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RESEARCH ARTICLE

Assessing sugarcane clones' resilience to waterlogging stress and comprehending the physiological and morphological processes

Anitha R[1], Jeyakumar P[2], Sassikumar D[1*], Vijayalakshmi D[3], Arul L[4], Manimekalai R[5], Thirumurugan T[6*], Nageswari R[7], Jayachandran M[1], Vanitha K[8] & Sritharan N[3]

[1]Sugarcane Research Station, Cuddalore 607 001, India

[2]Directorate of Planning and Monitoring, Tamil Nadu Agricultural University, Coimbatore 641 003, India

[3]Department of Crop Physiology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

[4]Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore 641 003, India

[5]ICAR- Sugarcane Breeding Institute, Coimbatore 641 003, India

[6]Department of Genetics and Plant Breeding, Anbil Dharmalingam Agricultural College & Research Institute, Trichy 620 001, India

[7]Tamil Nadu Rice Research Institute, Aduthurai 612 101, India

[8]Department of Fruit Science, Horticultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore 641003, India

*Email: sassikumar@tnau.ac.in , tthirumurugan@gmail.com

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Abstract

Waterlogging is a significant abiotic stressor that severely hampers sugarcane production worldwide. To address this issue, experiments were conducted at the Sugarcane Research Station, Cuddalore, TNAU, during 2022-2023 to evaluate the waterlogging tolerance of sugarcane clones. The experiment followed a factorial completely randomised design (FCRD) with three replications. Treatments included two water levels (control and waterlogging) and thirteen sugarcane nine clones and four varieties (Co 86032, C 2015 095, G 11035, C 2014 516, C 16338, C 30010, Co 15020, Co 62175, C 2015 021, Si 2014 047, CoC 13339, C 2015 006, CoG 7). After 70 days of seedling, plants were subjected to 20 days of waterlogging. Waterlogging stress significantly reduced total dry matter production, soluble protein, stomatal density, leaf area, above-ground fresh and dry weight, shoot length, root length and SPAD values in all sugarcane clones compared to the control. Conversely, the extent of aerenchyma and aerial roots increased. However, CoC 13339, C 16338, C 2014 516 and Co 62175 demonstrated superior waterlogging tolerance among the 13 sugarcane clones/varieties. These sugarcane clones/varieties have more adventitious roots at the base of their stems, which most likely made it easier for the plants to survive waterlogging.

Keywords

anatomical studies; clones; stomatal density; sugarcane; tolerance; waterlogging

Introduction

Sugarcane (*Saccharum officinarum* L.), a perennial plant of the family Poaceae (Graminae), is the primary source of sugar worldwide. China, India and Brazil grow the majority of the world's sugarcane, which supplies over 76 % of the world's sugar needs (1). After the plant is used to make sugar, molasses and its by-product bagasse are used as fuel. They are also used as feed for domestic animals and as a starting point for the synthesis of acids and alcohols. Sugar is produced by crystallising condensed cane juice. In 2022–2023, India's sugar production exceeded 35 million metric tons. In that year, more than 29 million metric tons of sugar were consumed domestically. The annual production of sugarcane in Uttar Pradesh is 177.43 million tons. This amounts to 45.89 % of the country's total agricultural production (2). Sugarcane contributes approximately 79 % of the global sugar production, with sugar beets providing most of the remainder, particularly in cooler regions. The mature stalk has 12–16 % soluble sugars, 2–3 % non-sugars, 11–16 % fibre and 63–73 % water.

Abiotic stresses that impact sugarcane crop productivity and juice quality include waterlogging. Waterlogging threatens almost 0.22 million of land in India, particularly in coastal regions of Andhra Pradesh, Karnataka, Bihar, Odisha, Maharashtra and Uttar Pradesh (3). Substantial modifications to society and improved efficiency at every stage of food production are necessary to feed 9 billion people by 2050. Maintaining yield under stressful circumstances is crucial to addressing such significant difficulties, especially in light of the effects of climate change. It will be necessary to take every action that can be taken, from developing tolerant crops to using optimal agronomic techniques to lessen the consequences of waterlogging (4). Examining and sugarcane phenotyping germplasm in a variety of settings from pot systems to field research is necessary to investigate crop adaptation to stress. Waterlogging reduces the amount and quality of biomass produced by crops at nearly every stage of growth, including germination, tillering and grand growth (5). Waterlogging causes physiological factors such as transpiration rates to decrease due to stomatal closure and photosynthesis rates to drastically decrease due to a decreased efficient leaf area. As a result, crop growth rates drop and the respiration rate of fully submerged organs increases more quickly than that of leaves.

Consequently, the energy generated by the respiration of roots is limited, which results in a significant reduction in plant development (6). When plants are stressed by waterlogging, their root systems are first to be impacted. Anoxia causes inadequate respiration for roots to operate normally and impaired root development (7, 8). To counteract the waterlogging stress on sugarcane, specific aerenchymatous floating roots and aerotropic root growth under oxygen scarcity are also natural characteristics (9). Although it has been demonstrated that the implementation of management strategies can lessen the effects of waterlogging (10), creating varieties that can withstand waterlogging is seen to be the most cost-effective method of minimising losses (11). Waterlogging-tolerant plants modify their morphological, metabolic and /or anatomical processes and systems to cope with waterlogging stresses, but tolerance varies from species to species (12). Restoring the supply of oxygen to the roots is a top priority for a plant once it senses waterlogging and this can be accomplished by changing the morphology and the structure of the roots (13).

One typical morphological response in plants under waterlogging stress is the production of aerenchyma in adventitious roots (11). In contrast to primary roots, adventitious roots arise from places like stem nodes and hypocotyls, while they can also form a component of the root system (14). In soggy settings, adventitious roots replace and support primary roots to increase gas diffusivity across and along with roots (15) through aerenchyma, which are gas gaps. A strategic breeding endeavour begins with determining the mechanisms of tolerance and the variance in tolerance between and within species.

Research on the effects of waterlogging on the development of grass and biomass production has been extensive in both glasshouse and field settings (16). However, the root morphological changes response, which has been identified as a critical adapted trait for waterlogged conditions (6, 16), has received less attention. Root morphological adaptations, including aerenchyma and adventitious root development, play a critical role in waterlogging tolerance. However, these traits remain underexplored in sugarcane.

Materials and Methods

Seed materials and preparations

In 2022 and 2023, a pot culture experiment was carried out at the Sugarcane Research Station in Cuddalore, Tamil Nadu. Co 86032, C 2015 095, G 11035, C 2014 516, C 16338, C 30010, Co 15020, Co 62175, C 2015 021, Si 2014 047, CoC 13339, C 2015 006, and CoG 7 were the thirteen sugarcane varieties/clones employed in this study (Table 1). For 25 days, setts were grown in raised beds. After 25 days, seedlings were transplanted into pots filled with potting mix. Each pot had a diameter of 70 mm and a depth of 210 mm, with one plant per pot. During the five weeks of establishment, pots were placed in shallow trays with water maintained at a depth of 30 mm under glasshouse conditions. Before applying waterlogging treatments, all pots were transferred to tanks outside the glasshouse for ten days.

