Accepted: 7.06.2019

ABSTRACT

Waterlogging or submergence or flooding is one of the important environmental constraints that reduces both growth and survival of sugarcane, which cause 15–45 % reduction in cane yield and sugar recovery. Extent of injury/damage due to waterlogging depends upon the genotypes, environmental conditions, stage of development and the duration of stress. The response of sugarcane for agro-morphological, physiological, biochemical and anatomical traits decide its tolerance under waterlogging conditions. The development of root and stalk aerenchyma, adventitious roots stimulated by ethylene and auxin help the plant to cope anoxia and hypoxia like conditions. Involvement of related species of Saccharum genera carrying stress tolerance genes can be utilized in on-going sugarcane breeding programmes. Moreover the genomic approaches in sugarcane has opened new avenues for the use biotechnology i.e. in identification of genes/quantitative trait loci, to create transgenic related to the abiotic stress and to develop molecular markers for integrating marker assisted selection (MAS) in sugarcane improvement. This article provides an insight of recent advances in strategies for sugarcane response to waterlogging stress.

Key words: *Sugarcane, Agro-morphological traits, Abiotic stress, Waterlogging, Quantitative traits.*

INTRODUCTION

Sugarcane belongs to the genus *Saccharum* L., of the tribe *Andropogoneae* in the grass family (*Poaceae*). Commercial sugarcane is arised through intensive breeding of *S. officinarum* with *S. spontaneum*, *S. barberi* followed by backcrossing is termed as ‘noblization’ resulted in complex polyploidy[16,68]. The chromosome number among varieties varies from 2n = 80 to 120 that’s why the sugarcane varieties are botanically described as *Saccharum* spp. complex hybrid. It is an important agro-industrial crop, ranking among the top most planted crops in the world. It is grown mainly for sugar contributing nearly 80% of the sugar (sucrose) produced worldwide[64,84,118].

Cite this article: Sanghera, G.S. and Jamwal, N.S., Perspective for Genetic Amelioration of Sugarcane towards Water Logged Conditions, *Int. J. Pure App. Biosci.* **7(3):** 484-502 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7473

More recently its use in production of ethanol, a bio-fuel alternative to currently used fossil fuel. Brazil used 47% of the sugarcane crop for ethanol production and use 40% of ethanol in vehicles[86]. It is also used for other processed products (gur and khandsari) or may be consumed raw.

Sugarcane is cultivated in both tropical and subtropical parts of the world. There are around 110 countries in the world growing sugarcane over an area of about 19.2 million hectare producing 1214 million tons of sugarcane with an average production of 63.3 tons per hectare. India stands second in the world both in area (5.3 million hectare) and production (366.8 million tons) after Brazil[101]. Sugarcane plays a significant role in world economy and the area cultivated yields observed have progressively increased to remarkable levels in the last decade. The climatic conditions have changed over years, which have also affected the sugarcane due to various biotic and abiotic stresses. Among them waterlogging or flood or submergence or soil saturation or anoxia or hypoxia is one of the abiotic stresses which limit the potential productivity of crop plants. In India, 11.60 million ha area is affected due to waterlogging out of which 10–30 % comes under sugarcane cultivation and considered as major constraint to the productivity. The south west monsoon brings 73.30 % of the annual rainfall in India and cause floods and waterlogging problems which expose the sugarcane crop to stagnant water stress in several parts of India i.e. Assam, Bihar, and West Bengal, and eastern Uttar Pradesh, coastal region of Andhra Pradesh, Tamil Nadu, Kerala and Karnataka[101].

Sugarcane is fairly tolerant to flooding or waterlogging or submergence. However, the severity of the problem depends on the duration of waterlogging, the condition of water *viz*., stagnant or moving, adopted varieties, soil type and drainage facilities available. Generally, the growth of sugarcane has divided into four phases: germination and emergence (1 month after planting), tillering and canopy establishment (at 2–3 months), grand growth (at 4–10 months), and maturation or ripening (at 11–12 months)[27]. It was observed that sugarcane crop was most susceptible to waterlogging in the first 3–4 months, comparatively tolerant at 5–9 months age and cause early maturity of sugarcane in response to flooding stress at later stages. Thus, waterlogging at this time may affect growth and, thus, the yield and quality of sugarcane produced during the maturation or ripening phase[91]. Deren and Raid[18] and Gilbert *et al.*[29] reported yield losses of 12-16% for a 10 days flood three days after planting and continuous three-month flood caused an 18-37% yield reduction in plant cane respectively. Even high sub-surface water level also reduces the yield of sugarcane, 54-64% loss in second ratoon to plots with natural water tables of 11-18 cm[29]. Glaz *et al.*[31] reported a yield reduction of 8% in a field with water table levels 13-17 cm below the soil surface compared with a field with water table levels 29-39 cm below the soil surface. Hence submergence tolerance is one of the desirable traits of sugarcane to make it more favorable for cultivation in the marginal lands. The breeders can exploit sugarcane on this aspect by screening desirable clones and genetically improving it. The literature pertaining to work conducted on this aspect in sugarcane is not complied properly.

EFFECT OF WATERLOGGING STRESS ON AGRO-MORPHOLOGICAL TRAITS

Waterlogging stress is also known to cause a number of morphological changes in plants. These changes can be utilized to screen the clones for the waterlogging stress tolerance. Gomathi[35] reported 14.50 % reduction in plant height over control and result of correlation analysis also revealed that, the shoot population and plant height are highly positively correlated with waterlogging tolerance during formative phase of the crop (90-170 DAP) (Fig 1). The most common approach utilized by the breeders for breeding of sugarcane against abiotic stresses is through screening of the desirable/potential germplasm for agro-morphological traits under stressed conditions[72]. The germplasm screening approach was employed by Hasan *et al.*[40]; Dey and Singh[22]; Bakshi[9]; Gilbert *et al.*[29]; Islam *et* *al.*[45,46,47,48,49]; Kumari *et al.*[66]; Kumar *et al.*[63] and Khaiyam *et al.*[58] where flooding stress tolerant sugarcane clones were identified based on morphological traits affecting sugarcane productivity like length and girth of cane, internode length, number of millable cane, single cane weight and Brix (%), commercial cane sugar (CCS kg/plot), yield and quality of sugarcane. Glaz *et al.*[32] also used green leaf weight and brown leaf weight but found no consistent effect of water table. Flooding inhibited leaf expansion and decreased leaf area (LA), leaf area index (LAI), and leaf weight[28,37,52]. Anitha *et al.*[3] analysed COC 24 sugarcane variety under normal and waterlogged conditions and reported reduction in plant height (7.22%) and stalk diameter (16.38%), but considerable improvement was observed in number of nodes (14.8%), internodal length (17.65%), nodes carrying roots (42.86%), number of roots (62.72%) and root volume (49.29%) conferring tolerance to waterlogging stress. While marginal increase of 7.69%, 5.76% and 8.53%, observed in leaf area index, leaf production and specific leaf weight respectively. Long term flooding reduced primary root length, leaf weight and yield of sucrose among susceptible sugarcane clones[29,88].

Fig. 1: Screening for waterlogging tolerance conducted in a concrete water tank under induced flood stress condition with control. (Source: Begum *et al.*[10])

EFFECT OF WATERLOGGING STRESS ON PHYSIOLOGICAL PARAMETERS

Beside agro-morphological traits, workers employed physiological traits to access the waterlogging stress because it has been found that the application of periodic flooding every month leads to a 50% reduction in the photosynthesis rate[111]. Some other physiological effects are found under waterlogging are the plant tends to decrease transpiration rates are due to stomatal closure and chlorosis banded chlorosis has also been seen in sugarcane growing in flood waters[2]. Plant continued transpiration under periodic flooding condition had either no effect or sometimes slightly increased stomatal conductance[34,15]. Besides in sugarcane, the periodic flooding conditions slightly reduced CO_2 assimilation whereas stomatal conductance and transpiration increased, but in case of prolonged flooding stomatal conductance and transpiration rate reduced in the same way as CO_2 assimilation[42,52]. In contrast positive response of sugarcane gas exchange rates to periodic flooding[33] and another study which reported that periodic flooding did not affect CO_2 assimilation in tolerant sugarcane clones but reported 48% reduction in ratoon cane[111]. Reduction in photosynthesis is due to the decrease in effective leaf area, there is one fourth reduction in leaf area. As a result crop growth rates are drastically reduced during waterlogging. The higher respiration rate of submerged organs compared to leaves. A shift in respiratory metabolism from aerobic to anaerobic pathways is one of the main effects of oxygen deficiency caused during waterlogging. Roots of the plants are usually in contact with oxygen at a partial pressure which is equivalent to the gaseous atmosphere. The various alterations happens during water logged conditions in plants and their mitigating approaches are summarized (Fig 2).

