

Physio- biochemical and molecular changes associated with waterlogging stress in sugarcane: A Review

Abstract

The term waterlogging refers to a condition of short/ long term water stagnation due to reduced capacity of the drainage system. In India, physical degradation of soil due to waterlogging has been estimated to be 8.5 million ha. It usually occurs due to rise in water table in almost all the canal irrigated areas with poor drainage system. Effects of waterlogging are augmented by secondary salinization and it aggravated the incidence of certain pests and diseases. In sugarcane, waterlogging is one of the major abiotic stresses affecting cane and sugar productivity. In many parts of the India, like eastern UP, northern Bihar and deltaic region of Tamil Nadu, sugarcane suffers from waterlogging during elongation phase because of heavy monsoon rains and poor drainage facilities. Higher water table during grand growth phase adversely affects cane weight and shoot population which occurs due to shift in respiratory metabolism from aerobic to anaerobic condition. Waterlogging not only reduced root growth, leaf emergence rate but also caused destruction of root function, hormone balance and nutrient availability. Sugarcane crop affected with waterlogging stress showed aerial rooting, cane lodging and decreased cane and sugar yield. Under water-logging condition, some morphological, anatomical, physiological and biochemical changes take place in plant for the sake of adaptation/survival. Present paper is an overview of physiological, morphological, anatomical, biochemical and molecular changes associated with waterlogging tolerance in sugarcane.

Introduction :

Sugarcane (*Saccharum officinarum*) has been widely known as raw material for white sugar production (Arif et al., 2019). Sugarcane juice is relished as a refreshing drink as it is nutritious and rich in vitamins, carbohydrates, and amino acids. Sugarcane is a tall perennial true grass belonging to the genus *Saccharum* and tribe Andropogoneae (Islam & Bose, 2022). It originated in Southeast Asia and is now cultivated in tropical and subtropical countries throughout the

world for sugar and by-products (Eggleston, 2018; Singh Kourav et al., 2022). Over the last few decades, waterlogging stress has increasingly threatened global crop production including sugarcane and as a result of change in climate, accelerated land degradation, and inadequate drainage systems, flooding or waterlogging incidents have become increasingly frequent and unpredictable worldwide in recent years (Zhang et al., 2021; Awal & Islam, 2020; Mondal et al., 2024). This study reviews physio- biochemical and molecular changes associated with waterlogging stress in sugarcane.

Morphological changes

Visible effects: Sugarcane plants showed leaf chlorosis, drying and aerial rooting in nodal region of cane stalk due to waterlogging (Fig 1&2).





July

August

September

Fig 1 Waterlogging affected sugarcane crop (July to September)





Fig.2: Visible symptoms

Changes in Physio-Biochemical attributes

Root Growth

Conversion of aerobic environment to anaerobic is the immediate effect of water logging in soil due to lack of aeration affecting growth and functioning of roots. In absence of oxygen, root hairs die and eventually the roots blacken and entire underground root system gets choked. Due to inadequate root system, absorption of nutrients and water is adversely affected leading to nutrient deficiency and physiological drought similar to the conditions observed during moisture stress. Waterlogged plants showed three different kind of root system (Tetsushi & Karim 2007). 1. Aerial roots emerged from aerial nodes 2. Roots developed from the pre-existing roots primordia 3. Secondary roots

initiated from the newly developed roots. These adventitious roots with high porosity help plant to absorb water and nutrient under waterlogging condition similar to older root system (Kozłowski et al., 1984). These adventitious roots are positioned near the aerated soil surface (Alam et al., 2013). Basic mechanism involved in the formation of these new root system is stimulation of the outgrowth of pre-existing root primordia in the shoot base.

Rooting pattern

To study the effect of waterlogging on rooting pattern in sugarcane, twenty three sugarcane genotypes viz., CoLK 94184, BO 91, CoS 767, CoJ 64, CoS 97264, UP 9530, CoLk 12204, CoLk 12202, CoLk 12206, CoLk 07201, CoLk 04238, LG 06605, LG 04439, LG 05350, LG 05020, LG 03040, A-46-11, B-44-12, A-27-12, D-12-9, D-6-13, S 5085/11, S 5087/11) were evaluated under waterlogged condition along with untreated control. Findings obtained indicated reduction in shoot dry mass in all the genotypes tested except CoLk94184, CoLk 12204, CoLk 12206, LG06605, LG04439, D-6-13 and S 5085/11. Under waterlogged condition, almost all the genotypes formed aerial roots at nodal region; some of the genotypes showed aerial roots up to the 12th nodes and dense aerial roots in CoLk 94184, UP 9530, CoS 97264, LG 04439, LG 05020, D-6-13, S5985/11, S 5087/11 genotypes (Fig.3). Due to aerial root formation, total root biomass was comparatively higher under waterlogged condition (Jain et al., 2018).

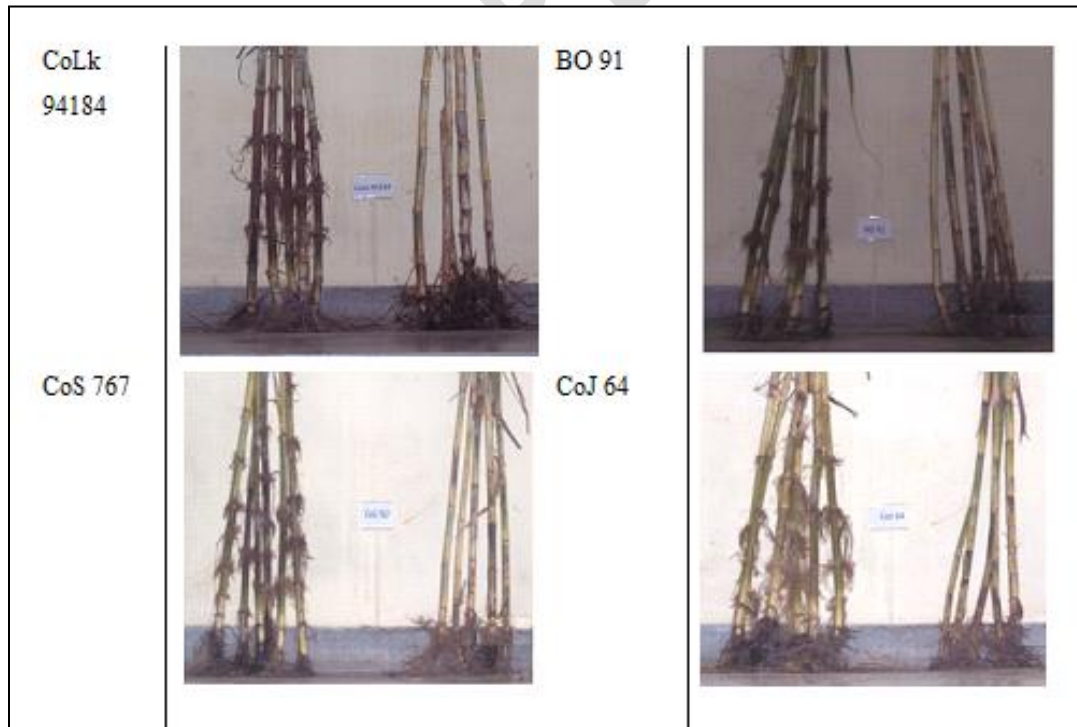


Fig.3: Root Anatomy of Sugarcane Genotypes Under Control and Waterlogged Conditions

Source: Jain et al., (2018)

Cortical cell distortion and loss of uniformity in endodermis was observed due to waterlogging in root tissues. The size and number of metaxylem vessel of waterlogged roots decreased (Fig. 4&5), while, their numbers increased in shoot roots and it showed positive association ($R^2= 0.392$) with shoot dry weight. Based on root parameters, seven genotypes, viz., CoLk 94184, UP 9530, CoS 97264, LG 04439, LG 05020, D-6-13, S5085/11 and S 5087/11 were grouped as tolerant lines (Jain et al., 2018).

