RESEARCH ARTICLE

SUGARCANE ROOT GROWTH AND DEVELOPMENT IN HYDROPONICS SYSTEM

K. Hari*, S. Vasantha, A. Anna Durai, Rajeshkumar, C. Brinda and P. Shruthi

Abstract

Sugarcane root system is distinct among crop plants since, it sustains the crop for several years through ratoons by its efficient root system and the capacity for nutrient and water uptake. The study of root system offers scope for exploiting the beneficial traits under adverse and normal situations for sustaining the cane yields with better economic returns. In the present investigation, sugarcane root growth, its development and root traits which have significance in the crop establishment were studied under hydroponic conditions. Twenty five commercial hybrids were assessed for root traits, lateral root development and for their response to injury. Observations on the lateral root initiation, root characteristics viz., root tip colour and shape warrant for detailed investigation. Among the varieties the root cap pigmentation varied from light pink (Co 62175) to deep violet (Co 99004) and root cap shape varied from simple triangle (Co 62175) to dome (Co 86032).The root injury studies revealed time dependent response among the three varieties *viz.*, Co 86032, CoC 671 and Co 06022. Faster root regeneration/lateral root initiation elucidated in limited varieties suggests significant variability for the traits and the inherent capacity of the variety. This has significance, as many of the commercial hybrids are vulnerable to soil biota. The root traits *viz.,* root weight, root volume and root shoot ratio varied significantly among the commercial hybrids and had a close relationship with plant biomass and needs to be exploited for their role in abiotic stress tolerance.

Key words : Sugarcane, hydroponic system, root traits, root injury, root development

Introduction

Growth and development of above ground parts in sugarcane depends on the root system and its development as in other Poaceae grass family members. When the growing roots encounter mechanical barriers they tend to force their way, however when the barrier is impenetrable further root tip growth is directed suitably in sideways (*Monshausenet et al.* 2009). In addition to such response, mechanical stimulation of the root is also able to exert developmental changes. The deep and prolific root system ensures more nutrients and water availability as compared to shallow system. Mace et. al. (2012) reported the conserved genetic control of root shoot angle and its relation with yield in wheat and rice. Such genetic control suggest, potential for altering the root system based on the need in addition to agronomic practices that limits biotic and abiotic constraints to root growth (*Bonnett*, 2013).The water relations of sugarcane root systems have been well studied indicating the existence of considerable variation in root hydraulic conductivities. Root system of commercial types possess several characteristics which make them efficient for sustaining the crop for about 12 months in the field and further support the succeeding ratoons till the shoot roots of the ratoon crop takes over the function. In the present study, an attempt has been made to elucidate the varietal potential for the various root biometric traits, their response to inflicted injury and related phytochemical assays to understand the root

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behaviour under unstressed hydroponic system.

Materials and Methods

Hydroponic system was established in glass tanks in the dimension of (LxBxH= 20x20x50 cm) and purge provided with aeration by bubbling air from the bottom of the tank. Three replicates were maintained for each variety. Twenty five commercial hybrids were utilized in the study. Bud chips germinated in soil compost mixture for 30 days were washed thoroughly and placed in meshbasket (@3 plants/tank) fitted on the upper side of the tank (Fig.1). Care was taken to place the entire root in the hydroponic medium. Modified Hoaglands' nutrient medium was used in the study (Table 1).