Experimental design and treatments

Treatments (controls and waterlogging) were included in the Factorial Completely Randomised Design (FCRD) design of the experiment. The experiment consisted of four main tanks, each serving as a block. For waterlogging and control conditions, each main tank was divided into two sections, one for waterlogging and the other for control conditions. Throughout the trial, water in the waterlogging treatments was maintained at 10 cm above the potting mix surface (Fig. 1) for the duration of the trial. Twenty days after the waterlogging, the complete plant was removed.

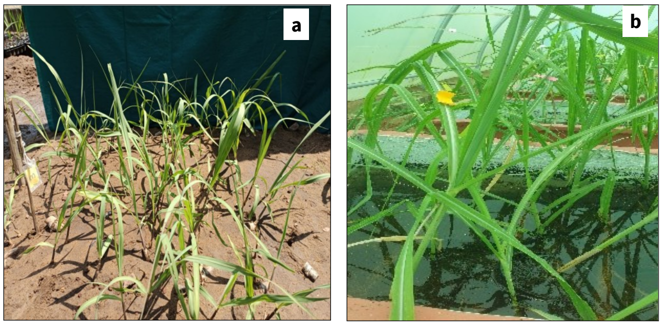
Measurements

At 28 days after the waterlogging treatment, one plant per cultivar was randomly selected for sampling from each tank. Four pots per cultivar were included for each

Table 1. Morphological parameters measured under control and waterlogged conditions as per clones/varieties (pooled data of 2022 and 2023)

S. No.	Varieties/clones	Shoot length (cm)		Root length (cm)	
		Control	Waterlogging	Control	Waterlogging
1.	Co 86032	127.22	103.00	22.147	16.00
2.	C 2015 095	117.11	107.33	24.67	19.06
3.	G 11035	125.25	116.33	22.67	17.55
4.	C 2014 516	152.05	132.66	47.09	31.50
5.	C 16338	138.17	126.33	46.26	28.67
6.	C 30010	123.89	118.00	25.33	17.94
7.	Co 15020	118.94	106.67	26.17	18.06
8.	Co 62175	126.55	123.00	32.50	26.50
9.	C 2015 021	149.89	129.67	44.00	21.93
10.	Si 2014 047	126.06	118.33	28.84	18.20
11.	CoC 13339	143.00	127.67	47.83	35.01
12.	C 2015 006	121.44	109.00	26.00	17.00
13.	CoG 7	148.61	130.33	46.00	26.27
	Mean	**132.16**	**119.10**	**67.62**	**22.59**
	SE (d)	2.664	1.045	1.275	0.9
	CD	5.346	2.097	2.562	2.210
	CxW (CD)	7.56		3.62	

Treatments (CXW) with the same letters do not differ significantly ($p < 0.05$) (C-Control and W-Waterlogging)



(a) Sett germination in the raised bed

(b) Transfer of 35th-day germinated healthy seedling to pot containing soil and vermicompost

(c) Transplanted pots for seedling growth under controlled conditions for 35 days

(d)70th-day seedlings were taken for experiment for both control and waterlogging for 25 days

(e) After the 95th day seedlings under waterlogging showed reduction in plant growth and aerial root growth

(f) After the 95th-day seedlings were removed from the pots, roots were washed carefully, root and shoots were separated to measure and take pho-

(g) Root aerenchyma analysis was done by using a Quanta 250 SEM machine at the Nano Technology Department. TNAU, Coimbatore

(h) Stomatal density was recorded through the microscope

Fig. 1. Experiment details of this research.

treatment.

Shoot and root measurement

Plants were removed from the pots to measure shoot and root parameters. A low-pressure hose was used to gently wash the roots of each plant. Adventitious roots were gathered and the length of the roots both main and adventitious was measured from the soil's surface to a depth that most of the root tip had reached. The roots and shoots were separated and dried in a fan-forced oven at 60°C for 48 hours to determine dry matter (DM) biomass.

Chlorophyll content (SPAD Reading)

A portable chlorophyll meter (the SPAD-502 Plus; Konica Minolta Sensing, Osaka, Japan) was used to measure the amount of chlorophyll and leaf greenness on the second youngest completely developed leaf of each plant before damaging measurements. The mean was then determined after three measurements were made from each plant: one at 1/3, one at 2/3 and one in the centre of the leaf length.

Aerenchyma formation

Shoot roots and aerial roots of sugarcane cultivars were removed, cleaned with distilled water to remove soil particles and stored at 4 °C for ultrastructural and anatomical analyses using Scanning Electron Microscopy (SEM). After cleaning the apical part of the roots (about 2.0 cm) that were attached to the stalk with distilled water to get rid of any contaminants, the cleaned sample was fixed in glutaraldehyde (2.5 % v/v in 200 mM PO buffer; pH 7.0) and kept in a refrigerator at 4 °C. The samples were moved twice in new fixative in a one-hour period prior to SEM preparation. To eliminate any remaining traces of glutaraldehyde, the fixed roots were washed five times with a solution of sucrose 7 % v/v in 3–100 mM PO buffer (pH 7.0). A series of dehydrated solutions (30, 50, 70 and 80 % alcohol for 15 min each; 90 % alcohol for 20 min; 100 % alcohol for 30 min; alcohol/amyl acetate (1:1) (v/v) and amyl acetate for 15 min each) were used to dehydrate the fixed samples before they were exposed to critical point drying. An ion-beam sputter coater was used to sputter a layer of gold over the dehydrated roots after they had been placed on stubs using double stick tape. The materials were analysed at Tamil Nadu Agricultural University's Nano Science Department in Coimbatore using an FEI Quanta 250 Scanning Electron Microscope. SEM images were obtained using an image analyser connected to the microscope at various magnifications (×500).

Assessing the density of stomata

An appropriate leaf material or plant portion was selected, gently washed under running water to cleanse the leaf material from any dust or dirt and let to dry. An appropriate fluid, such as a few drops of quick fix was applied to the leaf surfaces and allowed to dry completely. It is dried till the epidermal layer can be peeled off. The replica is removed carefully using fingers or forceps and positioned on the slide such that the imprinted surface is on the top. A cover slip is used to cover after adding a few drops of water or glycerol to ensure the replica spreads properly. The stomata visible in the microscope's circular view field at a particular magnification were determined. The visual field's diameter was determined using the ocular scale. The following formula was used to determine the area of the circle under a microscopic field of view: r^2, in which r is the circle's radius (view field) or ½ of its diameter. The number of stomata visible in the microscopic field was recorded to calculate stomatal density and expressed as stomata/mm^2.

Stomatal Density = Number of stomata counted / Area of the field of view in mm^2 (Eqn. 1)

Fresh and dry weight of seedlings (g)

The fresh weight was assessed after the seedlings from each replication and treatment were properly removed. The plants were then oven-dried for 48 hr at 80 °C before being weighed to determine their final dry weight. The fresh weight of the shoots and roots as well as their dried weight were determined independently and stated in grams.