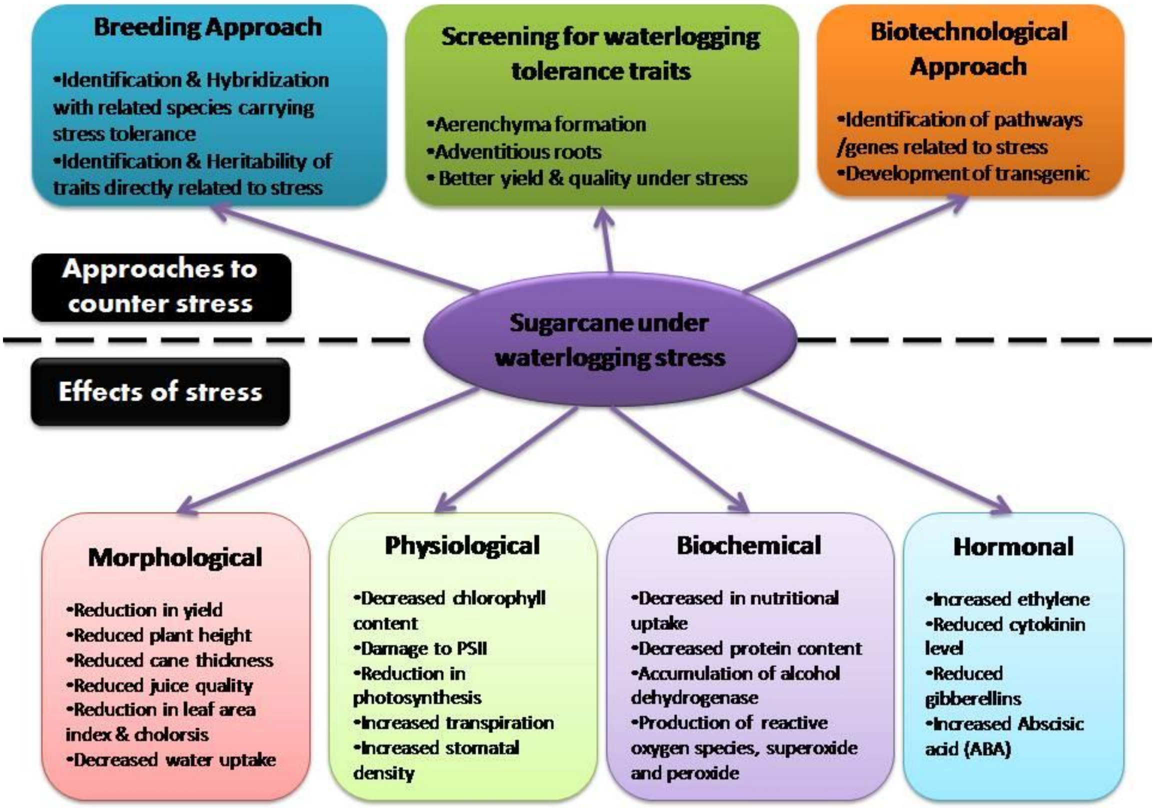

Fig. 2: Morpho-physiological and biochemical effects of waterlogging conditions in sugarcane and possible strategies to combat

Under waterlogging there are two conditions one is 'Hypoxia', reduction of oxygen below optimum level, is the most common form of stress which occurs during partial submergence of plant due to short-term flooding where the root goes under water and shoots remain in the atmosphere. Another condition is 'Anoxia' where plant goes under completely, hence complete absence of oxygen as a result of long term flooding[103]. Microbial flora of the soil may change by long time waterlogging which works in favour of anaerobic microorganisms that use, as alternative, electron acceptors to oxygen. In such condition soil tends to accumulate nitrite as it tends to accumulate more reduced and phytotoxic forms of mineral ions (from nitrate) and ferrous ions (from ferric) and very few number of plants are naturally adapted to grow in this kind of soil[49]. The effects of waterlogging on respiration rate depend on the varieties and its physiological age. The decrease in chlorophyll content could be taken as an indicator of plant adaptation during anaerobiosis for their maximum energy production as well as for the generation of reducing equivalents, which could be later utilized for the biosynthesis of carbohydrate by reductive pentose phosphate cycle[87]. Islam and Begum[44] and Begum *et al.*[10,11] used physiological traits like chlorophyll content for screening of waterlogging stress and reported less reduction in chlorophyll content among tolerant clones. The physiological traits i.e.

chlorophyll a, b, total chlorophyll and carotenoids have shown significant correlation with morphological traits like dry weight of leaf, leaf sheath, root and stalk and whole clump under waterlogging stress[51]. Further, waterlogging resulted in 13.0, 21.6, 26.5, 25.2, 24.0 and 42.0 % mean reductions in plant height, tiller production, leaf area index, nitrogen content in leaf, total chlorophyll content and total dry matter production, respectively. In an anther study the effect of waterlogging on growth and physiological changes of sugarcane clones under flooding condition at grand growth (180–240 DAP) and maturity phases of the crop (240–360 DAP) resulted in yellowing symptom in leaf, higher stalk mortality, faster drying of lower leaves, reduction in leaf number and size, reduction in cane height and thickness and emergence of aerial roots particularly up to water level are the morphological changes due to waterlogging stress[36]. Both cane and sugar yield have been reported to decrease under flood conditions as a result of a reduction in photosynthesis, root development, leaf area (LA), LA index, tiller production, stalk height, and sucrose yield[37,111,114] and reduced plant growth as a result of a decrease in the metabolic activity of the roots due to hypoxia[37]. The chlorophyll fluorescence is an excellent physiological marker that determine the primary processes involved in photosynthesis such as energy transfer due to excitation, absorption of light and photochemical reactions occurring in the PSII (photosystem II)[100]. Therefore, changes in chlorophyll fluorescence parameters determine the function and stability of photosystem II under waterlogging stress[1,54]. The plants subjected to waterlogged conditions exhibit certain alterations in this physiological marker. Another response that is correlated with waterlogging is rate of stomatal conductance. Stomatal closure reported had declined chlorophyll fluorescence[69] there was reported decreasing stomatal conductance under waterlogging[70]. Soleh *et al.*[105] reported chlorophyll fluorescence response as potential marker for screening of sugarcane clones under waterlogging conditions along with stomatal conductance for improving dry matter production in sugarcane.

EFFECT OF WATERLOGGING STRESS ON NUTRITIONAL UPTAKE

The loss of nutrients in prolonged flooding is one of the major issue which results in nutrient imbalance causes yellowing of leaves and giving a scorched appearance or large red–brown lesions on the leaves[37]. Waterlogging may reduce the oxygen availability for root system and inhibit the uptake of nutrients also. In waterlogged conditions, reduction in growth was accompanied with decrease in nitrogen, phosphorus and potassium contents but calcium and magnesium increased in the leaf lamina. The absorption of water and nutrients is influenced by the rate of root respiration and cell permeability. Since root respiration and cell permeability are reduced due to oxygen deficiency, water and nutrients absorption is also slowed down. Under such situation, plants exhibit nutrient deficiency and apparent wilting symptoms are observed even in soils supplied with available nutrients and water. Nitrogen is mobile in soil results in its leaching. In sugarcane waterlogging stress reported nearly 30 per cent reduction in its nitrogen contents compared to tolerant varieties. A study conducted by Jaiphong *et al.*[52] under waterlogging conditions reported the lower concentrations of various ions, such as ammonia, potassium, magnesium, chloride, phosphate and sulphate in the sugarcane juice and remained at a lower level than the control even once the water had been drained but the level of calcium and fluoride ions increased during flooding. By contrast, the concentration of sodium (Na^+) was higher in the flooded plants. Furthermore, during prolonged flooding, the sodium content of plants was 245.5% higher than in the control plants, representing a 71.3% increase as a result of flooding, which was maintained even after the water had been drained or the plants had been placed under prolonged drought conditions. The highest sodium content was found in the bottom part of the stem and its level increased during prolonged flooding, while the middle and top parts of the stem had similar sodium concentrations as the control plants. However phosphorous and potassium contents of leaf and stem were not affected by waterlogging. Morris *et al.*[83] reported negative correlation sugar yield and soluble organic carbon($r = -0.73$, $P<0.01$) and a positive relationship between sugar yield and depth to water table ($r = 0.84$, $P<0.01$), which suggests that carbon loss from roots has a detrimental impact on yield when water table levels are high.