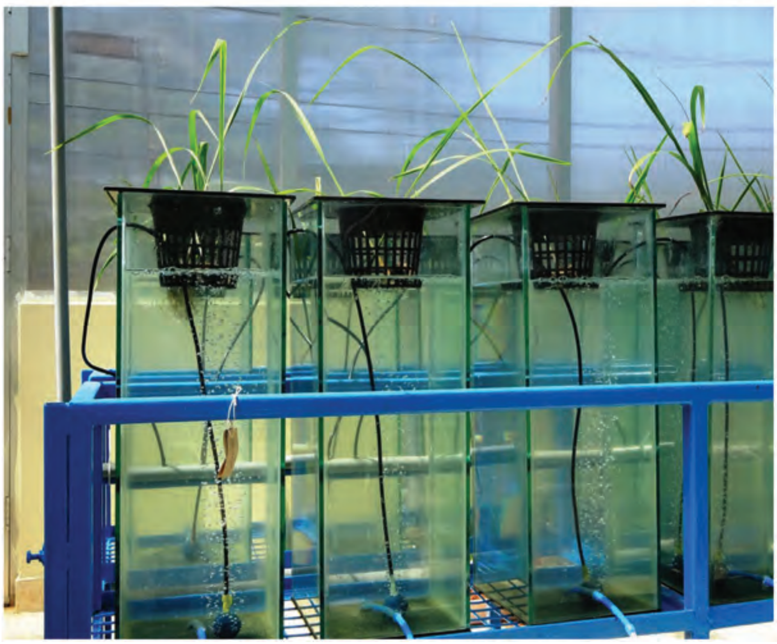

Fig. 1. Sugarcane in hydroponic set up

The root growth was monitored at weekly intervals and observations on root number length and volume were recorded at the end of three months as well as 10 months of crop age (Fig. 2). The root volume was recorded following liquid displacement technique and expressed in ml.

Root biochemical traits: Enzymes of ROS system assays include peroxidase, superoxide dismutase and total phenolics. Total phenolics was estimated with Folin-ciocalteau reagent. Plant phenolics react with

Table 1. Composition of modified Hoagland's nutrient solution

Sl. No.	Ingredients		Weight (g l^{-1})
1	Potassium nitrate	:	0.608
2	Calcium nitrate	:	1.416
3	Potassium dihydrogen phosphate	:	0.164
4	Magnesium sulphate	:	0.56
			Weight (g $250l^{-1}$)
5	EDTA- ferric monosodium salt	:	6.00
6	Boric acid	:	1.43
7	Manganese chloride tetradydrate	:	0.91
8	Zinc sulphate	:	0.11
9	Cupric sulphate	:	0.04
10	Molybdic acid	:	0.01

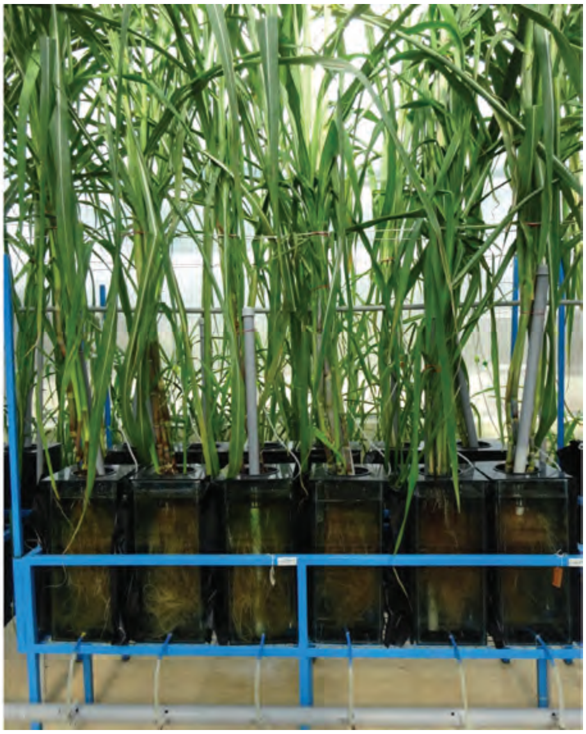

Fig. 2. Five month old sugarcane crop in hydro culture

phospho molybdic acid in Folin-ciocalteau's reagent in alkaline medium and produce blue coloured complex (Singleton and Rossilt 1965).

Peroxidase activity was assayed by the oxidation of o-dianisidine following the method of Malik and Singh (1980). Absorbance was read at every 30s interval up to 3 min. Increase in absorbance was plotted against time and from the linear phase of the graph the change in absorbance per minute was worked out. Enzyme activity was expressed in terms of rate of increased absorbance per unit time per gram tissue weight.