Leaf-soluble protein (mg g^{-1} of fresh weight)

The amount of soluble protein was calculated and expressed as mg/g of fresh weight (17).

Statistical analysis

Using Microsoft Excel and SPSS 9.4 software, three replications were used to calculate the mean, standard error and critical difference for the FCRD statistical analysis. Using Microsoft Excel, graphs were created. As necessary, % values were converted before analysis. Non-significant results were denoted by (NS) and significance testing was conducted using significance thresholds of 5 % (*) and 1 % (**). The critical difference was computed at a 5 % probability level for treatments that demonstrated significant differences according to the 'F' test and the corresponding values were given. To find out how different the treatments were, Duncan's test was used. The treatment effects can be accurately interpreted because of the careful statistical analysis of the experimental results from this methodological approach. GRAPES 1.0.0 was used to do the principal component analysis (PCA) and correlogram. GRAPES 1.0.0 was used for the box plot analysis.

Results and Discussion

Morphological characters under waterlogging stress

The commercially important part of the sugarcane plant is the stem. In the current investigation, flooding stress caused a considerable 12 % reduction in seedling length compared to the control (Fig. 2). This reduction was observed across all clones and cultivars tested (Table 1). Clone plants and varieties C 2014 516, CoG 7 and C 2015 021 had longer shoots under flooding (132.66 cm, 130.33 cm and 129.67 cm, respectively), followed by CoC 13339 (127.67 cm) compared to Co 86032 (103 cm). Water logging in the soil immediately affects root growth and function by converting an aerobic condition to an anaerobic environment as a result of inadequate aeration.

Fig. 2. The shooting and rooting canes of several clones and varieties under waterlogging stress.

Significant varietal diversity was seen in this study and flooding caused a 34 % decrease in settling root development. The tolerant cultivars CoC 13339 (35.01 cm) followed by C 2014 516, C 16338 and Co 62175 (31.50, 28.67 and 26.50 cm, respectively) had a greater growth in the length of the roots than Co 86032 (16.00 cm). Previous research has shown that sugarcane exhibits genotype variations in the length of the shoot and settling root length following floods (18). New roots are developed at the bottom of the shoot above the water level as a result of flooding, which raises auxin concentration and increases tissue sensitivity to auxin. The process of selection for spontaneous root growth may not boost sugarcane traits under flood. To identify cultivars that are flood tolerant, aerenchyma growth can be a useful screening method (18).

Leaf area under waterlogging stress

Waterlogging stress caused a significant reduction in leaf area compared to control conditions (Table 2). Variety Co 86032 exhibited the greatest reduction in leaf area (100.29 cm^2) under waterlogging stress, compared to CoC 13339 (217.07 cm^2), C 16338 (210 cm^2) and C 2014 516 (196 cm^2). Waterlogging reduced the leaf area of other sugar cane clones and types by 44 % to 48 % when compared to their respective control plants. Recovery of growth rates following root function degradation may be slower than recovery following leaf area reduction, consistent with findings reported by previous studies (19, 20). Similarly, other crops such as green gram (21), sesame (22, 23) and

Table 2. Physiological parameters measured under control and waterlogged conditions as per clones/varieties (pooled data of 2022 and 2023)

S. No.	Varieties /Clones	Leaf Area (cm^2)		SPAD index		Leaf Soluble protein (mg g^{-1} of fresh weight)	
		Control	Waterlogging	Control	Waterlogging	Control	Waterlogging
1.	Co 86032	193.33	100.29	38.18	17.90	91.05	98.35
2.	C 2015 095	229.33	126.18	35.50	26.46	91.17	119.79
3.	G 11035	240.66	108.29	35.21	24.97	90.77	120.75
4.	C 2014 516	388.66	196.07	38.57	34.55	94.30	131.95
5.	C 16338	367.00	210.00	36.31	34.47	94.20	131.93
6.	C 30010	243.33	176.01	35.13	27.13	90.72	120.02
7.	Co 15020	253.33	192.43	34.85	27.20	94.62	122.66
8.	Co 62175	288.00	159.92	34.80	31.52	94.05	124.31
9.	C 2015 021	306.00	180.26	39.16	33.16	93.65	126.95
10.	Si 2014 047	250.66	144.58	36.74	28.72	93.36	117.91
11.	CoC 13339	390.00	217.07	37.14	34.65	94.32	132.16
12.	C 2015 006	225.33	124.36	35.16	26.03	94.97	118.02
13.	Co G 7	318.67	181.47	37.28	27.70	94.35	126.93
	Mean	**284.17**	**162.84**	**36.46**	**28.80**	**93.19**	**122.44**
	SE (d)	5.63	2.209	1.015	0.398	1.584	0.621
	CD	11.31	4.436	2.039	0.8	3.181	1.248
	CxW (CD)	15.994		2.884		4.499	

Treatments (CXW) with the same letters do not differ significantly ($p < 0.05$) (C-Control and W-Waterlogging) mung bean (24) have shown similar reductions in leaf area under waterlogging stress, suggesting a consistent physiological response across different species.

Root and shoot dry weight and TMP

The results of the present investigation showed that root weight was relatively higher in waterlogged affected plants; CoC 13339 (4.19 g/plant) had the highest increase in root dry weight followed by C 2014 516 (3.73 g/plant) and C 16338 (3.56 g/plant) (Table 3). The increase in root dry weight might be due to higher root density as reported earlier (25). Aerial roots that are formed in response to waterlogging tolerant genotype CoC 13339 produced 23.43 aerial roots followed by C 16338 producing 22.96 aerial roots. However, the shoot dry weight was comparatively higher in CoC 13339 (14.70 g/plant) followed by C 16338 (14.50 g/plant) as compared to variety Co 86032 (10.27 g/plant) under waterlogged conditions. An increase in root and shoot weight and total dry matter production in genotypes CoC 13339 and C 16338 indicated tolerance to waterlogging stress.

Multiple studies showed that waterlogging reduced fresh weight and dry weight in soybeans (26). Studies have shown that waterlogging reduces chlorophyll content, reducing photosynthesis and resulting in a decreased rate of photosynthesis that inhibits plant growth and biomass accumulation (27). A waterlogged plant will disrupt plant physiology and catabolism, limiting stomatal conductance, gas transit and CO_2 metabolism. When CO_2 is reduced entering the leaf, transpiration is reduced entering the leaf. This leads to wilting of the leaves, a reduction in chlorophyll content and a consequent reduction in dry matter accumulation, which results in a decrease in fresh weight and then dry weight as well. According to the current findings, there was a reduction in dry matter accumulation due to reduced water absorption and inhibition of carbohydrate synthesis. Waterlogging-induced stomatal closure reduced CO_2 availability, affecting leaf water content, osmotic capacity and transpiration rates. These changes, along with reductions in RWC and biochemical constituents such as photosynthetic pigments, proteins and carbohydrates, collectively impaired photosynthesis (28, 29). Similar reductions in shoot dry weight due to flooding stress have been reported in other crops, including maize (30), green gram (21) and mung bean (24).