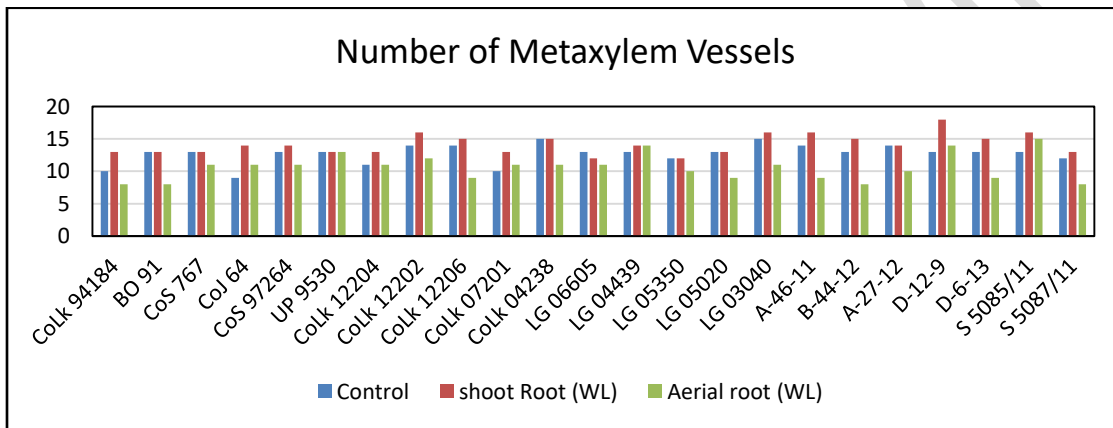
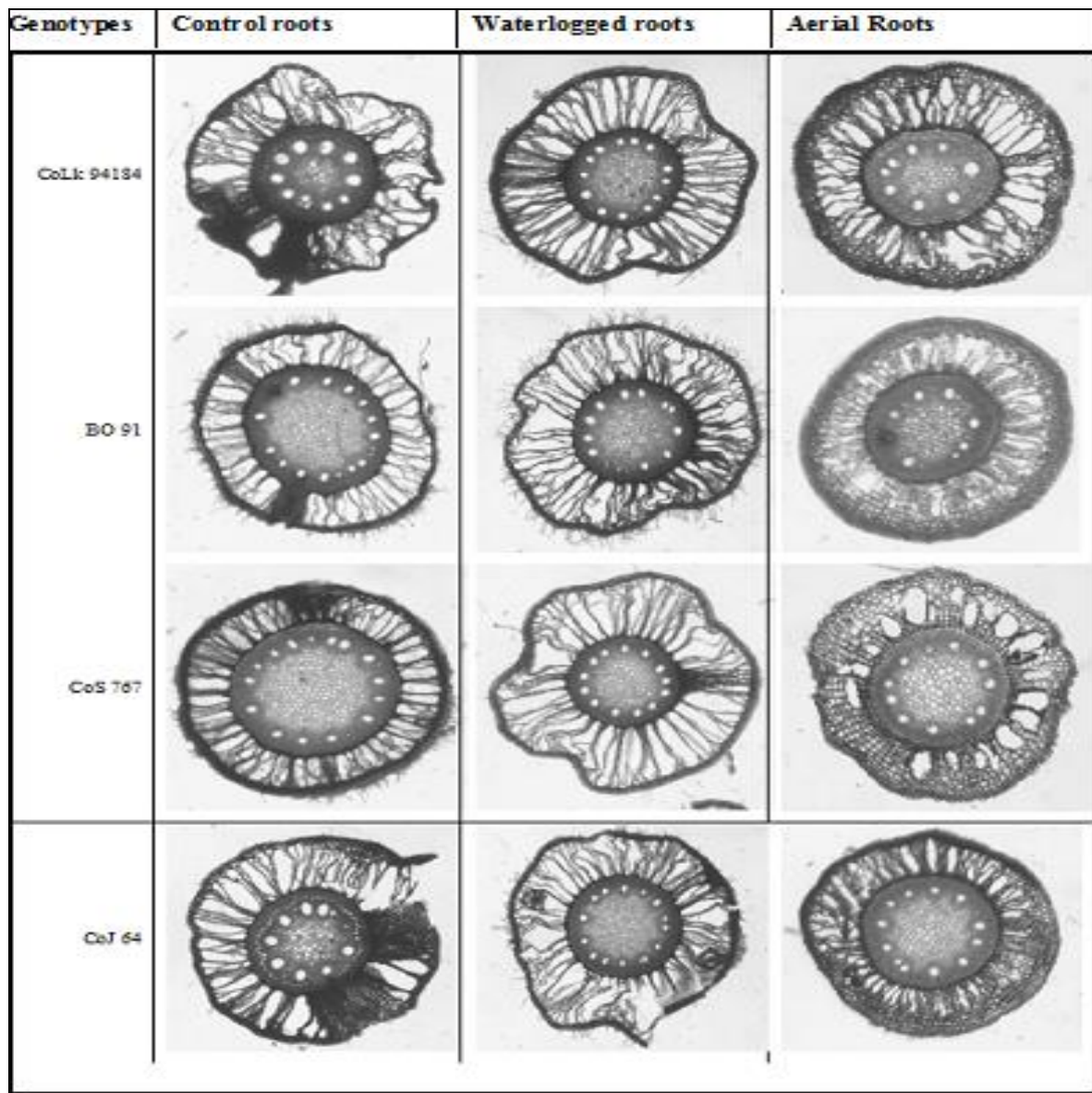


Fig 4: Effect of Waterlogging on Number of Metaxylem Vessels of Sugarcane Genotypes

(Jain et al., 2018)



Source: Jain et al (2018)

Fig.5: Root anatomy of different types of sugarcane roots in response to waterlogging
Aerenchyma Formation

Under waterlogged conditions, aerial roots have much larger intercellular spaces called aerenchyma (Kovar and Kuchenbuh 1994; Van der Heyden *et al.* 1998) which helps in transport of oxygen to the roots from shoots (Colmer 2003). Ethylene involvement in formation of adventitious roots and aerenchyma formation in several crops was summarized by Jackson (1990a, b). When ACC synthase concentrations increase ACC under flooded condition, which then diffuses to aerated parts of the root and is converted into ethylene by ACC oxidase (Pearce *et al.*, 1992). High ethylene level under waterlogged conditions makes the

tissues sensitive to auxin, which stimulates hypertrophy and adventitious root formation occurs at the base of stem just above the waterline (Visser *et al.*, 1996).

Scanning electron microscopy (SEM)

Scanning electron microscopy (SEM) were studied to observe alterations on the root surface and cross section upon WL exposure in 6 months old plant of *Saccharum* spp. hybrid cultivars CoLk 94184 and CoJ 64 grown under waterlogged and control conditions at Kharika Block, ICAR-IISR Lucknow farm (Jain *et al.*, 2019) (Fig 5 &6). The effect of waterlogging stress was more pronounced in root growth than on the shoot. Waterlogged affected plants showed aerial root formation in both the cultivars but the level was more in CoJ64. The SEM analysis of the roots indicated morphological changes and surface ultra structural modification. Both control and waterlogged affected shoot root and aerial roots showed aerenchyma formation in cortical region, size of aerenchyma was relatively more due to waterlogging. Compared to intact and uniform surface cells in the control roots, cells were irregular and distorted due to waterlogging. In WL affected roots, the number of root hairs increased manifold, showing dense growth, and these were apparently longer. Apart from the deformity in surface morphology and anatomy of the roots in response to waterlogging, considerable anatomical alterations were also observed. WL affected roots exhibited signs of injury in the form of cell distortion, particularly in the cortical cells. The endodermis and pericycle regions showed loss of uniformity due to waterlogging in both the cultivars, but level of deformation was more in CoJ 64, indicated more sensitivity for waterlogging stress.

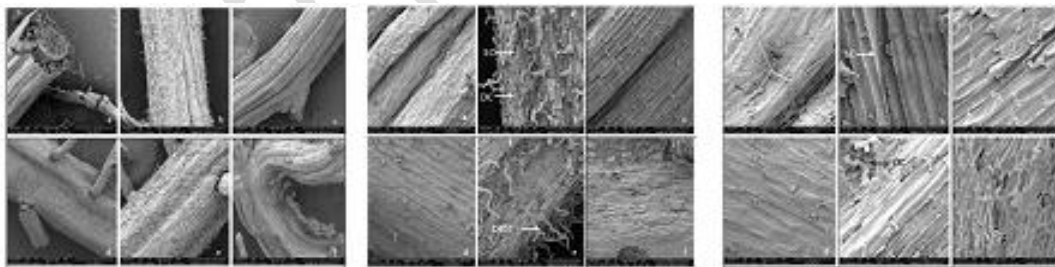


Fig.5: A.Scanning electron micrographs ($\times 50$) of the root surface of Sugarcane showing the effect of waterlogging treatment. a. CoLk94184 (control); b. CoLk94184 (waterlogged shoot root); c. CoLk94184 (waterlogged aerial root); d. CoJ64 (control); e. CoJ64 (waterlogged shoot root); f. CoJ64 (waterlogged aerial root). B. Scanning electron micrographs ($\times 250$) of the root surface of Sugarcane showing the effect of waterlogging treatment on root hair development. a. CoLk94184 (control); b. CoLk94184 (waterlogged shoot root); c. CoLk94184 (waterlogged aerial root); SC,

scrapped off cell; DC, damaged cells; DRH, dense root hairs. Increased number of root hairs in treated root (b and d) as compared to control. C. Scanning electron micrographs ($\times 500$) of the root surface of Sugarcane showing the effect of waterlogging treatment. a. CoLk94184 (control); b. CoLk94184 (waterlogged shoot root); c. CoLk94184 (waterlogged aerial root) SC, scrapped off cell; DC, damaged cells. Source: Jain et al (2019)

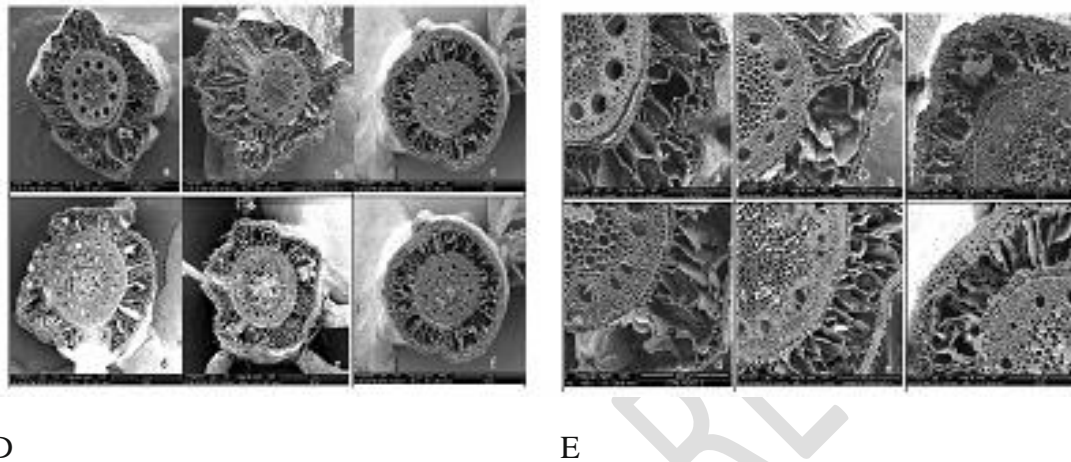


Fig.6 : D. Scanning electron micrographs ($\times 70$) of cross section root of Sugarcane showing the effect of waterlogging on ground cell and vascular bundle. a. CoLk94184 (control); b. CoLk94184 (waterlogged shoot root); c. CoLk94184 (waterlogged aerial root). Cx, Cortex; DC, damaged cortex. E. Scanning electron micrographs ($\times 170$) of cross section root of Sugarcane showing the effect of waterlogging on aerenchyma and pith region. a. CoLk94184 (control); b. CoLk94184 (waterlogged shoot root); c. CoLk94184 (waterlogged aerial root). Ar, aerenchyma; IC, intact cell; DC, damaged cell; Dp, depositions. Source: Jain et al (2019)