Superoxide dismutase (EC 1.15.1.1) was assayed by monitoring the inhibition of photoreduction of nitro blue tetrazolium (NBT) according to the method of Beauchamp and Fridovich (1971). Leaf samples were homogenized in four volumes (w/v) of an ice–cold buffer containing 0.1 M Tris-HCl, 1 M EDTA and 0.05% Triton-X 100. The homogenates were filtered through four layers of cheese cloth and centrifuged at 4^{o}C for 30 min at 15000 rpm. The supernatant collected was used for SOD assay. The reaction mixture contained 50mM phosphate buffer (pH 7.8), 0.053 mM NBT, 10 mM methionine, 0.053 mM riboflavin and an appropriate aliquot of enzyme extract. The reaction was initiated by switching on the light and allowed to run for 7 min. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm.

Root development: The lateral root formation was studied by taking serial sections and the stage wise structural /anatomical changes were observed through fluorescence microscope (Wild leitzgmbh, Type 020-503-030, Germany).

Root injury experiment: Response to root injury was studied in three popular commercial hybrids (Co 06022, CoC 671 and Co 86032) in hydroponic system. The injury was inflicted by longitudinal slicing of primary root (2cm) up to root tip using sterile surgical blade. The injured and intact root was maintained in the same nutrient medium. Observations on morphological changes and lateral root induction were recorded periodically.

Results and Discussion

Lateral root initiation and growth: Serial sections depict the developmental stages of lateral roots starting with the secondary root initiation and development (Fig. 2). The transverse section of the root samples shows the stage wise development of lateral roots. The outer layer of stellar region shows differentiation of few cells that form lateral roots. These differentiated cells gradually forms

Fig. 3. Lateral root initiation and development a) Differentiation of small group of cells with deep staining, b) A dome formation of root apex of lateral roots (LR), c) LR apex piercing the cortical tissue and rooting out, d) TS of main root and LS of LR showing the apex and root tip deeply stained.

small dome shaped structures (Fig. 3a-e) which is the root apex (primordium), passes through the cortical tissues and finally emerge out piercing the outer layer of epiblemma. Further, the lateral root primordium develops into a fully developed lateral root. Schiefeibeina and Benfeyb (1991) reported that a single cell or multiple cells (non pericycle) may simultaneously receive the signal which differentiate into a meristem and elaborates into a root which forces its way through cortex and exists as new root. Similar observation was recorded in the present study in Co 62175.

Root tip, cap and root hairs : The root tip pigmentation and prominent root cap is a natural gift for the protection of root system. Among the varieties the root cap pigmentation (Fig. 4) varied from light pink (Co 62175) to deep violet (Co 86032) and root cap shape varied from simple triangle (Co 62175) to dome (Co 86032). Root hair density also showed significant variation among the commercial hybrids. Moore and Botha, (2014) opined that root tip purple colouration is discerned once the root primordium is in a young root emerging out from a culm.

Root injury

The roots in general are prone for injury by both biotic as well as soil physical properties. The wound/injury thus created functions as gateway