Root anatomy studies

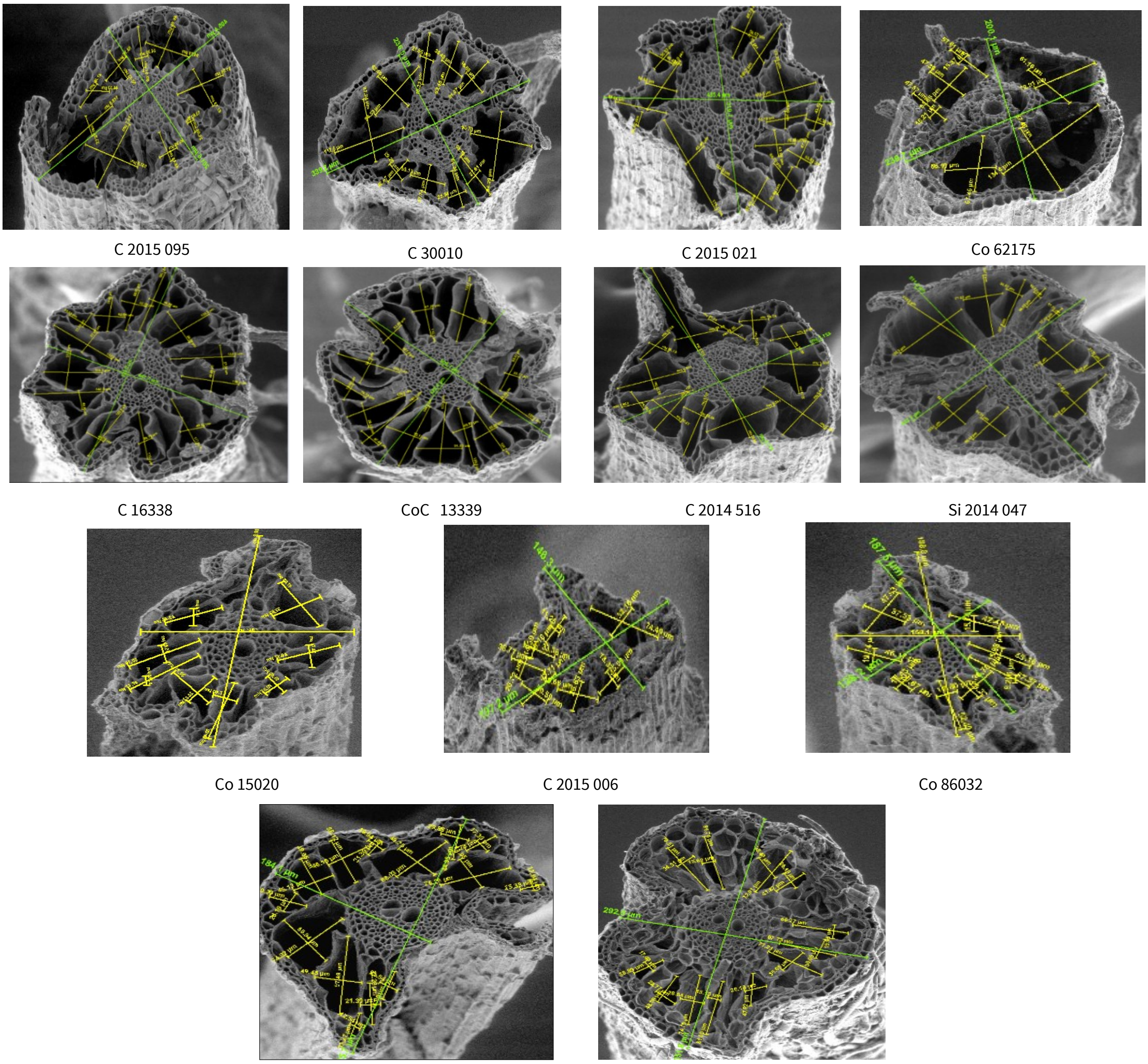
Because of hormonal imbalance brought on by hypoxia and the decreased oxygen availability to submerged tissues, the sugarcane crop develops adventitious roots when it is wet. It was discovered that clones that can withstand moist circumstances are linked to improved intercellular gaps in adventitious roots. One unique characteristic shared by all varieties that thrive in moist environments is the existence of a broad interconnecting intercellular gas-filled area (aerenchyma) that stretches from the shoots to the root tip (18). To find out how much of roots total area was occupied by aerenchyma cells, an anatomical examination was conducted (Fig. 3). The roots had a cross-sectional area that varied from 1.12 mm^2 (Co 86032) to 1.82 mm^2 (CoC 13339). The variety CoC 13339 had the largest total root area (1.82 mm^2). At $P > 0.05$, the clone's differences in total root area were statistically significant (Fig. 4). The cortical tissues took up between 1.1 and 1.66 mm^2 of space. The variety with the least amount of cortical tissue occupied was Co 86032 (1.1 mm^2), whereas the highest occupied area was CoC 13339 (1.66 mm^2), followed by C16338 (1.62 mm^2). For varieties Co 86032 and CoC 13339, the area of aerenchyma tissues on the cut surface varied from 0.036 mm^2 to 0.28 mm^2, respectively. The aerial roots of the variety CoC 13339 had the largest area of aerenchyma tissues, whereas C16338 had the most aerenchyma of the test clones. Aerenchyma tissues occupied a significantly smaller area in the clone CoC13339, which had a large total aerial root area. Aerenchyma tissues occupied the most area in the variation Co 62175. The percentage of aerenchyma tissues in relation to the cortical tissues varied between 3.2 (Co 86032) and 16.17 % (Co 62175) in check varieties and 2.6 % (C 2015 006) to 16.63 % (CoC 13339) in clones/varieties. In test clones and varieties, the percentage of aerenchyma tissue per aerial root area ranged from 2.5 % (C 2015 006) to 15.15 % (CoC 13339), while in check varieties, the range was 3.2 % (Co 86032) to 15.13 % (Co 62175).