Shoot Growth

The impact of water logging on shoot growth indicates changes in growth habit, visual health, internal anatomy, water relations, and hormonal and nutritional composition. Water logging can inhibit leaf and stem expansion and tiller production (Jackson, 1989). Plant height of the flooded plant was noticeably higher than that of the control plant in one month flooding (Tetsushi and Karim, 2007). The rapid elongation of plant apical meristems, juicy stems and internodes facilitates escape from the anoxic environment and contact with the air, thereby enabling normal respiration (Kuroha *et al.*, 2018). This mechanism is called as low oxygen escape syndrome (LOES). Internodes of submerged rice cultivars elongate rapidly. Waterlogging induces accumulation of ethylene (ET) and promotes synthesis of gibberellins (GAs) (largely GA_4), thus promoting internode elongation

(Kuroha *et al.*, 2018). Twenty four sugarcane genotypes (eighteen germplasms/lines and six commercial varieties) of sugarcane were evaluated for waterlogging tolerance based on leaf, stalk, root and whole clump dry weight, specific leaf weight, chlorophyll and carotenoid content for identifying waterlogging tolerant sugarcane genotypes based on tolerance indices at Kharika Block of Indian Institute of Sugarcane Research, Lucknow. Results of present investigation indicated lower leaf, stalk, and whole clump dry weight, specific leaf weight and area, chlorophyll and carotenoid, P and K contents in leaf tissues under waterlogged conditions. Waterlogging resulted in variation in rate of elongation and SR/AR ratio in different sugarcane genotypes (Jain *et al.*, 2017). Stalk elongation rate was ranged between 0.016-0.909 cm/day. Maximum SR/AR ratio (5.59) was observed in CoLk 07201 and minimum (0.795) in S 5087/11 sugarcane genotype. Tolerance indices for stalk dry weight showed significant positive correlation with tolerance indices for total clump dry weight, leaf weight, root weight, specific leaf weight, chlorophyll a, b, total chlorophyll, and carotenoids contents. Waterlogged affected plants showed reduction in SPAD index, an indicator of relative chlorophyll concentration of leaves; SPAD value < 40 indicates impairment of the photochemical process. Sugarcane genotypes had different tolerance indices for the studied parameters; D-6-13, S 5090/11 and S 5085/11 had greater tolerance indices for stalk and whole clump dry weight (Table 1). Based on relative cane weight CoLk 94184, S5085/11, CoLk 12206, LG 06605, LG 04439, UP 9530 and D-6-13 were identified the most waterlogging tolerant lines, while, CoJ 64 and A-27-12 was found most susceptible.

Table 1: Mean of Plant parameters measured under control and waterlogged condition

Parameters	Control	Waterlogging	% Numerical Increase/decrease
Leaf dry weight (kg)	0.178	0.131	-35.9
Leaf Sheath dry weight (kg)	0.107	0.089	-20.2
Stalk dry weight (kg)	0.695	0.678	-2.5
Root dry weight (kg)	0.022	0.029	24.1
Whole clump dry weight (kg)	1.002	0.938	-6.8
Chlorophyll a (mg/g fwt)	2.548	2.101	-21.3
Chlorophyll b (mg/g fwt)	0.710	0.630	-12.7
Total Chlorophyll (mg/g fwt)	3.256	2.720	-19.7
Carotenoids (mg/g fwt)	3.879	3.310	-17.2
SLW (gm dry wt/m ²)	109.9	106.1	-3.6
K content (%)	1.676	1.446	-15.9
P content (%)	0.386	0.331	-16.6

Single cane weight decreased under waterlogged condition in most of genotypes studied, very few genotypes viz., D-6-13, LG 05350, UP 9530 showed increase over control; highest in D-6-13 (Fig.7). Genotypes D-6-13, and S 5085/11 had greater tolerance indices for stalk and whole clump dry weight.

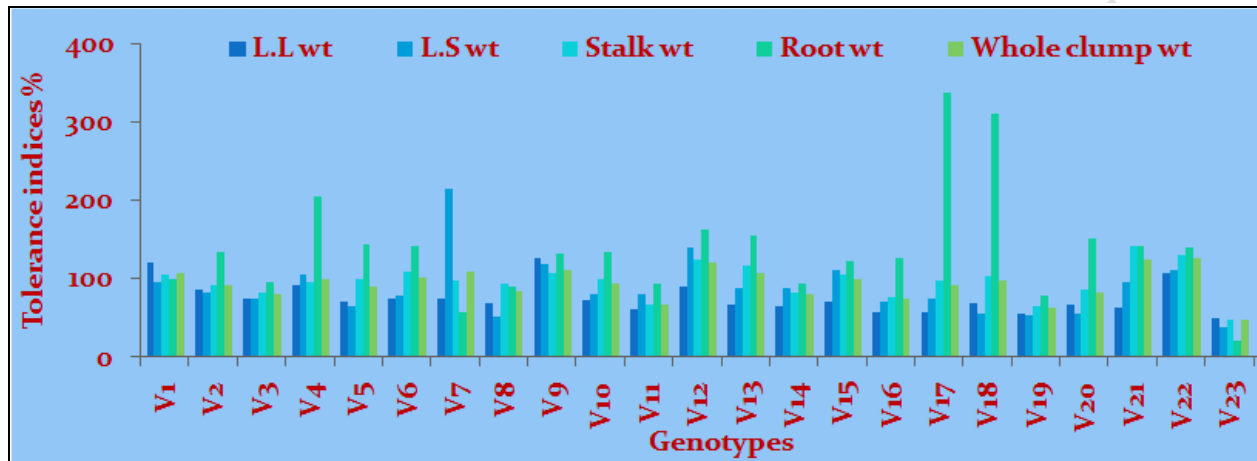


Fig.7: Mean comparison of tolerance indices for different plant parameters

V1-V23: CoLk 94184, BO 91, CoS 767, CoJ 64, CoS 97264, UP 9530 and seventeen advanced germplasm lines (CoLk 12204, CoLk 12202, CoLk 12206, CoLk 07201, CoLk 04238, LG 06605, LG 04439, LG 05350, LG 05020, LG 03040, A-46-11, B-44-12, A-27-12, D-12-9, D-6-13, S 5085/11, S 5087/11, LLwt- Leaf Lamina weight, LS wt- Leaf sheath weight (source: Jain et al., 2017)

Further, changes in growth pattern was studied using two sugarcane varieties (CoLk 94184- tolerant and CoJ 64 - sensitive) grown under waterlogged conditions at ICAR-IISR, Lucknow. Findings obtained indicated higher plant height of waterlogged plants, while, leaf parameters decreased markedly as compared to control (Table 2). Fresh and dry weight of different plant parts decreased due to waterlogging in both the varieties; level of decrease was more in sensitive variety CoJ 64 (Table 3). The earlier appearance of aerial rooting seems to be associated with greater shoot root injuries due to waterlogging.

Table 2: Changes in Growth attributes and fresh weight of sugarcane in response to waterlogging

Variety	Control	Waterlogging	%Decrease/increase
Cane Height			
CoLk 94184	185.96	207.00	11.31
CoJ 64	151.50	164.36	8.48
F wt. of leaf lamina (kg)			
CoLk 94184	0.566	0.488	-13.78
CoJ 64	0.280	0.172	-38.57
F wt. of leaf sheath (kg)			
CoLk 94184	0.478	0.400	-16.31
CoJ 64	0.288	0.210	-27.08
F wt. of Stalk (kg)			
CoLk 94184	2.437	2.846	16.78
CoJ 64	1.262	1.190	-5.54
F wt. of main root (kg)			
CoLk 94184	0.034	0.063	85.3
CoJ 64	0.015	0.031	106.7
Total no. of Internode/cane			
CoLk 94184	17	17	0
CoJ 64	16	18	12.5

Table 3: Changes in dry weight of sugarcane in response to waterlogging

Variety	Control	Waterlogging	% Decrease/increase
Total clump dry wt (kg)			
CoLk 94184	3.740	3.993	6.76
CoJ 64	2.029	1.753	-13.60
Leaf lamina dry wt (kg)			
CoLk 94184	0.1916	0.1692	-11.98
CoJ 64	0.0995	0.0583	-42.0
Leaf sheath dry wt (kg)			
CoLk 94184	0.1018	0.0973	-4.90
CoJ 64	0.0652	0.0433	-33.84
Stalk dry wt (kg)			
CoLk 94184	0.5005	0.6802	35.72
CoJ 64	0.2846	0.2569	-9.82
Total root dry wt(kg)			

CoLk 94184	0.0148	0.0201	54.73
CoJ 64	0.0073	0.0075	43.84

Tiller Production

In general waterlogging at any stage reduced the production of new tillers and rate of tillers elongation in post waterlogged period was greater than control conditions (Webster and Eavis, 1971). waterlogging causes greater tiller mortality resulting in reduced shoot population at later crop stage. Varietal differences have been observed in the production of new tillers as well as their elongation during long flooding conditions (Rahman *et al.*, 1989). Loss in yield appeared to be due to desiccation of cane stalk and was relatively more in the bottom portion of the cane than at the top. Studies conducted at SBI, Coimbatore, indicated that the waterlogging stress during formative phase (90–170 DAP) caused 21.63 -26.52 % reduction in tiller production and leaf area, respectively; however, reduction was less in resistant clones. Further it was observed that waterlogging stress had significant impact on biomass production, which results in 42.63, 45.16, 44.69 % reduction in leaf, stem and total dry matter production, respectively (Gomathi *et al.*, 2015). Waterlogging, in general causes tiller mortality, retard growth and reduces cane and sugar yield (Kumari *et al.*, 2013).