UNDERSTANDING OF ANATOMICAL ALTERATIONS DUE TO WATERLOGGING STRESS

Plants anatomy played a significant role in the deciding the tolerance level of plant towards abiotic stresses. After waterlogging many old roots die due to lack of oxygen supply, in place of that numerous adventitious roots with well-developed aerenchyma emerge from the base of the stem which depends mainly on the plant species and the flooding tolerance of the species[67]. Since the root elongation of sugarcane is closely related to oxygen concentration of the root zone, plant internal aeration might be achieved by increasing the root porosity. It has been observed that oxygen required for respiration reached from the aerial parts to the roots through the intercellular spaces of the stem and the roots. There are different kinds of roots developed from the flooded sugarcane, first type brown coloured roots initiated from the aerial nodes and remained above the water surface with no aerenchyma formation; second type adventitious roots initiated from the pre-existing root primordia which developed under water and having pink colour and third type of roots are white in colour and emerged from the newly developed root surface and having upward growth against the gravity. These roots also formed large interconnected gas filled spaces called aerenchyma[42]. Aerenchyma is an enlarged and interconnected intercellular spaces filled with gas. Some species that inhabit frequently flooded environments produce high volumes of aerenchyma in their root cortex and have high flood tolerance, whereas species that seldom inhabit flooded environments develop less aerenchyma and have low flood tolerance[67]. These anatomical differences have been observed in many plants[55,104], suggesting that aerenchyma is an important anatomical characteristic that helps plants to survive soil flooding. The aerenchyma which arises in shoot and roots from successive cell division by the phellogen, and is composed of white and porous (spongy) tissues within few weeks under flooded conditions are referred to as 'secondary aerenchyma[50]. The formation of aerenchyma is either constitutive, means its formation is included in the developmental program or it can be induced by abiotic stresses such as nutritional starvation and hypoxia. Roots that developed aerenchyma by the influence of flood, helped in maintaining root activity under flooding conditions by supplying necessary oxygen[23].

In sugarcane, the development of aerenchyma is constitutive. In species that are flood tolerant, aerenchyma formation is usually constitutive, meaning that it requires no external stimulus, such as flood[24]. Its formation seems to be a result of different combinations of developmental modules, which are activated by plant hormones and the environment. Leite *et al.*[71] reported that cell wall modifications in sugarcane roots produce a composite that apparently seals the gas spaces. They seem to function as chambers where gasses can be stored and used for respiration by the remaining living cells in the roots. Aerenchyma tissue in the shoots and roots may allow oxygen to diffuse from the stalk to the cells in the root (Fig 3). Glaz[34] found that, some genotypes had constitutive stalk aerenchyma, and some genotypes formed aerenchyma in stalks after they were exposed to flooding. In case of those clones which delay in aerenchyma formation after they were exposed to flooding reduced photosynthesis rate that caused yield losses. Presence of root aerenchyma facilitates the sustained root activity in flooded soil. The roots of 40 sugarcane genotypes examined contained aerenchyma[41,93]. Aerenchyma formation is also triggered by ethylene and auxin level in the plant their interplay also played significant role. Tavares *et al.*[109] reported that in the aerenchyma formation zone, the concentration of ethylene is lower in comparison to the concentration in maize. The correlation between level of auxin hormone and aerenchyma formation in root cortex has observed inversely correlated (r = −0.96) in sugarcane. Besides, the ratio between both hormones (ethylene and auxin) was around 1:1.

Under flooding condition, adventitious roots developed at or below the water level are more marked once the floods recede. These roots are adapted to waterlogging conditions than the original roots because they have much larger intercellular spaces[41,59]. Studies examining genetic correlation of sugarcane root traits under flood, found that selection for adventitious root development may not increase sugarcane yield under flooding[107]. Fazle *et al.*[25] screened six clones under waterlogging condition and identified that clones with more adventitious roots have better submergence tolerance. Gilbert *et al.*[28] reported a 38% reduction in leaf weight, 4-15 times greater adventitious root development, 108% greater aerenchyma extension, and 115% greater aerenchyma diameter under flood condition. Production of aboveground adventitious roots, however, was found under flooded conditions at the expense of belowground primary root mass, thus the formation of aboveground adventitious roots may offset losses associated with flooding. These traits i.e. adventitious roots with well-developed aerenchyma, proved to be a useful screening tool to identify flood tolerance in sugarcane cultivars[29].

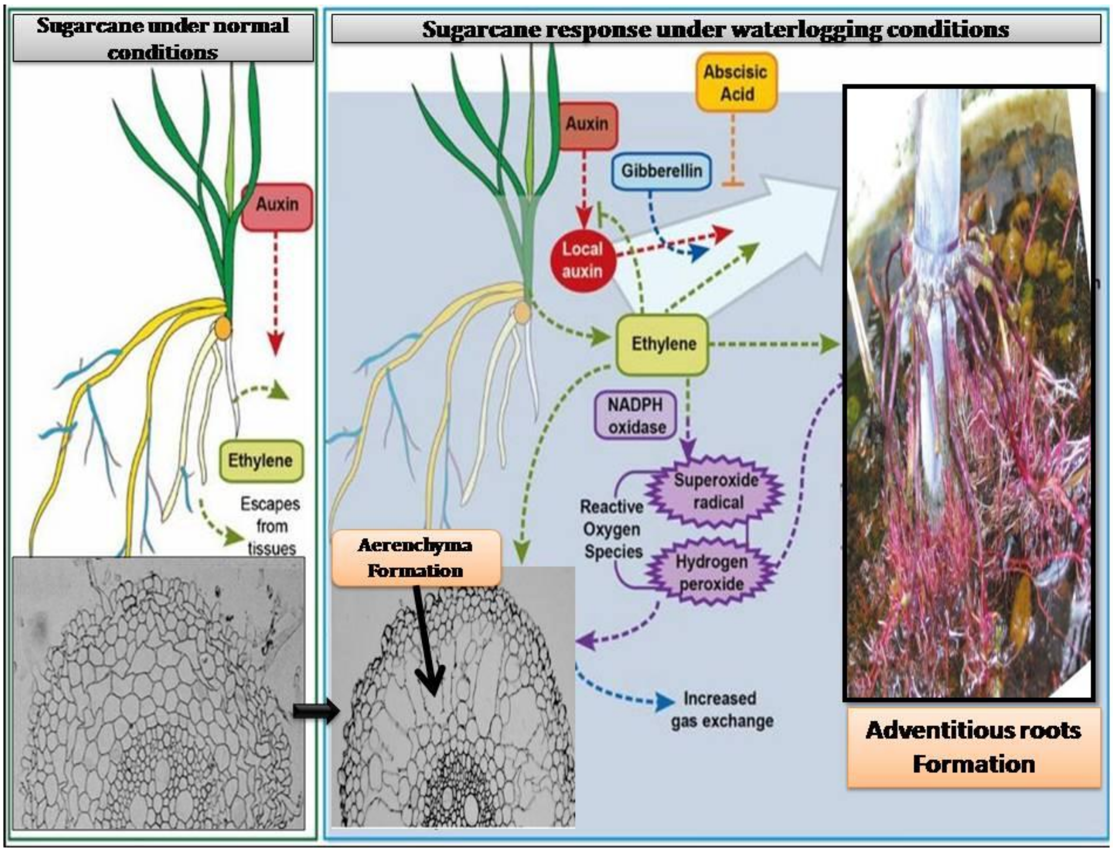

Fig. 3: Effect of waterlogging stress on sugarcane plant at anatomical and biochemical levels (Modified from and Rasmussen[106])