However, the size of the aerenchyma was larger in waterlogged seedlings. Waterlogged impacted plants had aerenchyma production in the cortical region. This extra

Table 3. Dry matter parameters measured under control and waterlogged conditions as per clones/varieties (pooled data of 2022 and 2023)

S. No.	Varieties/clones	Shoot dry weight (g/plant)		Root dry weight (g/plant)		Total dry matter (g/plant)	
		Control	Waterlogging	Control	Waterlogging	Control	Waterlogging
1.	Co 86032	18.45	10.27	5.35	2.24	20.69	15.62
2.	C 2015 095	18.18	10.53	5.41	2.25	20.43	16.74
3.	G 11035	18.90	11.42	6.40	3.17	22.07	17.82
4.	C 2014 516	19.13	14.46	7.77	3.73	22.86	22.23
5.	C 16338	20.18	14.50	8.46	3.56	23.74	22.96
6.	C 30010	17.96	11.67	6.48	3.30	21.26	18.15
7.	Co 15020	18.78	12.64	5.92	3.16	21.94	18.56
8.	Co 62175	20.46	14.39	7.98	3.58	24.04	22.37
9.	C 2015 021	21.45	13.46	7.08	3.30	24.72	20.54
10.	Si 2014 047	19.48	13.13	5.78	2.55	22.03	18.91
11.	CoC 13339	21.86	14.70	8.73	4.19	26.05	23.43
12.	C 2015 006	18.81	11.64	6.21	3.25	22.06	17.85
13.	CoG 7	20.78	14.29	7.02	3.52	24.30	21.31
	Mean	**19.57**	**12.85**	**6.81**	**3.22**	**22.78**	**19.73**
	SE (d)	0.432	0.17	0.13	0.331	0.529	0.207
	CD	0.868	0.341	0.261	0.666	1.062	0.417
	CxW (CD)	1.228		0.941		1.502	

Treatments (CxW) with the same letters do not differ significantly ($p < 0.05$) (C-Control and W-Waterlogging)



C 2015 095 C 30010 C 2015 021 Co 62175

C 16338 CoC 13339 C 2014 516 Si 2014 047

Co 15020 C 2015 006 Co 86032

Co G 7 G 11035

Fig. 3. Scanning electron micrographs (×500 mm) of cross-section of several clones and varieties of sugarcane root under waterlogging stress.

aerenchyma was presumably created by cell lysis. Aerenchyma was also generated in the cortex region of aerial roots grown in wet conditions, however, it was less pronounced in Co 86032, C2015 006, C 2015 095, G 30010, Co G 7, Si 2014 047 and Co 15020 (Fig. 4).

Aerenchyma formation is necessary for plants that experience waterlogging to survive and operate. The aerenchyma helps in gas ventilation (e.g. CO and methane) and oxygen transfer from shoots to roots. Through the concentration and movement of CO from root respiration to the intercellular spaces of leaves, aerenchyma may contribute to photosynthesis benefits. Aerenchyma that forms in the cortex of roots in the current study is comparable to that of rice in well-drained soil and can be further strengthened during soil waterlogging (7).

In waterlogged plant roots, this characteristic is a typical adaptive shift in morphology (15, 31). It is a tactic to increase accessibility to oxygenation at or above the wet soil surface to supply oxygen to the roots and shoots (32). However, adventurous roots with aerenchyma volume are necessary for the efficient transfer of oxygen. Compared to less tolerant species, more waterlogging-resistant species frequently have more aerenchyma in their newly produced roots. Waterlogged CoG 7 and Co 2015-021 plants had more adventitious roots in their root systems than Co 13339 and C 16338 plants had more aerenchyma. The increased aerenchyma development of CoC 13339 and C 16338 plants may boost waterlogging tolerance by improving inner circulation between or within roots and shoots via gas routes to increase gas diffusion (33).

Another way that plants morphologically respond to waterlogging is by forming root cortical aerenchyma. For instance, during waterlogging, the barley genotype with greater levels of root, cortical aerenchyma yielded more (34). Additionally, the spontaneous growth of roots and the generation of root cortical aerenchyma in barley have been found to be rather consistently correlated (35). Together with aerenchyma, a barrier that stops oxygen from leaking into the surrounding soil and promotes O_2 diffusion to the tips of the roots can also lessen the radial loss of oxygen from the roots (13). A higher cortex-to-stele ratio and a smaller area of surface-to-volume are two structural changes that some crops, like maize and wheat, have developed to deal with radial oxygen loss while being unable to establish the radial oxygen barriers (36). Both strategies encourage the dispersion of O_2 through the roots to get around the root shortage of energy (13).

SPAD index (chlorophyll content)

The pigment chlorophyll converts light energy into chemical energy, which is crucial to the process of photosynthesis. Chlorophyll processes solar energy to convert water molecules into gaseous oxygen and reduce NADP molecules into NADPH. Additionally, ATP, NADP and ATP are the molecules formed by light energy and employed in processes to make glucose. Treatments with flooding tended to have lower levels of chlorophyll than those without floods (37). Varieties' effects on the amount of chlorophyll are shown in Table 2. It shows that the cultivars with the highest chlorophyll contents CoC 13339, C 2014 516 and C16338 have respective amounts of 34.65, 34.55 and 34.47. On the other hand, cultivar Co 86032 has the minimum chlorophyll content (17.90). Chlorophyll production is affected by several variables, including light, leaf size and the growing media's water content. Chlorophyll formation may have decreased because of excessive water, as seen by the reduction in leaf area and consequent deterioration in leaf size. The highest SPAD values over control were found in CoC 13339, C 2014 516 and C16338. The current study findings regarding the decrease in chlorophyll and carotenoids were consistent with those of previous studies (25, 38). The way sugarcane reacts to waterlogging is delicate and demanding. Following a 72-hour waterlogging stress, the colour of the soybean leaves changed from green to pale green, which is caused by a decrease in the amount of chlorophyll in sugarcane leaves. When the chlorophyll content significantly decreased after seven days of waterlogging, a change in the colour of the leaves was observed (39). The percentage drop in the SPAD reading, a measure of the leaf's chlorophyll content, differed from clones to varieties as a result of waterlogging stress (Table 2). The waterlogging in soybeans causes a decrease in photosynthetic activity (40). Waterlogging decreased the SPAD value by 10-38 % in the KY16 variety and 5-30 % in the DMY1 variety of maize (30).

Stomata density

The data gathered shows that water stress has led to denser stomata in certain varieties, as shown in the images. Stomatal densities were highest in the varieties CoC 13339 (24,639 stomata/mm^2) and C 16338 (23,577.67 stomata/mm^2) compared to other varieties (Fig. 5 & 6). Stomata play a crucial role in maintaining leaf hydration because the majority of water in plants diffuses through them (41). The size and density of the stomata determine whether they have a high or low conductivity. The effect of an increase in photosynthetic rate is indicated by a density increase in stomata. In general, leaves with high stomata and a lower gas exchange rate have smaller stomata.

Soluble protein content

Plant tissues contain proteins that serve a variety of purposes, including transporting electrons for respiration and photosynthesis. Protein is made from nutrients, particularly nitrogen, that plants absorb. Nitrogen is a component of protein, chlorophyll and other amino acids that are beneficial to plants (42). The soluble protein level of the cultivars was calculated to ascertain their photosynthetic capacity under waterlogging stress. When compared to the control condition, the waterlogged conditions showed the highest levels of leaf-soluble protein in CoC 13339 (132.16 mg/g), C 2014 516 (131.95 mg/g) and C 16338 (131.93 mg/g), while the lowest levels were found in Co 86032 (98.35 mg/g) (Table 2). Short-term flooding stress caused sugarcane roots and leaves to express anaerobic proteins, suggesting that these proteins may play a part in tolerance (18). Numerous investigations have demonstrated that hypoxia-responsive anaerobic proteins sustain energy generation in anaerobic environments and their increased transcription promotes fermentative respiration and glycolysis. The low protein content is attributed to the reduced nitrogen uptake capacity of roots under water stress.