Leaf development

Experiments were conducted at ICAR-IISR, Lucknow to observe changes in growth attributes in response to waterlogging. Findings indicated reduction in leaf length, width, number, area, fresh and dry weight in sugarcane subjected to natural waterlogging during grand growth stage (Table 4) (unpublished).

Table 4: Changes in leaf attributes in response to waterlogging in sugarcane

Variety	Control	Waterlogging	% Decrease
Leaf length(cm)			
CoLk 94184	177.33	174.33	1.69
CoJ 64	120.41	103.33	14.18
Leaf Width (cm)			
CoLk 94184	3.98	3.76	5.52
CoJ 64	3.73	3.12	16.35
Leaf area (cm²)			
CoLk 94184	441.1	410.0	7.12
CoJ 64	280.7	201.5	28.12
No. of Leaves			
CoLk 94184	50	39	22
CoJ 64	40	29	27
Leaf lamina fwt (kg)			
CoLk 94184	0.566	0.488	13.78
CoJ 64	0.280	0.172	38.57
Leaf lamina dry wt (kg)			
CoLk 94184	0.1916	0.1692	11.98
CoJ 64	0.0995	0.0583	42.00

Photosynthetic Pigments

Destruction of chlorophyll due to waterlogging has been reported widely by several researchers (Jackson *et al.*, 1991; Huang *et al.*, 1994; Ashraf *et al.*, 2011) which adversely affects the photosynthetic capacity of plants. During the period of submergence, however, there was a reduction of chlorophyll content both in tolerant and susceptible cultivars; reduction rate was relatively higher in susceptible cultivars (CR 383-10 and IR 42) as compared to tolerant FR 13A rice cultivar (Das and Sarkar, 2001). An effort was also made to assess the changes in chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total Chl (Chl *a*+Chl *b*), carotenoids (CAR), the ratio of Chl *a* and Chl *b* (Chl *a/b*) at ICAR-Indian Institute of Sugarcane Research, Lucknow by Jain and Pathak (2022) using four sugarcane cultivars. Chl *a* content (mg/g fresh wt) ranged from 1.277 to 2.30 with average 1.949 in control and 1.057- 2.150 with average 1.716 in leaves of waterlogged plants. Chl *b* contents ranged from 0.319- 0.607 with mean value of 0.518 in control and 0.290 to 0.571 with mean value of 0.453 mg/g fresh wt in waterlogged plants, respectively. In waterlogged plants, Chl *a/b* ratio was slightly higher (3.794) as compared to control (3.789) except in CoJ 64, while Chl/CAR ratio was relatively lesser (Table 5). SPAD index was in the range of 30.1 - 35.3, mean: 34.0 in control and 22.4-34.1, mean: 29.6 in waterlogged plants. Carotenoids content was relatively lower in waterlogging treatment and it ranged from 0.477- 0.787 (in control) and 0.467-0.797 mg/g fwt (in waterlogging); cultivars BO91 and CoS767 showed slightly higher content. Significant positive correlation was observed among different attributes except chlorophyll *a/b* ratio (Table 6&7)). Cultivar CoJ 64 showed highest reduction in Chl *a/b*, Chl/CAR ratio and CAR contents reflecting high sensitivity towards waterlogging as compared to other cultivars tested. Decrease in chlorophyll contents during different periods of waterlogging has also been reported in sugarcane (Subha 2008; Gowri Manohari, 2009; Gomathi *et al.*, 2015).

Table 5: Changes in Chl a, b, total Chl, carotenoids (mg g⁻¹ fwt), Chl a/b ratio due to waterlogging in sugarcane (Source: Jain and Pathak, 2022)

Cultivar	Treatment		% Numerical increase/decrease over control
	Control	Waterlogged	
Chlorophyll a			
CoLk 94184	2.070 ±0.04	1.540 ±0.30	-28.91
BO 91	2.150 ±0.12	2.117 ±0.07	-4.03
CoS 767	2.300 ±0.18	2.150 ±0.27	-7.91
CoJ 64	1.277 ±0.01	1.057 ±0.01	-9.09
Mean	1.949	1.716	
Chlorophyll b			
CoLk 94184	0.550 ±0.02	0.391 ±0.08	-25.60
BO 91	0.595 ±0.01	0.571 ±0.02	-1.55
CoS 767	0.607 ±0.02	0.559 ±0.08	-6.52
CoJ 64	0.319 ±0.00	0.290 ±0.01	-17.23
Mean	0.518	0.453	
Total Chlorophyll			
CoLk 94184	2.620 ±0.06	1.930 ±0.38	-26.3
BO 91	2.747 ±0.13	2.690 ±0.04	-2.1
CoS 767	2.910 ±0.20	2.710 ±0.35	-6.9
CoJ 64	1.593 ±0.01	1.347 ±0.01	-15.5
Mean	2.468	2.169	
Carotenoids			
CoLk 94184	0.740 ±0.02	0.570 ±0.12	-22.97
BO 91	0.737 ±0.02	0.750 ±0.03	1.81
CoS 767	0.787 ±0.05	0.797 ±0.08	1.27
CoJ 64	0.477 ±0.01	0.467 ±0.01	-2.10
Mean	0.685	0.646	

Table 6: Changes in total Chl/ Carotenoids ratio and SPAD index due to waterlogging in sugarcane(Source: Jain and Pathak 2022)

Cultivars	Treatment		% Numerical Decrease over control
	Control	Waterlogged	% Numerical increase/decrease over control
Total Chl/ CAR ratio			
CoLk 94184	3.541	3.386	-4.37
BO 91	3.716	3.587	-3.49
CoS 767	3.684	3.388	-8.04
CoJ 64	3.313	2.872	-13.29
Mean	3.563	3.308	
Chl a/b ratio			
CoLk 94184	3.770 ±0.05	3.957 ±0.07	4.95
BO 91	3.610 ±0.13	3.717 ±0.26	2.95
CoS 767	3.780 ±0.18	3.863 ±0.09	2.20
CoJ 64	3.997 ±0.05	3.640 ±0.04	-8.92
Mean	3.789	3.794	
SPAD Index			
CoLk 94184	35.2	30.2	-14.20
BO 91	35.2	34.1	-3.13
CoS 767	35.3	31.9	-9.72
CoJ 64	30.1	22.4	-25.58
Mean	34.0	29.6	

Table 7: Correlation among photosynthetic pigments and SPAD index in sugarcane (Source: Jain and Pathak, 2022)

Parameters	SPAD index	Chl a/b ratio	Total Chl/ CAR ratio	Chl a	Chl b	Total Chl	CAR
SPAD index	1.000						
Chl a/b ratio	-0.018	1.000					
TotalChl /CAR ratio	0.977	-0.072	1.000				
Chl a	0.894	-0.242	0.872	1.000			
Chl b	0.875	-0.345	0.863	0.994	1.000		
Total Chl	0.890	-0.266	0.871	1.000	0.996	1.000	
CAR	0.818	-0.275	0.781	0.986	0.980	0.986	1.000

Photosynthesis and Partitioning of Assimilates

Under anaerobic condition, photosynthesis declined due to slow diffusion of CO₂ in water and reduced availability of light, as a result decreased flow rate of assimilates to the roots. In sugarcane, chlorophyll content, photosynthetic rate and leaf dry matter accumulation reduced under submergence; the reduction was more in susceptible varieties (Gomathi *et al.*, 2015). An increase in the total dry matter per unit area is the first indicator for high crop yields. Carbon compounds account for 80–90 % of the total dry matter produced by the plants. Reports are available on reduced rate of photosynthesis at the end of saturation causing 40–50 % reduction in biomass as compared control treatment (Glaz *et al.*, 2004a; Glaz, 2006; Glaz and Gilbert, 2006). Flooding leads to a 50% reduction in the rate of photosynthesis and reduced plant growth which might be due to decreased metabolic activity of roots under hypoxia (Viator *et al.*, 2012). Stomatal conductance declined due to reduction in root size which lowers root hydraulic conductance under waterlogging condition (Srivastava *et al.*, 2010). During waterlogging the stomatal pore closed to reduce water loss (Jaiswal and Srivastava, 2015). Photosynthetic rate is decreased due to less leaf area which leads to reduced production of photosynthates and less biomass and harvest index (Avivi *et al.*, 2016). Photosynthesis rate was decreased by flooding but stomatal conductance and intercellular CO₂ concentration were increased thus, indicated a non-stomatal limitation to photosynthetic rate (Tetsushi and Karim, 2007).