EFFECT OF WATERLOGGING STRESS ON BIOCHEMICAL TRAITS

Plant tends to change its biochemical pathways to emerge tackle abiotic stress. Avivi *et al.*[8] reported The role biochemical pathways is significant in case of waterlogging because of 'anoxia' like situation lead to anaerobic respiration instead of aerobic. Plants also respond to anoxia by altering the pattern of root protein synthesis. The proteins which are synthesized as a specific response to anaerobiosis are called the anaerobic polypeptides (ANPs)[99]. In sugarcane, expression of anaerobic polypeptides in leaf and root in response to short term flooding stress was reported in tolerant clones and indicating their possible role in tolerant behaviour[37]. Plant also synthesize proline, glycine betaine, as an important osmotic adjustment substance, exists in free state in the cells and possesses low molecular weight, high water solubility, and no net charge in the physiological pH range, under various environmental stress. Glycine betaine has role in improvement of growth, net photosynthesis and the apparent quantum yield of photosynthesis of the waterlogged plants. The profound effects of waterlogging on the glycine betaine accumulation in sugarcane which serve as an intercellular osmoticum provided the basal metabolism of the plant[4]. In addition, catalase and peroxidase are the key enzymes involve in decomposition of hydrogen peroxide into water and oxygen and helps in protecting the cell organelle like mitochondria and chloroplast from oxidative damage by reactive oxygen species (ROS). Nilkantha[85] analzed six sugarcane clones and reported significant differences for proline, glycine betaine, reducing and non-reducing sugar, catalase and peroxidase activity among clones under waterlogging conditions. The oxidative stress generated during waterlogging and promotes the production of reactive oxygen species (ROS) including superoxide (O_2^-), singlet oxygen, hydroxyl anion (OH^-) and hydrogen peroxide (H_2O_2) which can be detrimental to proteins, lipids and nucleic acids (Fig 2). Gomathi *et al.*[38] analysed the activity and isozyme pattern of antioxidant enzymes i.e. activities superoxide dismutase (SOD), peroxidase (POX) and ascorbate peroxidase (APX) and reported their significant role in protection of cell from oxidative damage and control the reactive oxygen species (ROS) to protect the cell membrane damage induced during flooding stress. The cell membrane leakage was significantly lower in tolerant clones Co 99006 (37.00 %) followed by Co 99004 (40.00 %), Co 8371 (42.50 %) as compared to susceptible clone Co 86032 (52.00 %). Another significant enzyme involve in anaerobic stress is alcohol dehydrogenase (ADH). It is considered essential in anoxia survival, because it recycles NAD^+ for continuing glycolysis in the absence of oxygen[57,98]. In sugarcane, its activity was traced by Gomathi *et al.*[37] and reported the high alcohol dehydrogenase activity among tolerant clones (Co 99006-36.25 %) compared to sensitive genotypes (Co 86032-14.50 %). These biochemical enzymes can be utilized as potential markers for identification of flood tolerant and susceptible clones.

BREEDING SUGARCANE FOR WATERLOGGING STRESS TOLERANCE

There is no alternative for abiotic stress tolerance besides synthesizing varieties with genetic tolerance for them. The narrow genetic base of modern sugarcane varieties are the major bottleneck of sugarcane improvement for biotic and abiotic stress tolerance which further complicated by the highly heterozygous, complex polyploidy (10–12 copies of the genome), aneuploidy hybrids (100–120 chromosomes) and often with four species of *Saccharum* in their ancestry[43]. As a result modern cultivars have chromosome numbers ranging from 2n = 99–130[12]. In polyploids such as sugarcane, the haploid chromosome number (1C value = n) is not the same as the monoploid number (x)[13]. The monoploid genome size for *S. officinarum* (x = 10) is approximately 926Mbp (mega base pair), and for *S. spontaneum* (x = 8) 760Mbp[13]. Selective breeding across many generations in a limited parental population has slowed down the rate of genetic gain in recent years. Genes for tolerance to biotic and abiotic stresses of serious economic concern (e.g. frost and drought) are limited or unknown in the current parental populations. An introgression program would address these concerns in a more effective manner than using only core breeding material which has a narrow genetic base. Hence, sugarcane breeders have tried to exploit the genetic variation within the *Saccharum* complex, which shows great variation for a range of traits of interest like waterlogging to the breeders. The genetic variation can be created by utilizing *S. spontaneum, Miscanthus sinensis, Erianthus arundinaceus,* and *Erianthus rockii* in introgression initiatives. Especially, the genus *Erianthus*, one of the *Saccharum* complex, has become important as a genetic resource in sugarcane breeding for biomass production because of its tolerance to extreme conditions, such as drought and flooding. The root development of *Erianthus* in saturated soil goes deep as 250 cm from the ground. Meanwhile, the pot experiment reported *Erianthus* increased biomass production and had well-developed aerenchyma on the roots of plants grown under waterlogged conditions[53]. Moreover, inter-generic and inter-specific *Saccharum* hybrids of commercial sugarcane with *Erianthus arundinaceus* and *S. spontaneum* grew well under a flooded condition for 6 month[19,20,21]. Thus, Erianthus is a potential genetic resource in sugarcane breeding not only for higher biomass production but also for flood tolerance[53]. In another study, Reed *et al.*[95] screened 29 *Saccharum barberi* and 20 *Saccharum sinense* accessions under short term flooded conditions and reported not significant effect of flood on accessions. Shrivastava and Srivastava[102] reviewed various species and genera of *Saccharum* related to various abiotic stresses along with various sugarcane varieties carrying abiotic stress tolerance.

Breeding for any trait is possible if there is heritability of that trait. In case of waterlogging stress Roach and Mullins[97] reported that tolerance to high water tables is probably a heritable character in sugarcane. Narrow sense heritabilities for sugar production and cane biomass were estimated to range from 0.29 to 0.51 for sugarcane produced under flooding as a per cent of a control. Several clones maintained productivity after 6 months of flooding in the plant crop and 5 months of flooding in the first-ratoon crop. Clones that produced high biomass and sugar in non-flooded controls also were productive under flooded conditions. Krishna *et al.*[61] reported that sugar yield, cane yield, leaf area index after waterlogging exerted high genetic advance as percentage of mean coupled with high heritability indicated that these traits were controlled by additive gene action. Sukhchain and Saini[107] reported that flooding enhanced the direct effect of individual stalk weight on total cane yield, which has a strong influence on the total amount of sugar produced. Glaz *et al.*[31] tested nine sugarcane cultivars using 15- and 38-cm water tables and reported that one cultivar had the same sugar yields at both water table levels, and some genotypes could tolerate high water tables. The significant and positive correlation of cane yield (t/ha) with germination percentage, number of shoots, number of millable canes, sugar yield, plant height, single cane weight, cane diameter, leaf area index before and after waterlogging, number of fully emerged leaves at 30 and 60 days after waterlogging was reported by Krishna and Kamat[60]. Kang *et al.*[56] reported that some sugarcane clones produced greater biomass under a high water table (29.8 cm) than under a low water table (56.8 cm). Krishna *et al.*[62] analysed the genetic diversity among 16 clones based on 25 agro-morphological traits and grouped them in five clusters with 21 to 85 inter cluster distance with major contribution from CCS% and leaf area index towards genetic diversity.

BIOTECHNOLOGICAL APPROACHES TOWARDS WATERLOGGING STRESS TOLERANCE

Conventional breeding in sugarcane has several limitations i.e. different proportions of chromosomes and varying chromosome sets (aneuploidy) cause complex re-combinational events[116], breaking genetic linkage due to highly polyploidy nature, long duration of crop and breeding cycle, sexually compatibility between species is prerequisite of conventional breeding which is lacking in sugarcane. These limitations can be addressed by biotechnological approaches. Reference genome sequences have become key platforms for genetics and breeding. Sugarcane is one of the largest crops produced in the world in term of weight of crop harvested which lacks a reference genome sequence. Sugarcane has one of the most complex genomes in crop plants due to the extreme level of polyploidy. The genome of modern sugarcane hybrids includes sub-genomes from two progenitors *Saccharum officinarum* and *S. spontaneum* with some chromosomes resulting from recombination between these sub-genomes. Advancing DNA sequencing technologies and strategies for genome assembly are making the sugarcane genome more tractable[80]. Advances in long read sequencing have allowed the generation of a more complete set of sugarcane gene transcripts[110]. Recently, the genome sequencing of sugarcane (R570) has opened a whole new vista for biotechnological applications[26,118]. Even structural and functional characterization of environment stress induced genes has contributed to a better understanding of how the plants respond and adapt to different abiotic stresses. Studies have shown the specific proteins accumulate in response to imposition of stress conditions, *viz*., drought, low temperature, high temperature oxidative stresses and flooding etc. Low oxygen induced genes have been characterized by the presence of anaerobic response element in the promoter[112]. A transcription factor, AtMYB2 is induced in response to hypoxia (as also in other abiotic stresses). This gene may enable a transgenic plant to recognize anaerobic conditions early and synthesize ANPs so as to impart greater tolerance to hypoxia[17]. In a recent study, Tavares *et al.*[108] sequenced two independent genes *epg1* and *rav1* encoding an endopolygalacturonase (EPG), a glycosyl hydrolase related to homogalacturonan hydrolysis from the middle lamella and a RAV transcription factor, from the ethylene response factors superfamily, and reported that the two isolated genomic regions (*rav1* and *epg1* loci) contain essential genes that control early pectin degradation during aerenchyma formation in sugarcane roots. The functional diversity and expression profiling of water logging tolerant genes *viz*., zinc finger protein, alcohol dehydrogenase (Adh), sub1A-1, sub1C-1 and xyloglucan endo-transglycosylase, aldehyde dehydrogenase (ALDH5F1), ACC oxidase, submergence induced proteins (ANP's) and G-box binding factor-1 (GBF1) in the commercial sugarcane cultivars[37]. Transgenic related abiotic stress tolerance in sugarcane reported by Augustine *et al.*[5,6,7] where number of genes *EaHSP70*, *PDH45* and *EaDREB2* from pea and *Erianthus arundinaceus* has been successfully introduced in sugarcane variety Co 86032 through Agrobacterium-mediated transformation. In another study, Pereira-Santana *et al.*[89] utilized total of 16 sugarcane libraries sequenced on a HiSeq-Illumina platform and identified A total of 536 genes and 750 genes up-regulated under abiotic stress, while 1093 and 531 genes were differentially down-regulated in leaves and roots, respectively. Development of molecular markers associated with sugarcane waterlogging tolerance and marker assisted selection (MAS) could enhance the breeding programs for waterlogging tolerance. Quantitative trait loci (QTLs) analysis has proven to be very useful in identifying the genetic components of the variation for important economic traits[81]. Number of QTLs related to waterlogging has been identified in maize[90,115] and other crops but limited studies has been reported in sugarcane so far (Table 1). In Sorghum, four major QTLs that control stay-green and grain yield (*Stg1–Stg4*) have been identified under water stress[39]. Further, identification of specific transcripts that are expressing in response to anoxia and hypoxia situation, cloning and characterization of those specific genes are crucial for manipulating the sugarcane genome for improved stress tolerance through genetic transformation.