Principal Component (PC) analysis

Using principal component (PC) analysis, the contribution of evaluated variables to each PC was calculated in order to analyse the effect of waterlogged streets on the growth, physiological and root anatomical features of several sugarcane clones. The results of the analysis indicated that the first PC would account for up to 84 % of the variance, with the second PC accounting for 5.87 per cent of the variance during waterlogged street stress. Soluble protein (0.974), shoot length (0.898), root length (0.908), SPAD index (0.948), leaf area (0.877) and stomatal density (0.891) were the morpho-physiological to dry matter production variables that showed the highest values at waterlogging. The second PC during waterlogging, however, showed the highest weightage for the following: soluble protein (0.171), leaf area (033), stomatal density (0.115) and SPAD index (0.115). It also showed a negative correlation with shoot length (-0.36), root length (-0.331) and dry matter production. The first PC might account for up to 86.34% of the variance, while the second PC could account for 9.56%. The first PC accounted for the highest weighting of variables like dry matter production (0.9577), aerial root length (0.931), aerial root total area (0.902), aerenchyma tissue area (0.973), cortex tissue area (0.87), aerenchyma/total aerial root area percentage (0.928) and aerenchyma/cortical tissue percentage (0.94). The second PC at waterlogging, however, showed significant importance for dry matter production (0.023), total aerial root length (-0.006), aerenchyma tissue area (-0.174), the percentage of aerenchyma area compared to total aerial root area (-0.359) and the percentage of aerenchyma area compared to cortex tissue area (-0.326) (Fig. 7). According to the PC analysis's biplot, there was a positive correlation between waterlogging and every measured variable that was located in both the first and

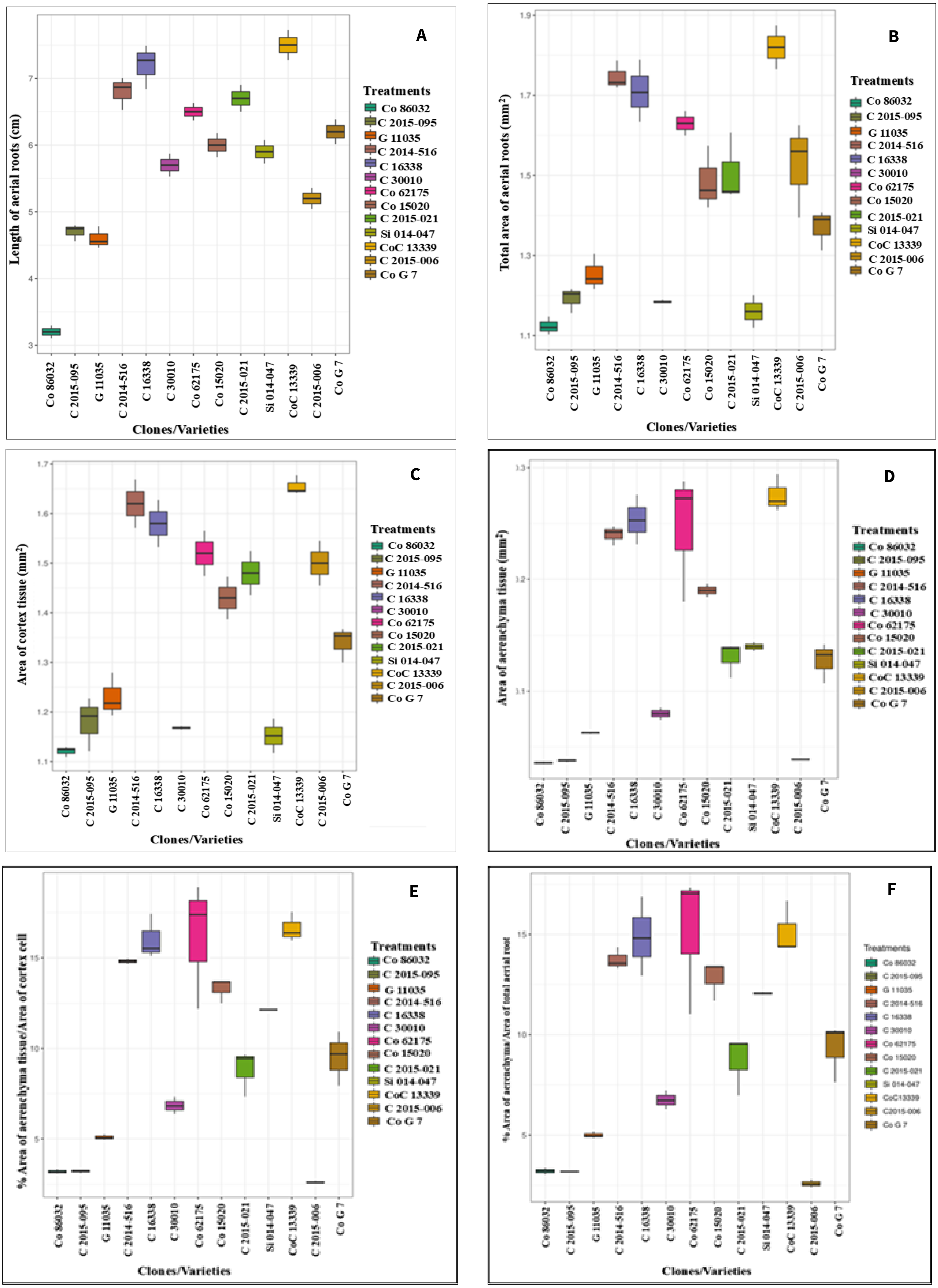
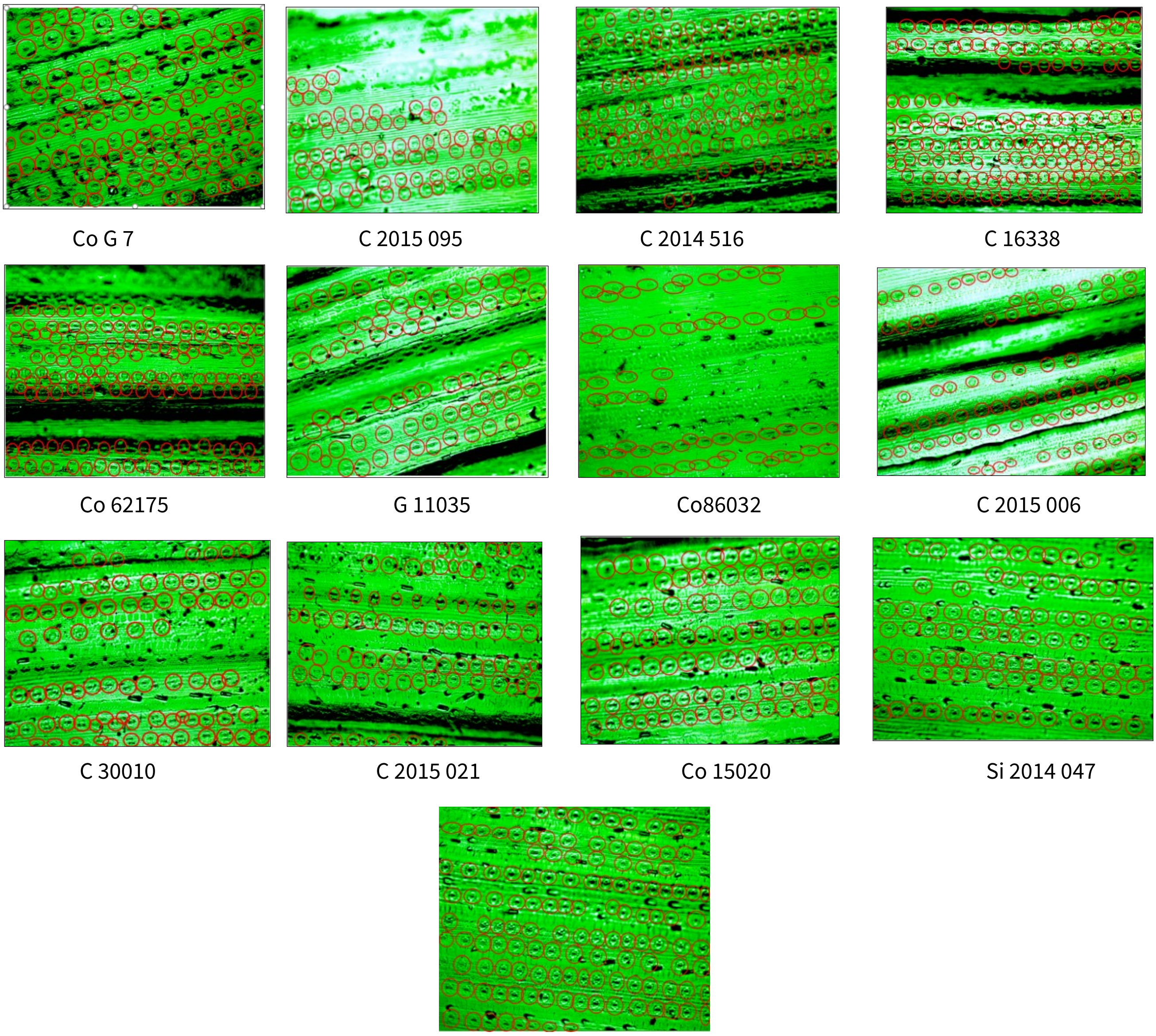