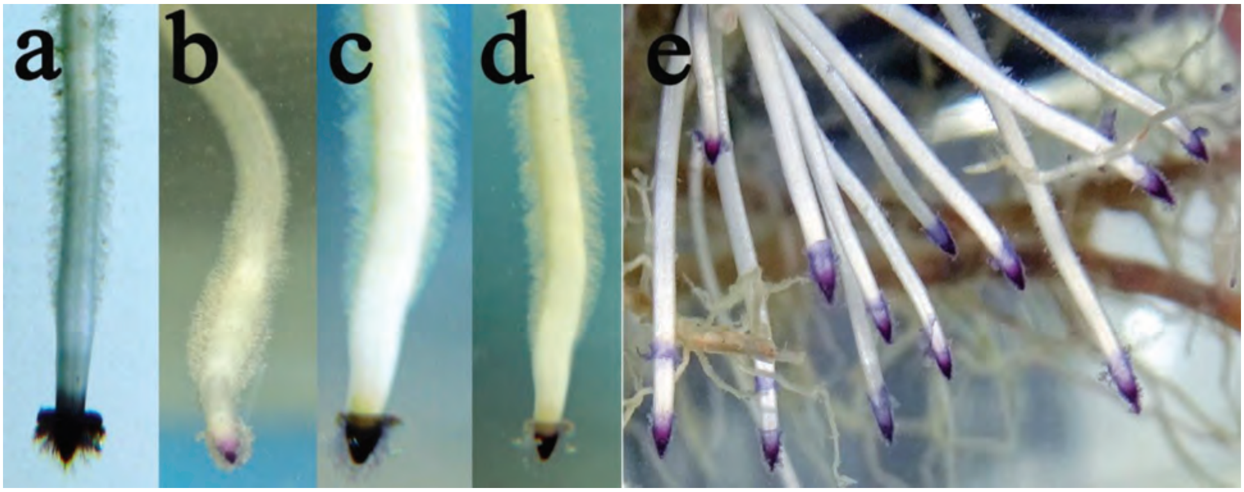

Fig. 4. Root tip, cap, hairs and colouration in hydroponically grown sugarcane varieties. a-c) Root tip triangle to dome shape. d) High root hair density and e) Deep purple colour root tip.

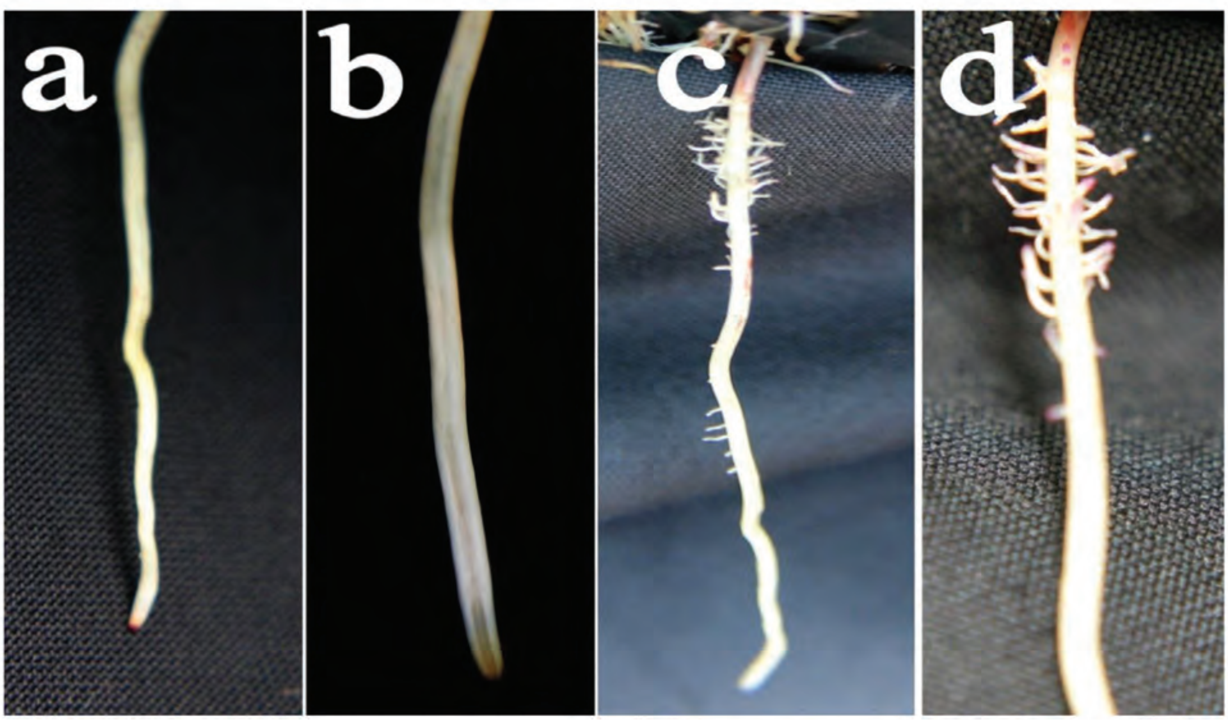

Fig. 5. Effect of root injury on sugarcane variety Co 86032, a) Uninjured root, b) Injured root, c) Three days after injury and d) Ten days after injury

for pathogens and nematodes. The regeneration potential as well as the senescence of the injured tissue contributes substantially for maintaining the crop health. The wound induced production of number of phenolic compounds imparts defense reaction. The varieties showed differential response on root injury. Up to three days of injury there was no change in coloration of the injured region in two of the varieties studied. A dense red/ pink pigmentation was observed in CoC 671 in the senesced root terminal tissue, while in Co 86032 the injured root remained apparently healthy and