Table 1: Quantitative trait loci (QTLs)/genes associated to waterlogging stress tolerance in sugarcane and related crops

Characters	**Crop**	**QTLs/gene**	**Reference**
Leaf Cholorsis	Maize	*Qft-rd 1.03-4*	74
	Maize	*Qft-rd4.07-4.11*	73
Leaf senescence	Maize	*Subtol6*	14
	Sorghum	*Stg1*	39
	Sorghum	*Stg2*	39
	Sorghum	*Stg3*	39
	Sorghum	*Stg4*	39
Adventitious root formation	Maize	*Qarf8.05*	75,77, 78
	Maize	*Qarf8.03*	75,77
	Maize	*Qarf5.03*	75,77
	Maize	*Qarf3.04*	77
Ethylene Pathway	Sugarcane	*ALDH5F1*	37
	Sugarcane	*Adh1*	37
	Sugarcane	*ACC oxidase*	37
	Sugarcane	*ANP*	37
	Sugarcane	*GBF1*	37
ROS signalling	Sugarcane	*AtBI-1*	92
	Sugarcane	*AtDREB2A CA*	96
	Sugarcane	*ZFP-Mo17*	37
	Sugarcane	*XET*	37
	Maize	*ROL-3*	113
Aerenchyma formation	Maize	*Qaer1.07*	79
	Maize	*Qaer1.02-3*	79
	Maize	*Qaer1.06*	73
	Maize	*Qaer1.05-6*	77
	Maize	*Qaer1.11*	73
	Maize	*Qaer2.06*	76
	Maize	*Qaer5.09*	73,79
	Maize	*Qaer5.05-6*	76
	Maize	*Qaer8.05*	77
	Maize	*Qaer8.06-7*	79
Chlorophyll fluorescence (PSII)	Sugarcane	*TSase*	117
	Sugarcane	*P5CS*	82
	Maize	*QY2*	115
Brace roots	Maize	*Br.7*	115
	Maize	*Br.8*	115
Root dry weight	Maize	*rdw1*	90
	Maize	*Rdw3*	90
Root length	Maize	*rl1*	90
	Maize	*rl3-1*	90
	Maize	*rl3-2*	90
	Maize	*rl3-3*	90
	Maize	*rl7-5*	90
	Maize	*rl10*	90
Osmotic regulation	Sugarcane	*AVP1*	65, 94
Nucleic acids metabolism	Sugarcane	*HSP70*	6
Cellular stabilization	Sugarcane	*PDH4*	5
	Sugarcane	*PDH45*	7

FUTURE OUTLOOK

Waterlogging is significant abiotic stress for sugarcane production in low lying, high rainfall areas and areas along the rivers[58]. The short term flooding is also most common, but prolonged flooding is also a serious problem for crops like sugarcane[30]. The tolerance in plant for waterlogging stress is controlled by cascades of molecular networks involved in stress perception, signal transduction, and expression of specific stress-related genes and metabolites. Waterlogging significantly affect various agro-morphological, physiological and biochemical traits *viz*., light interception, degradation of chlorophyll pigment, photosynthetic rate, reduction of key enzyme activity (RNase) and soluble protein content and nutrient uptake and finally shoot and root growth behaviour. The tolerance of plant depends upon duration as well as intensity of flood it can survive without losing economic yield. Flood tolerance is genetic trait and various between genotype to genotype hence breeders exploit this by screening large number of clones under stress for various yield related parameters to identify tolerant genotype. The flood tolerant plants could able to survive in high water table, through formation of anatomical traits which triggered during the stress and changing the biochemical pathways aerobic to anaerobic respiration. In sugarcane adventitious roots arise from the nodal region above the water and formation of aerenchyma which helps for functioning of the plant processes under anoxia conditions. These anatomical features of sugarcane are both constitutive as well as induced in nature. The involvement of ethylene and auxin in adventitious root formation and aerenchyma formation was well reported in sugarcane and constitutive formation of stalk and root aerenchyma possibly enables sugarcane to tolerate periodic floods. Breeding for waterlogging stress in sugarcane is complicated as trait is quantitative and crop is genetically complex. However, genus *Saccharum* has many species which carry genes for flood tolerance and these can be used for improving sugarcane. Recently genome of sugarcane is sequenced which has opened whole new possibilities for biotechnological improvements in sugarcane number of genes identified related to flooding stress i.e. endo-polygalacturonase (*epg1*), RAV transcription factor (*rav1*), zinc finger protein, alcohol dehydrogenase (*Adh*), sub1A-1, sub1C-1 and xyloglucan endotransglycosylase, aldehyde dehydrogenase (*ALDH5F1*), ACC oxidase, submergence induced proteins (ANP's) and G-box binding factor-1 (*GBF1*) these genes could further be utilized in development of molecular markers for marker assisted selection (MAS) and transgenic in sugarcane to develop flooding tolerant sugarcane clones.

REFERENCES

1. Abdeshahian, M., Nabipour, M. and Meskarbashee, M., Chlorophyll fluorescence as criterion for the diagnosis salt stress in wheat (*Triticum aestivum*) plants. *Int. J. Chem. Biol. Eng.,* **4:** 184-186 (2010).
2. Ahmed, F., Rafii, M. Y., Ismail, M. R., Juraimi, A. S., Rahim, H. A., Asfaliza, R. and Latif, M. A., Waterlogging tolerance of crops: breeding, mechanism of tolerance, molecular approaches, and future prospects. *BioMed Res. Int.,* **963525:** 1-10 (2013).
3. Anitha, R., Mary, P. C. N. M. and Purushothaman, R. S., Biometric and physiological characteristics of sugarcane ratoon under waterlogging condition. *Plant Archives,* **16(1):** 105-109 (2016).
4. Ashraf, M. A., Waterlogging stress in plants: A review. *African Journal of Agric. Res.,* **7(13):** 1976-1981 (2012).
5. Augustine, S. M., Narayan, J. A., Syamaladevi, D. P., Appunu, C., Chakravarthi, M., Ravichandran, V., Tuteja, N. and Subramonian, N., Overexpression 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 Reports,* **34(2):** 247–263 (2015b).
6. Augustine, S. M., Narayan, J. A.,

Syamaladevi, D. P., Appunu, C., Chakravarthim, M., Ravichandran, V., Tuteja, N. and Subramonian, N., Introduction of pea DNA *helicase* 45 into sugarcane (S*accharum* spp. hybrid) enhances cell membrane thermostability and upregulation of stress-responsive genes leads to abiotic stress tolerance. *Molecular Biotechnology,* **57(5):** 475-488 (2015c).