Fig. 4. Responses of sugarcane clones/varieties under waterlogging conditions: (a) length of aerial roots (cm), (b) total area of aerial roots (mm^2), (c) area of cortex tissue (mm^2), (d) area of aerenchyma tissue(mm^2), (e) % area of aerenchyma tissue/area of cortex cell and (f) % area of aerenchyma/area of total aerial root. For each variable, the least squares mean sharing the same letters is not significantly ($p > 0.05$) different.



Co G 7

C 2015 095

C 2014 516

C 16338

Co 62175

G 11035

Co86032

C 2015 006

C 30010

C 2015 021

Co 15020

Si 2014 047

CoC 13339

Fig. 5. Microscopic image of stomata of clones and varieties of sugarcane under waterlogging stress.

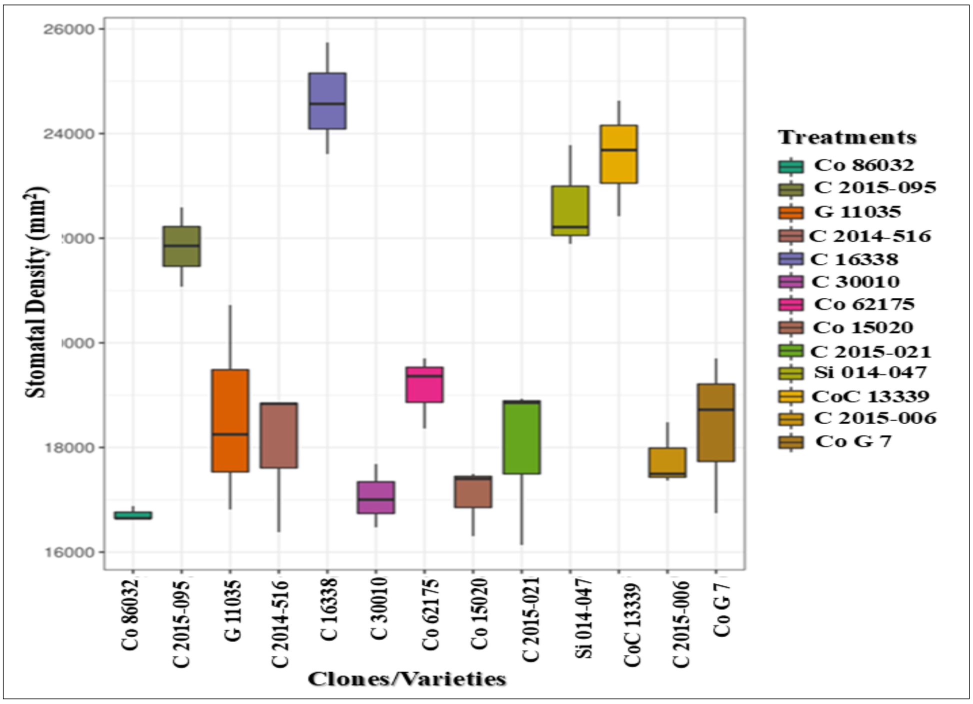


Fig. 6. Stomatal density of clones and varieties of sugarcane under waterlogging stress.