Nutrient uptake

In waterlogged conditions, root respiration and cell permeability are reduced due to oxygen deficiency which slowed down water and nutrients absorption. Under such situation, plants exhibit nutrient deficiency and apparent wilting symptoms even in soils supplied with available nutrients and water. In sugarcane waterlogging stress induced 28.07 and 29.53 % reduction in leaf and stem nitrogen contents, respectively. However, in both leaf and stem, reduction in nitrogen was comparatively less in resistant clones suggesting that nitrogen content in index leaf could be used as one of the markers to screen waterlogging resistance in sugarcane (Gomathi *et al.*, 2015). Research conducted at Florida on flooding in sugarcane found 10–78 % reduction in leaf N, P, K, Ca, Mg, Fe, Mn, Zn and Cu concentration which indicated decreased cane growth due to reduced nutrient uptake (Gilbert *et al.*, 2007). Na, K, Ca contents estimated in dry leaf tissues of waterlogged sugarcane plants indicated varying concentrations among twenty three sugarcane genotypes; Na

ranged from 0.126 to 1.275, K from 0.375 to 2.363 and Ca from 0.176 to 0.508 percent (Table 8) (Jain et al, unpublished).

Table 8: Nutrient Analysis

	Parameters	Range	Mean
% Dry Wt	Na	0.126-1.275	0.269
	K	0.375-2.363	1.543
	Ca	0.176-0.508	0.310
	Soluble silica	0.23-2.23	0.960
mg/kg Dry Wt	Fe	37.0-453.9	138.0
	Mn	17.3-79.6	40.0
	Cu	0.84-7.13	3.32
	Zn	4.40-25.80	11.6
Nutrient ratio	Na/K	0.066-0.828	0.193
	K/Na	1.21-15.11	8.085
	K/Ca	1.04-9.92	5.185
	Ca/K	0.101-0.963	0.235

Source: Jain *et al.* 2022(unpublished)

Further an experiment was conducted under soil pot culture condition using eleven sugarcane genotypes (CoLk 94184, BO 91, CoS 767, CoJ 64, CoLk 12204, CoLk 07201, LG 04439, LG 03040, A46-11, A-27-12 and UP 9530 w) grown under control and waterlogged conditions at IISR , Lucknow (Singh et al., 2023). Findings obtained indicated reduction in cane weight to the range of 5.3% (A-46-11) to 32.3% (CoJ 64) and significant decrease in N, P, K, S, Zn and Cu contents and increase in l Fe, Al, Mn and Na in leaf tissues of sugarcane genotypes due to waterlogging.

Biochemical changes

Antioxidant system

Waterlogging modulates plant metabolic activity and root antioxidant system. Waterlogging generates oxidative stress 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 induce damage to proteins, lipids, pigments and nucleic acids (Biemelt *et al.*, 2000; Garnczarska and Bednarski, 2004). H_2O_2 accumulation under hypoxic conditions has been shown in the roots and leaves of barley (*Hordeum vulgare*) (Kalashnikov *et al.*, 1994), sugarcane (Gomathi *et al.*, 2012) and wheat roots (Biemelt *et al.* 2000). Tolerance of varied varieties to

environmental stress conditions has been correlated with increased activity of antioxidant enzymes and levels of antioxidant metabolites (Davies, 1987; Srivalli *et al.*, 2003). Waterlogging treatment waterlogging-resistant lines showed significant increase in activities of catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), as well as polyphenol oxidase as compared to sensitive ones. Changes in activities of these enzymes may impart resistance to environmental stresses (Allen, 1995). The mechanisms related to cellular adaptation in response to flooding tolerance was only recently reported in sugarcane (Gomathi *et al.*, 2012). Waterlogged plants showed increased activity of antioxidant Enzymes SOD, ADH and APX in most of the genotypes tested at ICAR-IISR, Lucknow (Jain *et al.*, 2015).

Alcohol Dehydrogenase (ADH)

A significant increase in ADH activity was reported in sugarcane due to short term flooding (Gomathi *et al.*, 2015); increase was high in tolerant genotypes (Co 99006-36.25 %) as compared to sensitive genotypes (Co 86032-14.50 %) providing more energy to plant under anaerobic condition. Foliar application of potassium nitrate reduced the up regulated ADH activities in hypoxic root of the waterlogged maize seedlings (Goyal *et al.*, 2020).

Nitrate Reductase (NR)

NR is a key enzyme of nitrogen assimilation and sensitive to changes in environmental conditions (Kaiser and Huber, 1994; Ren *et al.*, 2017). Leaf N content in index leaf and NRase activity are identified as key physiological markers for screening water logging tolerance in sugarcane (Gomathi *et al.*, 2015). The reduction of NR activity in leaves of waterlogged plants is due to rapid depletion of NO_3^- and oxygen under anaerobic conditions (Sung and Sun, 1990; Hoff *et al.*, 1992). Under waterlogging stress, the greatest reduction in the activities of NR (60%), GS (50%), GDH (33%), and GOGAT (26%) took place in maize crop (Ren *et al.*, 2017). Additional foliar N fertilization improved root respiratory activity, leaf chlorophyll content, photosynthesis, and increased nitrate reductase (NR) activity of waterlogged wheat, resulting in improved plant nutrition (Wu *et al.*, 2014). Compared to the well watered condition, waterlogging significantly reduced shoot biomass, N accumulation, harvest index, N harvest index, nitrate reductase activity, N use efficiency, and N partial factor productivity at all the N application rates and enhanced these parameters at higher N treatments (Qi and Pan, 2022).

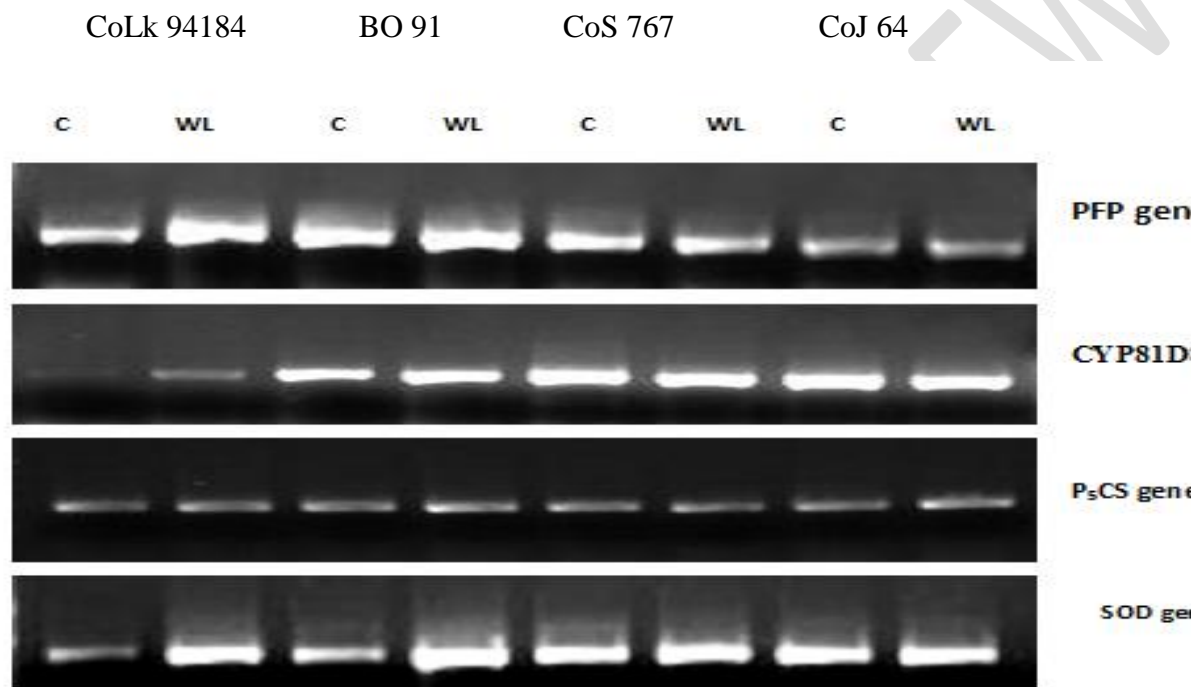
Anaerobic Root Proteins (ANPs)

Waterlogging can cause hypoxia due to oxygen deficiency in the soil environment and anoxia, a condition that refers to complete absence of oxygen in a defined soil environment, leading to complete arrest of root respiration and adversely affecting plant metabolism. Under partial oxygen deficiency due to waterlogging, genes coding for anaerobic proteins (ANPs protein) are up-regulated at transcriptional and post-transcriptional levels (Jackson 2003). These ANPs are grouped in different categories; 1. Enzymes involved in starch breakdown, 2. Enzymes implicated in pH regulation, 3. Enzymes involved in aerenchyma formation 4. Free radical scavenging enzymes 5. Proteins involved in signal sensing and transduction (ethylene receptor) 6. Other enzymes of unidentified function. The proteins which are synthesized in response to anaerobiosis are called the anaerobic polypeptides (ANPs). In maize, a set of 20 polypeptides designated as ANPs is synthesized in the primary root (Nover 1989), and distinguished as alcohol dehydrogenase (Ferl *et al.*, 1980), pyruvate decarboxylase (Lazlo and Lawrence, 1983), glucose phosphate isomerase and aldolase (Kelley and Freeling, 1984) and glyceraldehyde-3- phosphate dehydrogenase (Russell and Sachs, 1992). The root and leaf protein contents in maize seedlings showed decreased level under waterlogging treatment. Sinha *et al.* (1995) observed a repression of the normal protein synthesis during anaerobiosis but not a complete inhibition of the gene expression, although complete dissociation of polysomes has been observed. Das and Sarkar (2001) found that a negative correlation existed between the duration of waterlogging and primary metabolites on total soluble protein and RNA. In tolerant sugarcane genotypes, specific expression of ANP's viz., 66, 98 and 132 kDa proteins was reported in relation to waterlogging exhibiting their possible role in tolerant behavior (Gomathi *et al.* 2015).