7. Augustine, S. M., Syamaladevi, D. P., Premachandran, M. N., Ravichandran, V. and Subramonian, N., Physiological and molecular insights to drought responsiveness in *Erianthus* spp. *Sugar Tech,* **17(2):** 121–129 (2015a).
8. Avivi, S., Slameto, S. S. and Ramadhan, R. A., Physiological characters of sugarcane after stress. *Agri. and Agricultural Sci. Proc.,* **9:** 31-39 (2016).
9. Bakshi, A. N., Biochemical characters of sugarcane after flooding stress. *Agric. and Agric. Sci. Proc.,* **8(6):** 31-39 (2003).
10. Begum, M. K., Alam, M. R. and Islam, M. S., Adaptive mechanisms of sugarcane genotypes under flood stress condition. *World J. Agril. Sci.,* **1(2):** 56-64 (2013).
11. Begum, M. K., Miah, M. A. S., Islam, M. S., Hossain, M. A. and Alam, M. R., Effects of periodic flood stress on sugarcane yield and yield contributing characters *Sugar Tech,* **10(4):** 355-358 (2008).
12. Bremer, G., Problems in breeding and cytology of sugarcane. *Euphytica,* **10:** 59–78 (1961).
13. Butterfield, M. K., D'Hont, A. and Berding, N., The sugarcane genome: a synthesis of current understanding and lessons for breeding and biotechnology. *Proceeding of the South African Sugar Technologists Association,* **75:** 1–5 (2001).
14. Campbell, M. T., Proctor, C. A., Dou, Y., Schmitz, A. J., Phansak, P., Kruger, G. R., Zhang, C. and Walia, H., Genetic and molecular characterization of submergence response identifies *subtol6* as a major submergence tolerance locus in maize. *PLoS ONE,* **10:** e0120385 (2015).
15. Chabot, R., Bourafa, S. and Zimmer, D., Sugarcane transpiration with shallow water-table: Sap flow measurements and modelling. *Agricultural Water Management,* **54:** 17–36 (2002).
16. Cox, M., Hogarth, M. and Smith, G., Cane breeding and improvement. In: Hogarth, M., Allsopp, P., eds. Manual of cane growing. Bureau of Sugar Experimental Stations Indooroopilly, Australia, pp 91-108 (2000).
17. Dennis, E. S., Dolferus, R., Ellis, M., Rahaman, M., Wu, Y., Hoeren, F. U., Grover, A., Ismod, K. P., Good, A. G. and Peacoock, W. J., Molecular strategies for improving waterlogging tolerance in plants. *Journal of Experimental Botany,* **51:** 89–97 (2000).
18. Deren, C. W. and Raid, R. N., Yield Component of sugarcane subjected to flood at planting. *Journal of American Society Sugarcane Technology,* **17:** 28-36 (1997).
19. Deren, C. W., Cherry, R. H. and Snyder, G. H., Effects of flooding on selected sugarcane clones and soil insect pests. *Journal of American Society Sugarcane Technology,* **13:** 22–27 (1993).
20. Deren, C. W., Snyder, G. H., Miller, J. D. and Porter, P. S., Screening for and heritability of flood tolerance in the Florida (CP) sugarcane breeding population. *Euphytica,* **56:** 155–160 (1991a).
21. Deren, C. W., Snyder, G. H., Tai, P. Y. P., Turick, C. E. and Chynoweth, D. P., Biomass production and biochemical methane potential of seasonally flooded intergeneric and interspecific Saccharum hybrids. *Bioresource Technology,* **36:** 179–184 (1991b).
22. Dey, P. C. and Singh, S. N., Effect of waterlogging on growth, yield and juice quality performance of sugarcane genotypes. *Cooperative Sugar,* **25(12):** 547-550 (1994).
23. Drew, M. C., Oxygen deficiency in the root environment and plant mineral nutrition, In Jackson, M. B., (Ed.) Plant

life under oxygen deprivation. Academic Publishing, The Hague, The Netherlands, pp. 301–316 (1991).

24. Drew, M. C. and Sisworo, E. J., Early effects of flooding on nitrogen deficiency and leaf chlorosis in barley. *New Phytologist,* **79(3):** 567–571 (1977).
25. Fazle, H. E., Fazlul, A. A. and Yunus, M. O., A study of sugarcane genotypes under flood stress condition and adaptive mechanisms. *African Journal of Crop Science,* **3(7):** 206-213 (2015).
26. 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. *Nature Communications,* **9:** 2638 (2018).
27. Gascho, G. J., Water–sugarcane relationships. *Sugar Journal,* **48:** 11–17 (1985).
28. Gilbert, R. A., Rainbolt, C. R., Morris, D. R. and Bennett, A. C., Morphological responses of sugarcane to long-term flooding. *Agronomy Journal,* **99:** 1622–1628 (2007).
29. Gilbert, R. A., Rainbolt, C. R., Morris, D. R. and McCray, J. M., Sugarcane growth and yield responses to a 3-month summer flood. *Agricultural Water Management,* **95:** 283–291 (2008).
30. Glaz, B. and Gilbert, R. A., Sugarcane response to water table, periodic flood, and foliar nitrogen on organic soil. *Agronomy Journal,* **98:** 616–621 (2006).
31. Glaz, B., Edme, S. J., Miller, J. M., Milligan, S. B. and Holder, D. G., Sugarcane cultivar response to high summer water tables in the Everglades. *Agronomy Journal,* **94:** 624–629 (2002).
32. Glaz, B., Morris, D. R. and Daroub, S. H., Sugarcane photosynthesis, transpiration and stomatal conductance due to flooding and water table. *Crop Science,* **44:** 1633–1641 (2004a).
33. Glaz, B., Morris, D. R. and Daroub, S. H., Periodic flooding and water table effects on two sugarcane genotypes. *Agronomy Journal,* **96:** 832–838 (2004b).
34. Glaz, B., Sugarcane growth, morphological, and photosynthetic responses to water-table depths. *Journal of Sustainable Agriculture,* **28:** 77–97 (2006).
35. Gomathi, R., Physiological basis of waterlogging resistance in sugarcane. In Sugarcane physiology, ed. Gururaja Rao, P. N., Published by Sugarcane Breeding Institute (ICAR) and Directorate of open and distance learning, TNAU, Coimbatore, pp 53-61 (2009).
36. Gomathi, R. and Chandran, K., Effect of waterlogging on growth and yield of sugarcane clones. Sugarcane Breeding Institute (SBI-ICAR). *Quarterly News Letter,* **29(4):** 1–2 (2009).
37. Gomathi, R., Gururaja, P. N., Chandran, K. and Selvi, A., Adaptive responses of sugarcane to waterlogging stress: An overview. *Sugar Tech,* **17:** 325-338 (2015).
38. Gomathi, R., Manohari, N. G. and Rakkiyappan, P., Antioxidant enzymes on cell membrane integrity of sugarcane varieties differing in flooding Tolerance. *Sugar Tech,* **14:** 261–265 (2012).
39. Harris, K., Subudhi, P. K., Borrell, A., Jordan, D., Rosenow, D., Nguyen, H., Klein, P., Klein, R. and Mullet, J., Sorghum stay-green QTL individually reduce post-flowering drought-induced leaf senescence. *Journal of Experimental Botany,* **58:** 327–338 (2007).
40. Hasan, M. F., Alam, M. R., Jabber, M. A., Begum, M. K. and Miah, M. A. S., Effect of waterlogging on juice quality and yield of sugarcane. *Pak. J. Biol. Sciences,* **6(13):** 1151-1155 (2003).
41. Heyden V. D., Ray, C. J. D. and Nable, R., Effects of water logging on young sugarcane plants. Aust. Sugarcane, **2:** 28-30 (1998).
42. Hidaka, T. and Karim, M., Flooding tolerance of sugarcane in relation to growth, physiology and root structure. *South Pacific Studies,* **28:** 11–21 (2007).
43. Hogarth, D. M., Genetics of sugarcane. In: Heinz, D. J., (ed.) Sugarcane improvement