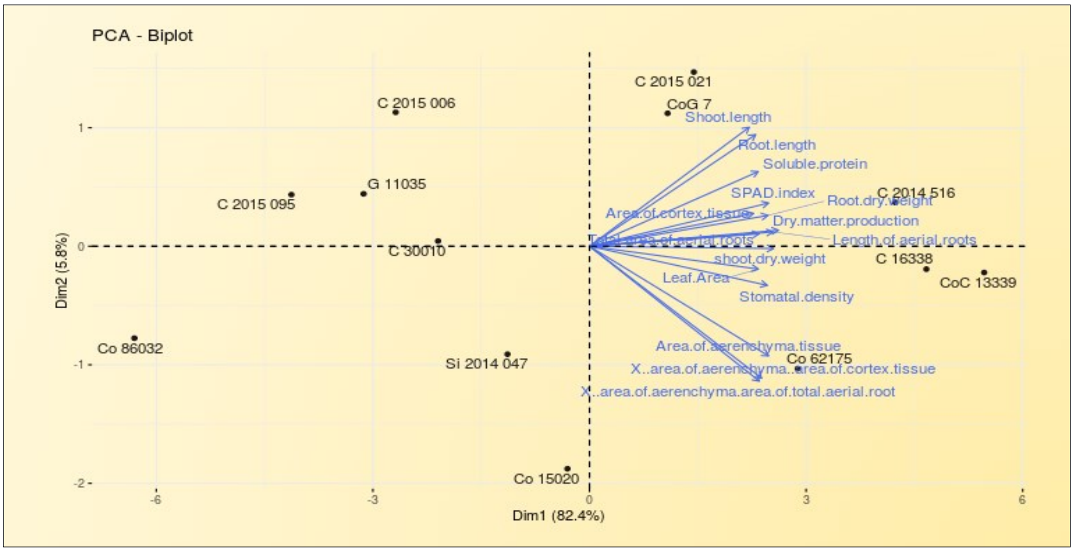


Fig. 7. PCA analysis of sugarcane clones/varieties under waterlogging condition.

second zones (the top and bottom quadrants, respectively). Furthermore, under waterlogging conditions, the genotypes in the biplot's first quadrant (right top quadrant) behaved well.

Correlation studies on anatomical and morpho-physiological traits

Dry matter production was positively correlated with the aerial root's aerenchyma tissue area. Of the clones and varieties, the clone C 2015 006 and variety Co 86032 with the smallest area of aerenchyma tissue produced the least amount of dry matter. However, aerenchyma growth is a helpful criterion for selecting clone areas occupied by the aerenchyma tissues of the aerial root (43).

The waterlogging-tolerant clones and varieties CoC 13339 and C 2014 516 have long and numerous aerial roots on the node, according to studies on the adaptation traits of sugarcane clones to waterlogging stress. Root anatomical qualities and morphophysiological attributes under waterlogging conditions were found to be positively correlated, according to the correlation coefficients. As a result, these traits can be efficiently used to generate varieties that are tolerant of waterlogging. Shoot length, root length, SPAD index, leaf area, soluble protein and stomatal density greatly and favourably correlated with the tolerance index for dry matter production. A positive connection with aerenchyma is also shown in Fig. 8.

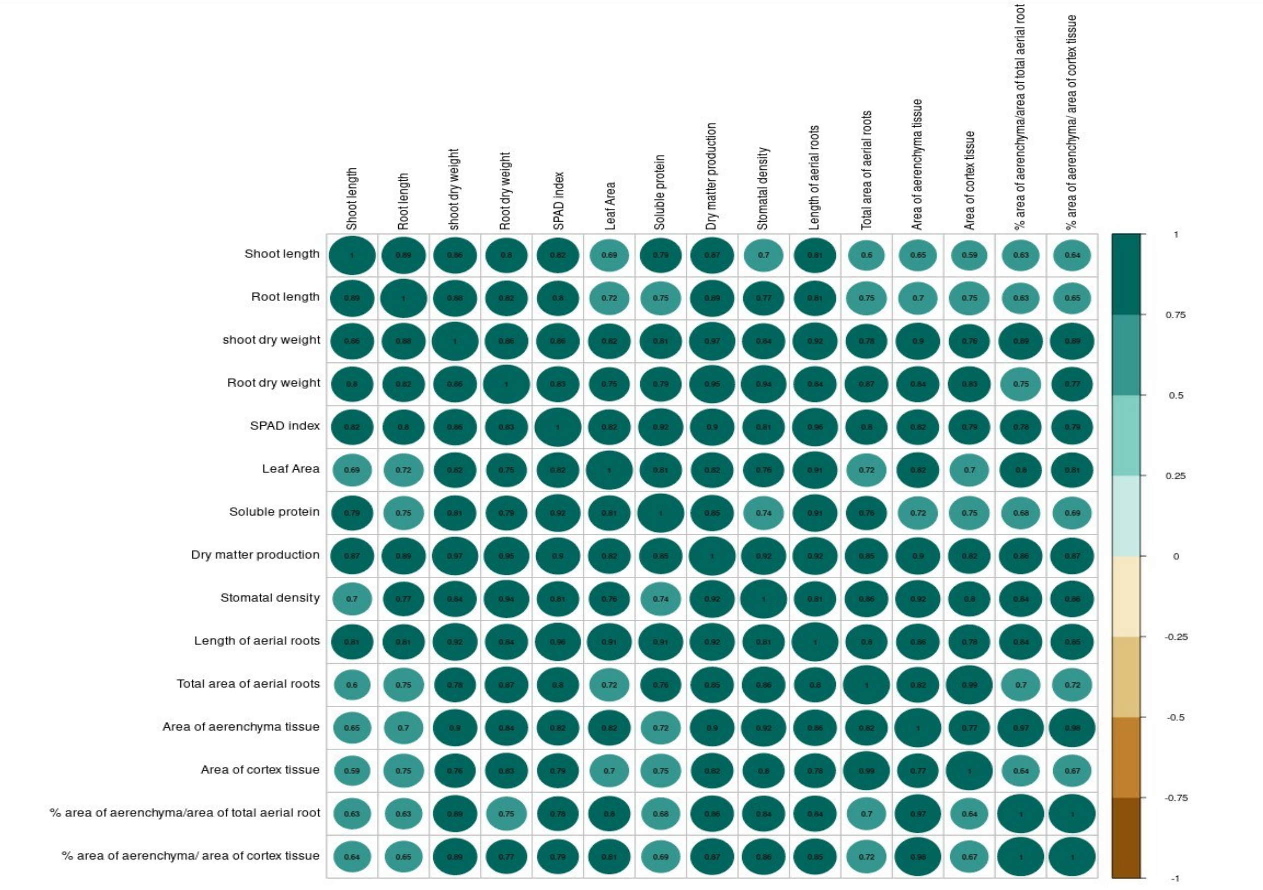


Fig. 8. Correlation correlogram of morpho-physiological traits and root anatomical traits under waterlogging condition; Significance at P ⩽ 0.05.

Conclusion

The sugarcane plant's ability to withstand waterlogging was largely attributed to the establishment of aerenchyma in adventitious roots. While waterlogging caused adventitious roots to grow in waterlogged sugarcane crops, it encouraged the development of more aerenchyma in plants that could withstand waterlogging. Although there were no appreciable decreases in biomass in this study, waterlogging had a major impact on the root mechanism, which could have long-term repercussions on plant growth if it continues. Since just thirteen varieties/clones were evaluated, more research is needed to determine whether waterlogging tolerance varies, whether the findings apply to other types going forward and to find germplasm that may be utilised in breeding initiatives.

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Authors' contributions

AR, JP, SD presented the idea and conceptualisation of the research article. VD, AL, MR collected the scientific materials and contributed to writing the manuscript. Writing and editing were done by NR, VK. JM, SN reviewed the manuscript. All the authors have contributed to the preparation of the final edited draft of this paper.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues : None

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