Gene expression

Differential gene expression pattern was studied using four sugarcane varieties, CoLk 94184, BO 91, CoS 767, CoJ 64 grown under waterlogged conditions at ICAR-IISR, Lucknow by Jain et al (2020). In leaves, the transcript of PFP enzyme (phosphor- fructokinase) which is involved in glycolytic

reactions was up-regulated in two varieties (90.5% in CoLk 94184), (2.9% BO 91) but down-regulated in others. In waterlogged plants, CYP81D8 (ROS related proteins) gene showed marked increase in variety CoLk 94184 (+110%) and BO 91 (13.6%) but slightly decrease in CoS 767 (-7.7%) and CoJ 64 (-5%). P₅CS gene expression was relatively increased under waterlogged conditions as compared to untreated control. Waterlogged plants showed higher SOD gene expression; highest increase (+154%) was in CoLk 94184 variety. Expression analysis of these genes exhibited strong correlation with shoot and leaf attributes (Jain et al., 2020) (Fig.8).



UNDER

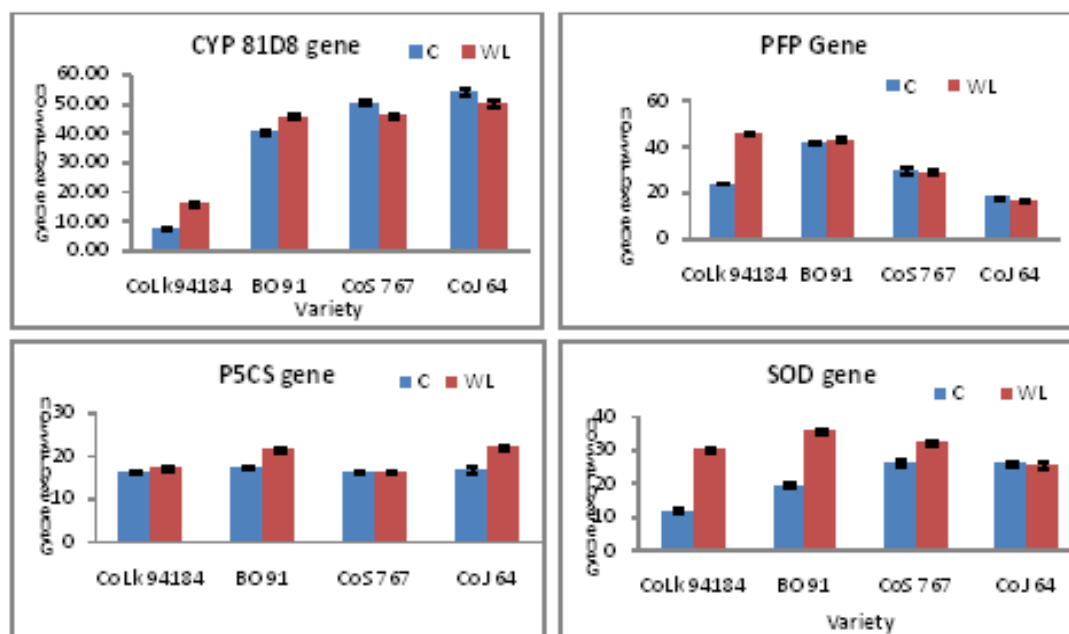


Fig. 8: Differential gene expression in relation to waterlogging Source: Jain et al 2020

Influence of Waterlogging on Yield and Quality attributes

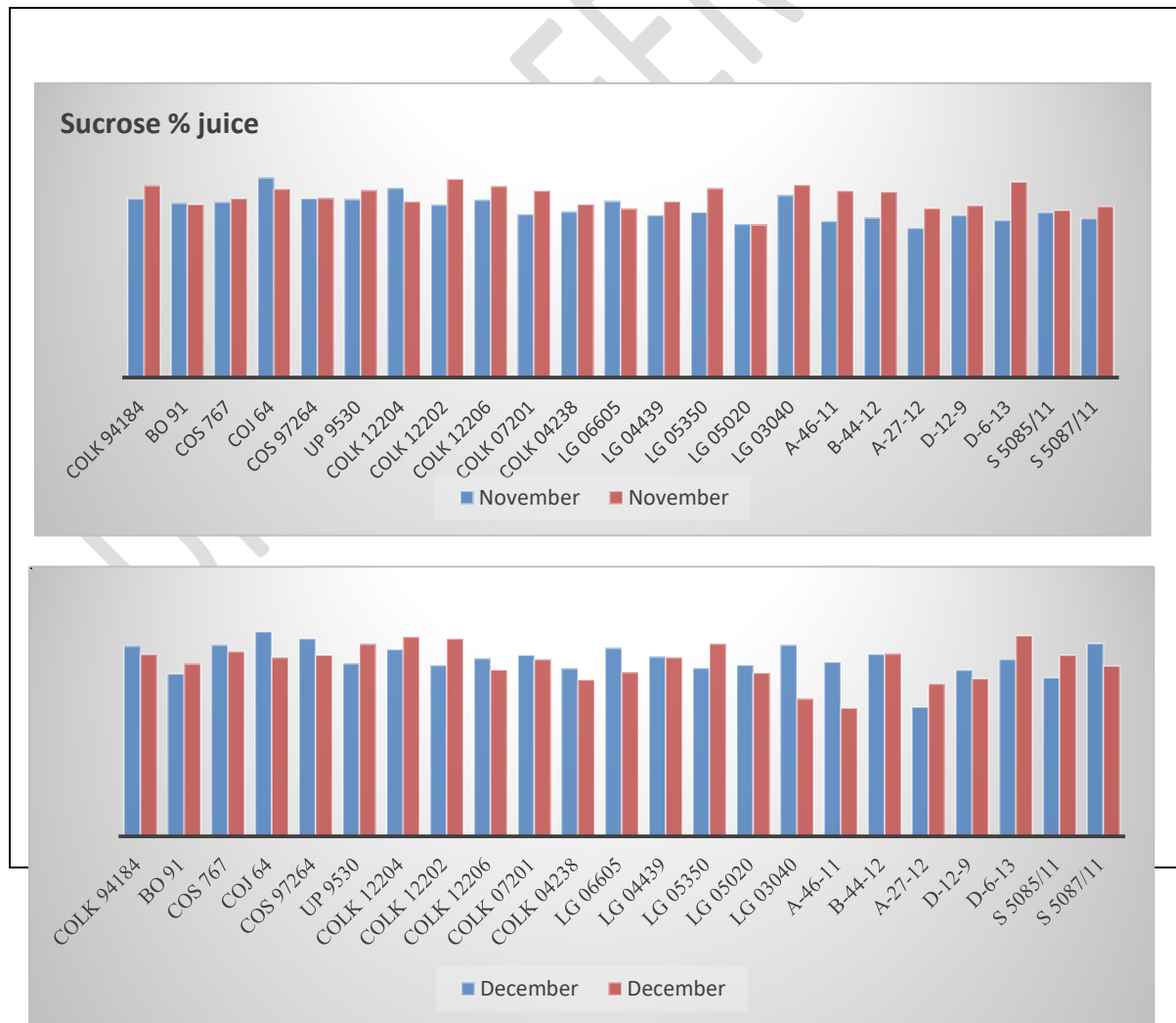
Waterlogging reduces shoot and root growth, dry matter production and total crop yield; rate of reduction depends upon water logging duration, growth stage and management practices before, during and after water logging. A study conducted at ICAR-IISR, Lucknow on yield variation among 24 sugarcane genotypes indicated reduced single cane weight, girth and increased in cane length and internode number under waterlogged conditions (Table 9).

Table 9: Effect of waterlogging on yield attributes (mean value of 24 genotypes)

Treatment	Single cane wt (kg)	Cane length (m)	Cane girth (cm)	Internode no.
Control	0.856	2.31	2.2	20
Waterlogged	0.821	2.38	2.1	23

Twenty three genotypes were evaluated for juice quality attributes at early stage of cane ripening at ICAR-IISR, Lucknow (Jain et al., 2017). Data obtained indicated relatively higher juice quality parameters, viz., degree Brix, sucrose percent juice, juice purity, CCS% juice and S/R ratio at early stage of cane ripening (in the month of November) and lower at later stage of waterlogging stress

(December and February) as compared to control plants (Fig 10). Sucrose% juice was ranged from 16.19 (CoLk 12202) to 12.48 (LG 05020) in November, 16.54 (CoLk12204) to 10.63 (A-46-11) in December, 17.33 (UP 9530) to 12.83 (BO 91) in February under waterlogging. Highest increase in CCS% juice (14.97% over control) was observed in D-6-13 and highest decrease was observed in CoJ 64. Reducing sugar content in cane juice was comparatively higher in waterlogged affected plants.