through breeding. Elsevier, Amsterdam, pp. 255–272 (1987).
44. Islam, M. S. and Begum, M. K., Comparative studies of chlorophyll content, yield and juice quality of eight sugarcane varieties. *J. Agro. Env,* **6(1):** 121-124 (2012).
45. Islam, M. S., Miah, M. A. S., Begum, M. K., Alam, M. R. and Arefin, M. S., Effects of waterlogging stress on growth and yield of five sugarcane varieties. *Int. J. Sustain. Agril. Tech.* **5(4):** 92-97 (2009a).
46. Islam, M. S., Miah, M. A. S., Begum, M. K., Alam, M. R. and Arefin, M. S., Biochemical studies of juice quality and yield performance of some promising sugarcane clones under water-logging stress condition. *J. Agrofor. Environ,* **5(1):** 87-90 (2011a).
47. Islam, M. S., Miah, M. A. S., Begum, M. K., Alam, M. R. and Arefin, M. S., Growth, yield and quality of some selected sugarcane clones under water-logging stress condition. *World Journal of Agricultural Sciences,* **7(4):** 504-509 (2011b).
48. Islam, M. S., Miah, M. A. S., Begum, M. K., Alam, M. R. and Arefin, M. S., Effects of water-logging stress on yield and yield contributing characters of sugarcane. *Int. J. Sustain. Agril. Technology,* **5:** 23-27 (2009b).
49. Islam, M. S., Miah, M. A. S., Begum, M. K., Alam, M. R. and Hossain, M. A., Performance of sugarcane grown under water-logging stress condition. *Bangladesh J. Sugarcane,* **29:** 96-105 (2007).
50. Jackson, M. B. and Armstrong, W., Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biology,* **3:** 274–287 (1999).
51. Jain, R., Singh, A., Singh, S., Singh, S. P., Srivastava, V. K., Chandra, A., Pathak, A. D. and Solomon, S., Physio-biochemical characterization of sugarcane genotypes for waterlogging tolerance. *World Journal of Agricultural Sciences,* **13:** 90-97 (2017).
52. Jaiphong, T., Tominaga, J., Watanabe, K., Nakabaru, M., Takaragawa, H., Suwa, R., Kawamitsu, Y., Effects of duration and combination of drought and flood conditions on leaf photosynthesis, growth and sugar content in sugarcane. *Plant Production Science,* **19:** 427–437 (2016).
53. Jaiphong, T., Tominaga, J., Watanabe, K., Suwa, R., Ueno, M. and Kawamitsu, Y., Changes in photosynthesis, growth, and sugar content of commercial sugarcane cultivars and *Erianthus* under flood conditions. *Plant Production Science,* **20:** 126-135 (2017).
54. Jimenez, A., Hernández, J. A., del Rio, L. A. and Sevilla, F., Evidence for the presence of the ascorbate-glutathione cycle in mitochondria and peroxisomes of pea leaves. *Plant Physiology,* **114:** 275-284 (1997).
55. Justin, S. H. F. W. and Armstrong, W., Evidence for the involvement of ethylene in aerenchyma formation in adventitious roots of rice (*Oryza sativa*). *New Phytologist,* **118:** 49-62 (2003).
56. Kang, M. S., Snyder, G. H. and Miller, J. D., Evaluation of *Saccharum* and related germplasm for tolerance to high water table on organic soil. *J. Am. Soc. Sugar Cane Technol,* **6:** 59-63 (1986).
57. Kennedy, R. A., Rumpho, M. E. and Fox, T. C., Anaerobic metabolism in plants. *Plant Physiology,* **100:** 1–6 (1992).
58. Khaiyam, M. O., Islam, M. S., Ganapati, R. K., Uddin, M. J. and Hossain, M. I., Suitability of newly released sugarcane varieties in farmers field condition under high ganges river floodplain of Bangladesh. *Int J Plant Biol Res,* **6(2):** 1086 (2018).
59. Kovar, J. L. and Kuchenbuh, R. O., Commercial importance of adventitious rooting to agronomy. In Biology of adventitious root formation, ed. by Davis, T. D. and Haissig, B. E., New york, pp. 25–35 (1994).
60. Krishna, B. and Kamat, D. N., Character association among cane yield and their component traits in sugarcane under

waterlogged condition. *Int. J. Curr. Microbiol. App. Sci.,* **6(10):** 2331-2339 (2017).

61. Krishna, B., Kamat, D. N., Kumar, B., Ram, J. J., Kumar, G., Kumari, R., Bharati, S. P., Ranjan, R. and Kumar, S., Study of genetic variability among mid late maturing sugarcane clones for different yield and juice quality traits under waterlogged condition. *Int. J. Curr. Microbiol. App. Sci.,* **6(7):** 1390-1397 (2017).
62. Krishna, B., Kamat, D. N., Kumari, J. and Prakash, D., Genetic divergence of sugarcane under waterlogging conditions. *Int. J. Pure App. Biosci.,* **6(1):** 210-218 (2018).
63. Kumar, N., Singh, H., Kumari, R. and Singh, V. P., Comparative analysis of yield and quality in sugarcane genotypes under waterlogged and normal condition. *J. Life Sci*., **10(1):** 323-327 (2015).
64. Kumar, R., Kumar, B., Jain, J. and Sandhu, S. K., *In vitro* selection of variants for red rot resistance in the subtropical sugarcane cultivar CoJ64. *Pl. Dis. Res.,* **28:** 16-21 (2013).
65. Kumar, T., Khan, M. R., Abbas, Z. and Ali, G. M., Genetic improvement of sugarcane for drought and salinity stress tolerance using *Arabidopsis vacuolar pyrophosphatase* (*AVP1*) gene. *Mol. Biotechnol*., **56:** 199–209 (2014).
66. Kumari, R., Singh, V. P. and Kumar, N., Effect of waterlogging on quality constraints of sugarcane varieties. *Env. and Eco,* **31(2):** 546-550 (2013).
67. Laan, P., Clement, J. M. A. M. and Blom, C. W. P. M., Growth and development of Rumex roots as affected by hypoxic and anoxic conditions. *Plant and Soil,* **136:** 145–151 (1991).
68. Lakshmanan, P., Geijkes, R. J., Aitken, K. S., Grof, C. L., Bonnet, G. D. and Smith, G. R., Sugarcane Biotechnology: the challenges and opportunities. *In Vitro Cellular and Developmental Biology,* **41:** 345-363 (2005).
69. Lawlor, D. W. and Cornic, G., Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. *Plant Cell Environ,* **25:** 275-294 (2002).
70. Lawson, T., Guard cell photosynthesis and stomatal function. *New Phytol,* **181:** 13-34 (2009).
71. Leite, D. C. C., Grandis, A., Tavares, E. Q. P., Piovezani, A., Pattathil, S., Avci, U., Rossini, A., Cambler, A., De Souza, A. P., Hahn, M. G. and Buckeridge, M. S., Cell wall changes during the formation of aerenchyma in sugarcane roots. *Annals of Botany,* **120:** 693–708 (2017).
72. Malik, S. S. and Tomer, B. S., Sugarcane varietal performance under high waterlogging conditions. *Indian Sugar,* **53:** 585 (2003).
73. Mano, Y. and Omori, F., Flooding tolerance in interspecific introgression lines containing chromosome segments from teosinte (*Zea nicaraguensis*) in maize (Zea mays subsp. *mays*). *Annals of Botany,* **112:** 1125–1139 (2013).
74. Mano, Y., Muraki, M. and Takamizo, T., Identification of QTL controlling flooding tolerance in reducing soil conditions in maize (*Zea mays* L.) seedlings. *Plant Prod. Sci*., **9:** 176–181 (2006).
75. Mano, Y., Muraki, M., Fujimori, M., Takamizo, T. and Kindiger, B., Identification of QTL controlling adventitious root formation during flooding conditions in teosinte (*Zea may*s ssp. huehuetenangensis) seedlings. *Euphytica,* **142:** 33–42 (2005a).
76. Mano, Y., Omori, F., Kindiger, B. and Takahashi, H., A linkage map of maize × teosinte Zea luxurians and identification of QTLs controlling root aerenchyma formation. *Molecular Breeding,* **21:** 327–337 (2008).
77. Mano, Y., Omori, F., Loaisiga, C. H. and Bird, R. M., QTL mapping of above-ground adventitious roots during flooding in maize × teosinte *Zea nicaraguensis* backcross population. *Plant Root,* **3:** 3–9 (2009).