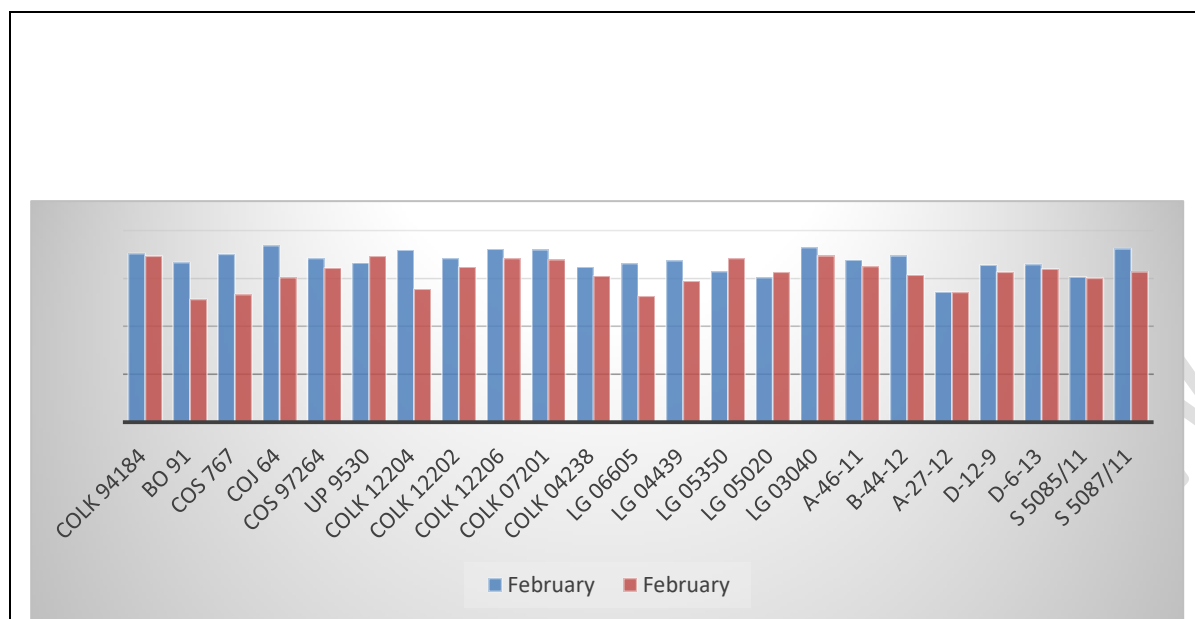


Figure 10: Sucrose % juice in sugarcane genotypes in response to waterlogging
(Source: Jain et al., 2017)

Performance of Ratoon crop under waterlogging

Ratoon crop of twenty four sugarcane genotypes, viz., CoLK 94184, BO 91, CoS 767, CoJ 64, CoS 97264, CoLk 12204, CoLk 12202, CoLk 12206, CoLk 07201, CoLk 04238, S 5085/11, S 5087/11, LG 06605, LG 04439, LG 05350, LG 05020, LG 03040, A-46-11, B-44-12, A-27-12, D-12-9, D-6-13, UP 9530, CoSe 96436 were evaluated for waterlogging response at IISR, Lucknow. Results obtained indicated higher root dry mass (sum of shoot root and aerial roots) and plant height in waterlogged affected plants. Aerial rooting pattern varied among genotypes; some of the genotypes showed aerial roots up to 9th nodes. A-46-11, UP 9530, LG 06606, D-12-9, CoLk 12206, CoS 767, CoLk 1220, D-6-13, genotypes showed dense aerial root growth. Leaf length, width, area and SPAD index decreased due to waterlogging (Jain et al 2023) (Table 10).

Table 10: Table Mean, range and changes in physiological parameters of waterlogged over control and standard error (SE ±)

Physiological parameters	Control		Waterlogged		Relative change (%)	(SE ±)	
	Mean	Range	Mean	Range		Control	Waterlogged
Plant height (m)	2.1	1.12 - 2.69	2.45	1.62 - 3.31	16.67	0.084	0.09
SPAD	30	23 - 38	28	10 - 40	-8.7	1.16	1.31

Leaf No	33	25 - 44	34	25 - 46	3.03	1.02	0.96
Leaf Length (cm)	140.8	93 - 169	139.6	93 - 165	-0.85	3.98	3.61
Leaf width (cm)	3.42	2.55 - 5.06	3.2	2.1 - 4.9	-6.43	0.119	0.116
Leaf area (cm ²)	303.6	194.8 - 494	282.4	122 - 474.1	-6.98	15.3	15.7
NMC	104	60 - 174	114	6 - 190	9.62	7.09	9.5
Cane yield (kg/plot)	62.5	21 - 116.9	77.43	1.45 - 119.9	23.89	5.66	6.65
Single cane weight (kg)	0.589	0.31 - 0.949	0.664	0.241 - 0.955	12.73	0.035	0.037
Cane length (m)	2.1	1.36 - 3.00	2.6	1.33 - 3.33	17.65	0.098	0.099
Brix	19.73	13.13 - 19.97	15.84	13.36 - 18.41	-8.86	0.39	0.29
Sucrose	14.36	10.56 - 17.89	13.63	11.1 - 16.02	-7.65	0.40	0.29
WCwt (kg)	2.784	1.285 - 4.342	3.306	1.663 - 5.976	18.75	0.183	0.227
Leaf wt (kg)	0.292	0.131 - 0.505	0.328	0.195 - 0.560	12.3	0.023	0.021
Stalk wt (kg)	2.25	0.925 - 3.56	2.675	1.27 - 4.935	18.9	0.158	0.193
Leaf sheath wt (kg)	0.22	0.12 - 0.33	0.24	0.094 - 0.41	9.1	0.013	0.014
Root wt (kg)	0.021	0.005 - 0.055	0.064	0.026 - 0.117	204.8	0.003	0.008

WCwt whole clump weight, NMC Number of Millable cane

Soil analysis & Microbial diversity

Soil samples of waterlogged plot along with control were analysed for nutrient composition. Soil data obtained indicated reduced PH, organic matter, available nitrogen and higher potassium, phosphorus and EC value in waterlogged affected soil as compared to control plot (Table 11).

Table 11: Soil data in relation to waterlogging

Crop type	Treatment	pH	EC	% organic matter	Available nitrogen (kg/ha)	Available P ₂ O ₅ (kg/ha)	Available K ₂ O (kg/ha)
Plant crop	Control	8.55	0.18	0.37	138.09	29.15	231.44
	Waterlogged	8.09	0.29	0.27	125.5	71.23	378.72
Ratoon crop	Control	8.40	0.18	0.28	138.09	27.54	236.70
	Waterlogged	8.08	0.28	0.26	100.4	80.4	510.22

Microbial diversity of rhizosphere soil of waterlogged and control plots of plant and ratoon crop were analysed based on the 16S rRNA gene. Out of total 154420 representative sequences used for taxonomic classification, 153066 sequences clustered as Operational Taxonomic Units (OTUs). Out of 153066 OTUs, 91-92 % were classified under kingdom Bacteria in waterlogged and 94% in control plots and 6 – 9 % were unknown species. Raw reads were deposited in NCBI metagenome SRA module. Accession number received as SRR26190803 (control ratoon crop), SRR26190804

(control Plant crop), SRR26190805 (waterlogged plant crop) and SRR26190806 (waterlogged ratoon crop), under Bio-project PRJNA1020738.

Transcriptome analysis

An Illumina-based comparative differential transcriptomic analysis was performed using leaf samples of two sugarcane varieties; CoLk 94184 and CoJ 64 subjected to waterlogging along with untreated control. Overall, a total of 447,196 transcripts were identified with an average length of 509 bp & N50 length of 621 bp (Table12). Transcriptome analysis using four samples (S1, S2, S3, S4) having leaf tissue of control and waterlogging induced plant, CoLk 94184 and CoJ 64 varieties revealed a total of 295618 Unigenes. These Unigenes were further processed using seven databases (Nr, Uniprot, GO, KOG, PFAM, KEGG and Transcription factor). Unigenes showed 49.2% similarity with *Sorghum bicolor*, 14.9%% with *Zea mays*, 2.1% with *Oryza sativa*, 4.1% with *Setaria italic*, 1.87% with *Saccharum hybrid* and 19.48% with others (Nr annotation)(Fig.11) .

Table 12: Transcript data

Type	Total no	Total length(bp)	Mean length(bp)	N50 (bp)	>500bp	>1000bp
Transcript	447,196	227,661,629	509	621	26049	46042
Unigene	296518	156,485,793	529	664	18928	32471

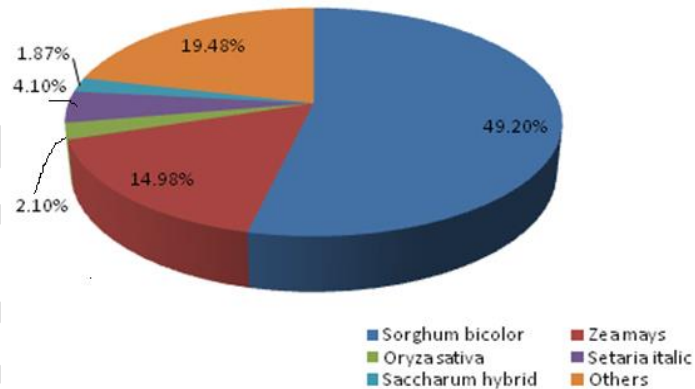


Fig.11: Species classification of Nr distribution

Table 13: Number of Unigenes annotated with different sets of databases

	No of Unigenes	Percentage (%)

Annotated in NR	66731	22.6
Annotated in Uniprot	47817	16.2
Annotated in GO	31893	10.8
Annotated in KOG	29933	10.1
Annotated in Pfam	29243	9.89
Annotated in Transcription factor database	33,082	11.19
Annotated in KEGG	10380	3.51
Annotated with all 4 databases	20261	6.85
Annotated with at least 1 database	66785	22.59
Total no of Unigenes	295618	

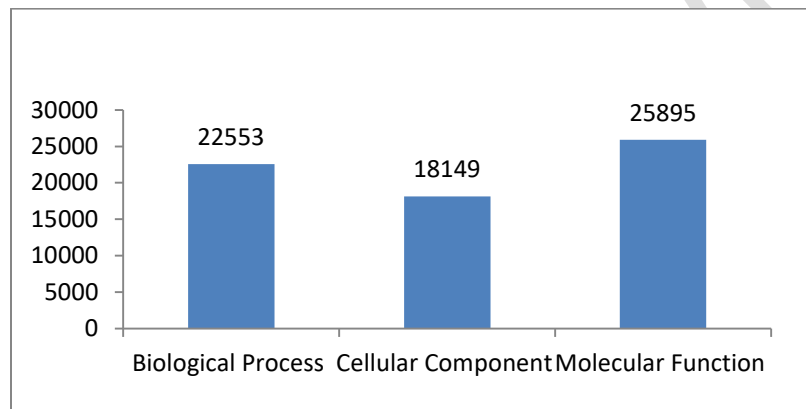


Fig. 12: Based on GO annotation, genes are grouped under three different components, Biological process, Cellular component and molecular function CDS.

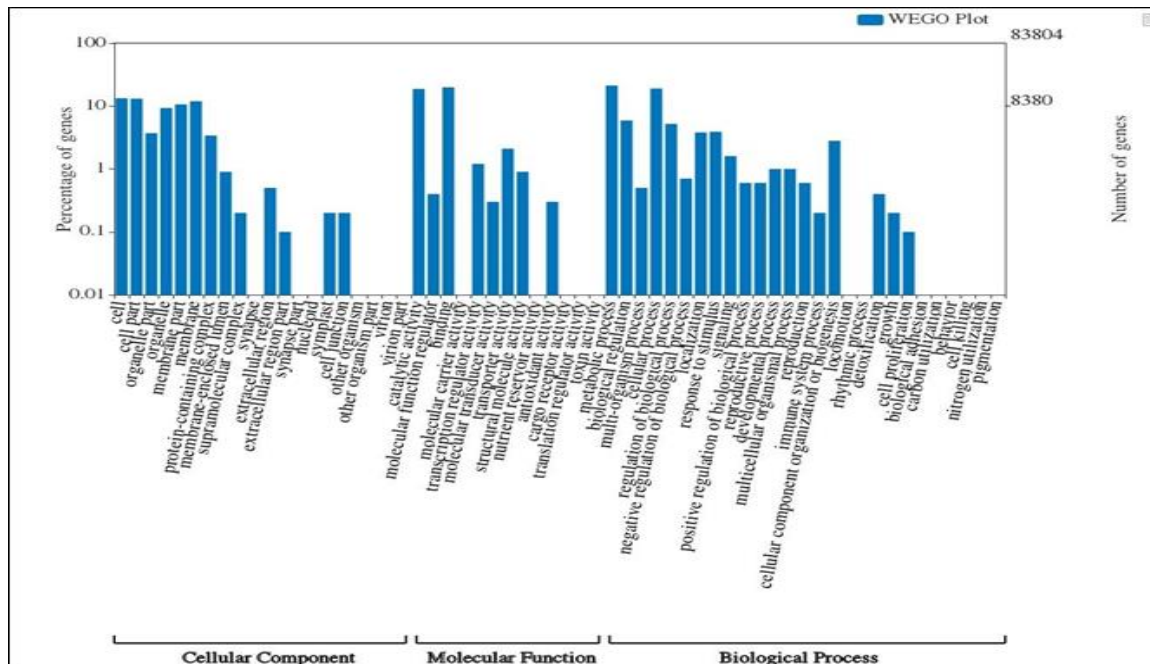


Fig.13: WEGO plots shows the number/ percentage of genes belonging to different functional groups

The most enriched KOG (Eukaryotic Orthologous Groups) category was “Signal transduction mechanisms (T)” followed by “General function prediction only (R)” and "Post-translational modification, protein turnover, chaperones (O). In Pfam analysis, most abundant domains identified were representing “Protein kinase domain” followed by “Protein tyrosine kinase”, “Cytochrome P450” and RNA recognition motif. The most abundant transcription factor families enriched were bHLH followed by WRKY, NAC and MYB_ related. Differentially expressed genes (DEGs) were identified in four sets of samples (S1 vs S2, S1 vs S3, S2 vs S4 and S3 vs S4) using total RNA of leaf tissues of both the varieties planted under control and waterlogged conditions, as per the DESeq R/Bioc package (Fig.14).

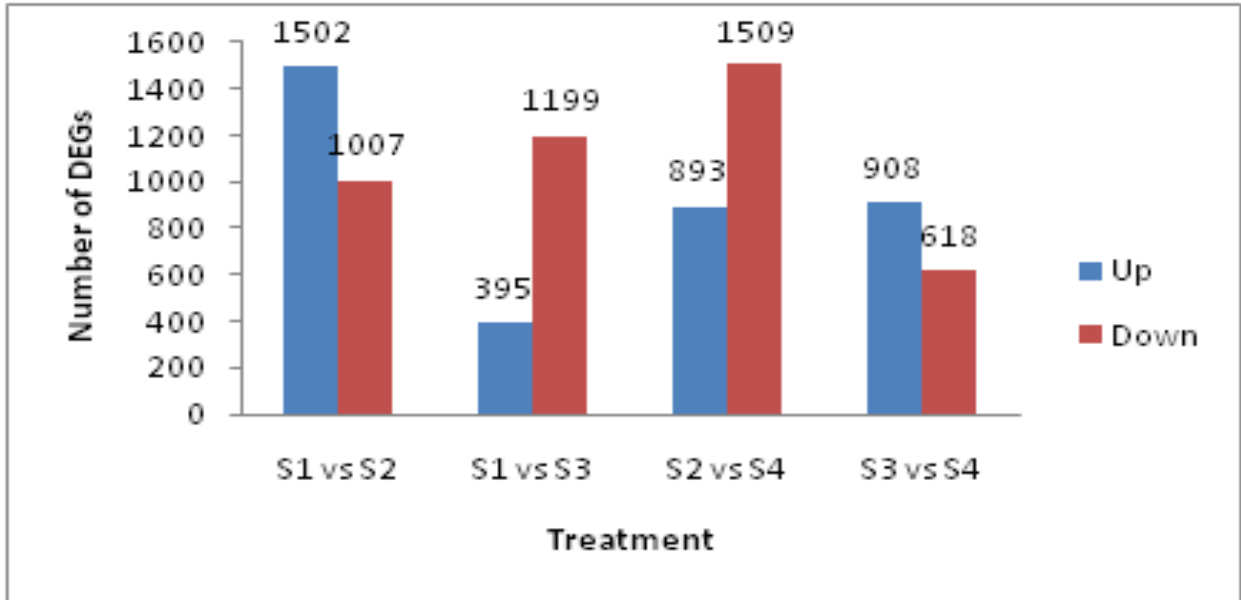


Fig.14: Differentially expressed genes (DEGs) of four sets of sample

S1 = CoLk94184 Control; S2 = CoLk94184 waterlogged; S3 = CoJ 64 control; S4 = CoJ 64 waterlogged (Jain et al., unpublished).

Conclusion

Short term or long term waterlogging significantly reduced various physiological and metabolic processes viz., light interception, degradation of chlorophyll pigment, photosynthetic rate, reduction of key enzyme activity (NRase) and soluble protein content and nutrient uptake and finally shoot and root growth behavior. Degree of tolerance depends upon the varieties, duration and intensity of waterlogging. In sensitive varieties, leaf senescence was associated with chlorophyll degradation, MDA accumulation, ethylene production, and decrease in activity of antioxidant enzymes under long term stress. Sugarcane plants have adaptation, avoidance, acclimation mechanism or a combination thereof to survive under waterlogging stress. Tolerant plants could able to survive in high water table through formation of aerial roots which are temporary source of oxygen for the respiratory activity. The tolerant species form aerenchyma, which helps in functioning of the plant processes under anoxia conditions. Key enzymes in ethylene biosynthesis like ACC synthase and oxidase were significantly accumulated during submergence. In contrast to adaptation, acclimation is a plastic response, often short lived and reversible because it does not involve permanent genetic changes which leads to transient changes in physiological and molecular process under waterlogging

condition. Waterlogging stimulated the synthesis of a small group of proteins known as anaerobic polypeptides (ANP's) which appear to play major role in ethanol fermentation under anoxia. Molecular mechanisms of waterlogging tolerance include changes in expression of genes involved in complex pathways, such as signal transduction, protein degradation, ion transport, carbon and amino acid metabolism, and transcriptional and translational regulation. Transcriptional factors (TFs) that constitute the signal transduction components play an important role in waterlogging tolerance. Molecular mechanism will be of relevance to identify candidate genes which can further be used for manipulating the sugarcane genome for improved stress tolerance.

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