78. Mano, Y., Omori, F., Muraki, M. and Takamizo, T., QTL mapping of adventitious root formation under flooding conditions in tropical maize (*Zea mays* L.) seedlings. *Breeding Science,* **55:** 343–347 (2005b).
79. Mano, Y., Omori, F., Takamizo, T., Kindiger, B., Bird, R. M., Loaisiga, C. H. and Takahashi, H., QTL mapping of root aerenchyma formation in seedlings of a maize × rare teosinte *Zea nicaraguensis* cross. *Plant Soil,* **295:** 103–113 (2007).
80. Mano, Y., Omori, F., Tamaki, H., Mitsuhashi, S. and Takahashi, W., DNA marker-assisted selection approach for developing flooding-tolerant maize. *Jpn. Agric. Res. Q*uarterly, **50:** 175–182 (2016).
81. Mazur, B. J. and Tingey, S. V., Genetic mapping and introgression of genes of agronomic important. *Current Opinion in Biotechnology,* **6(2):** 175–182 (1995).
82. Molinari, H. B. C., Marur, C. J., Daros, E., De Campos, M. K. F., De Carvalho, J. F. R. P., Pereira, L. F. P., Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp.): osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiol. Plant*. **130:** 218–229 (2007).
83. Morris, D. R., Tai, P. Y. P. and Struve, D., Sugarcane yield and rhizosphere characteristics in flooded organic soil determined from a pot study. *Journal American Society of Sugar Cane Technologists,* **24:** 18-30 (2004).
84. Nayak, S. N., Song, J., Villa, A., Pathak, B., Ayala-Silva, T., Yang, X., Promoting utilization of *Saccharum* spp. Genetic resources through genetic diversity analysis and core collection construction. *PLOS ONE,* **9:** e110856 (2014).
85. Nilkantha, K. A., Effect of waterlogging on physiological, biochemical, yield and yield attributing parameter of sugarcane (*Saccharum* spp.) Navsari Agricultural University Navsari Gujarat, pp. 396-450 (2016).
86. Orellana, C. and Neto, R. B., Brazil and Japan give fuel to ethanol market. *Nat Biotech,* **24:** 232 (2006).
87. Pandey, D. M., Changes in chlorophyll content of three sugarcane (*Saccharum officinarum* L.) cultivars in relation to water logging and planting methods. *Crop Research,* **21(3):** 360-363 (2001).
88. Paul, G. C., Rahman, M. K., Hossain, A. H. M. D. and Alam, M. J., Performance of some newly released sugarcane varieties in growers field under tista flood plain soils. *Bangladesh J. Sugarcane,* **16:** 131-133 (1994).
89. Pereira-Santana, A., Alvarado-Robledo, E. J., Zamora-Briseño, J. A., Ayala-Sumuano, J. T., Gonzalez-Mendoza, V. M. and Espadas-Gil, 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,* **12(12):** e0189271 (2017).
90. Qiu, F., Zheng, Y., Zhang, Z. and Xu, S., Mapping of QTL associated with waterlogging tolerance during the seedling stage in maize. *Annals of Botany,* **99:** 1067–1081 (2007).
91. Rahman, M. S., Islam, M. S., Amanullah, A. S. M., Islam, M. A. and Ohiduzzaman, M., Potentiality of seven sugarcane varieties in high ganges river flood plain soils of Bangladesh. *Int. J. Sustain. Agril. Tech.,* **6(2):** 04-07 (2010).
92. Ramiro, D. A., Melotto-Passarin, D. M., Barbosa, M. A., Santos, F., Gomez, S. G. P., Massola Júnior, N. S., Expression of *Arabidopsis bax inhibitor-1* in transgenic sugarcane confers drought tolerance. *Plant Biotechnology Journal,* **14:** 1826–1837 (2016).
93. Ray, J. D., Miller, J. D. and Sinclair, T. R., Survey of aerenchyma in sugarcane roots. In Fifth Symposium, Int. Soc. of Root Research. July 14–18, 1996. Clemson, SC, p. 118 (1996).
94. Raza, G., Ali, K., Ashraf, M. Y., Mansoor, S., Javid, M. and Asad, S., Overexpression of an H^+-PPase gene from Arabidopsis in sugarcane improves drought tolerance, plant growth, and photosynthetic

responses. *Turkish J. Biol*ogy, **40:** 109–119 (2016).
95. Reed, S., Ayala-Silva, T., Brown, S., Glaz, B. and Comstock, J., Screening *Saccharum barberi* and *sinense* accessions for flood tolerance and biomass production. *Journal of Agronomy and Crop Science,* **198(3):** 236-244 (2012).
96. Reis, R., da Cunha, B., Martins, P., Martins, M., Alekcevetch, J., Chalfun, A., Induced over - expression of *ArDREB2A* CA improves drought tolerance in sugarcane. *Plant Sci*ence, **221:** 59–68 (2014).
97. Roach, B. T. and Mullins, R. T., Testing sugarcane for water logging tolerance. *Proceedings of Australian Society Sugarcane Technology,* pp. 95–102 (1985).
98. Russell, D. A. and Sachs, M. M., Protein synthesis in maize during anaerobic and heat stress. *American Society of Biologists,* **99(2):** 615–620 (1992).
99. Sachs, M. M., Freelins, M. and Okimoto, R., The anaerobic proteins of maize. *Cell,* **20:** 761–767 (1980).
100. Saleem, M., Ashraf, M. and Akram, N. A., Salt (NaCl) - induced modulation in some key physio-biochemical attributes in okra (*Abelmoschus esculentus* L.). *J. Agron. Crop Science,* **197:** 202-213 (2011).
101. Sanghera, G. S. and Kumar, A., Recent perspectives towards enhancing drought tolerance in sugarcane. *J. Pl. Sci. Res.,* **34:** 23-34 (2018).
102. Shrivastava, A. K. and Srivastava, S., Diversity of the germplasm of Saccharum species and related genera available for use in directed breeding programmes for sugarcane improvement. *Current Science,* **111(3):** 475-482 (2016).
103. Singh, K., Sugarcane cultivated in water-logging areas. *Indian Sugar,* **10:** 537 (1990).
104. Smirnoff, N. and Crawford, R. M. M., Variation in the structure and response to flooding of root aerenchyma in some wetland plants. *Annals of Botany,* **51:** 237–249 (1983).
105. Soleh, M. A., Ariyanti, M., Dewi, I. R. and Kadapi, M., Chlorophyll fluorescence and stomatal conductance of ten sugarcane varieties under waterlogging and fluctuation light intensity. *Emirates Journal of Food and Agriculture,* **30(11):** 935-940 (2018).
106. Steffens, B. and Rasmussen, A., The physiology of adventitious roots. *Plant Physiology,* **170:** 603-617 (2016).
107. Sukhchain, S. D. and Saini, G. S., Inter-relationships among cane yield and commercial cane sugar and their component traits in autumn plant crop of sugarcane. *Euphytica,* **95:** 109-114 (1997).
108. Tavares, E. Q. P., De Souza, A. P., Romim, G. H., Grandis, A., Plasencia, A., Gaiarsa, J. W., Grima-Pettenati, J., Setta, N., Sluys, M. A. V. and Buckeridge, M. S., The control of endopolygalacturonase expression by the sugarcane RAV transcription factor during aerenchyma formation. *Journal of Experimental Botany,* **70(2):** 497–506 (2019).
109. Tavares, E. Q. P., Grandis, A., Lembke, C. G., Souza, G. M., Purgatto, E., De Souza, A. P. and Buckeridge, M. S., Roles of auxin and ethylene in aerenchyma formation in sugarcane roots. *Plant Signal Behav,* **13(3):** e1422464 (2018).
110. Thirugnanasambandam, P. P., Hoang, N. V. and Henry, R. J., The challenge of analyzing the sugarcane genome. *Front. Plant Sci.,* **9:** 616 (2018).
111. Viator, R. P., White, P. M., Hale, A. J. and Waguespack, H. L., Screening for tolerance to periodic flooding for cane grown for sucrose and bioenergy. *Biomass Bioenergy,* **44:** 56-63 (2012).
112. Walker, J. C., Howard, E. A., Dennis, E. S. and Peacock, W. J., DNA sequences required for anaerobic expression of maize *Adhl* gene. *Proceedings of National Academy Science,* **84:** 6624–6629 (1987).

113. Watanabe, K., Takahashi, H., Sato, S., Nishiuchi, S., Omori, F., Malik, A. I., Colmer, T. D., Mano, Y. and Nakazono, M. A., Major locus involved in the formation of the radial oxygen loss barrier in adventitious roots of teosinte *Zea nicaraguensis* is located on the short-arm of chromosome 3: Chromosomal region endowing a root ROL barrier. *Plant Cell Environ*ment, **40:** 304–316 (2017).
114. Webster, P. W. D. and Eavis, B. W., Effect of flooding on sugarcane growth stage. *Proceeding of International Society Sugarcane Technology,* **14:** 708–714 (1971).
115. Zaidi, P. H., Rashid, Z., Vinayan, M. T., Almeida, G. D., Phagna, R. K. and Babu, R., QTL mapping of agronomic waterlogging tolerance using recombinant inbred lines derived from tropical maize (*Zea mays* L.) germplasm. *PLoS ONE,* **10(4):** e0124350 (2015).
116. Zhang, J. S., Nagai, C., Yu, Q. Y., Pan, Y. B., Ayala-Silva, T. and Schnell, R. J., Genome size variation in three Saccharum species. *Euphytica,* **185:** 511–519 (2012).
117. Zhang, S. Z., Yang, B. P., Feng, C. L., Chen, R. K., Luo, J. P., Cai, W.W., Expression of the *grifola frondosa trehalose synthase* gene and improvement of drought-tolerance in sugarcane (*Saccharum officinarum* L.). *Journal of Integrated Plant Biology,* **48:** 453–459 (2006).
118. Zhou, D., Wang, C., Li, Z., Chen, Y., Gao, S., Guo, J., Lu, W., Su, Y., Xu, L. and Que, Y., Detection of bar transgenic sugarcane with a rapid and visual loop-mediated isothermal amplification assay. *Frontiers in Plant Science,* **7:** 279 (2016).