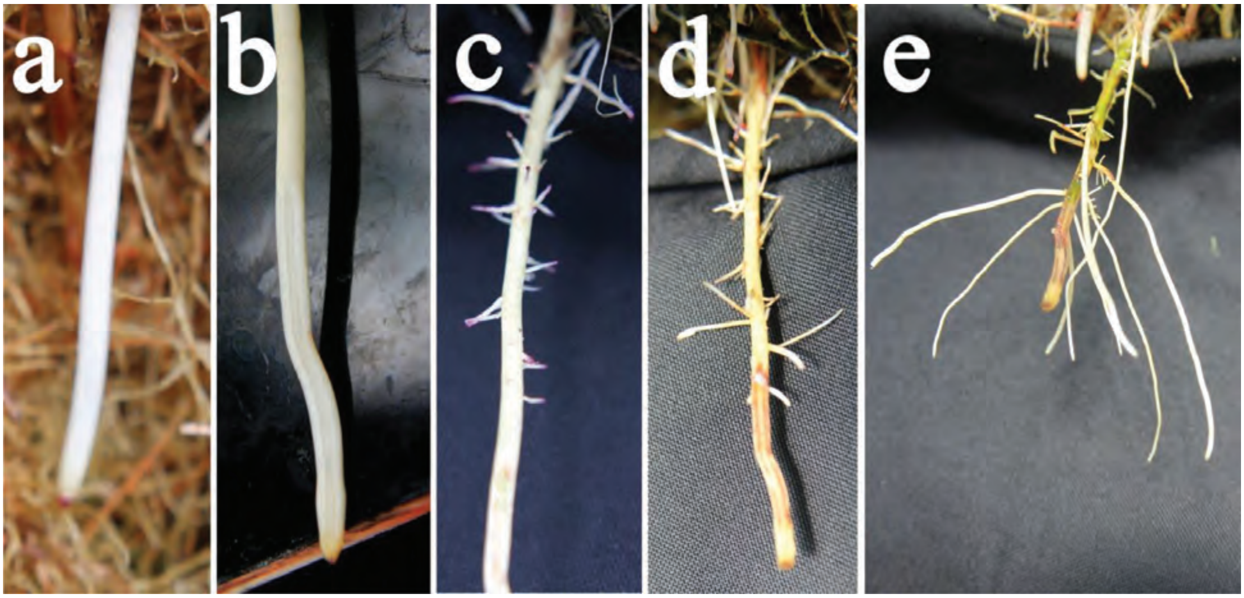

Fig. 6. Effect of root injury on sugarcane variety CoC 671, a)Uninjured root, b)Injured root, c)Three days after injury d) Ten days after injuryshowing senescence of primary root and development of secondary rootsand e) Initiation of tertiary roots

Fig. 7. Effect of root injury on sugarcane variety Co06022, a) Uninjured root, b) Injured root, c) Three days after injury and d) Ten days after injuryshowing senescence of primary root and development of secondary roots

free of symptoms of senescence and pigmentation even after 10 days of injury (Fig. 5) Perhaps, this one reason may explain the delayed secondary laterals initiation and development.

The secondary root initiation showed differential time lag in the varieties. In Co 06022, the secondary roots appeared after 10 days of injury (Fig. 7) while, in Co 86032 and CoC671, it took only three days. In CoC 671, even tertiary roots (roots from secondary roots) were initiated in about 10 days after injury of main shoot root (Fig.6), this quick response of replacing the damaged root by new roots is a positive and desirable step to explore the soil for water and nutrients for sustaining the crop. Laclau, (2009), through his studies opined that the two major factors associated to root branching are influenced by local environmental conditions. It was observed that irrigation reduced

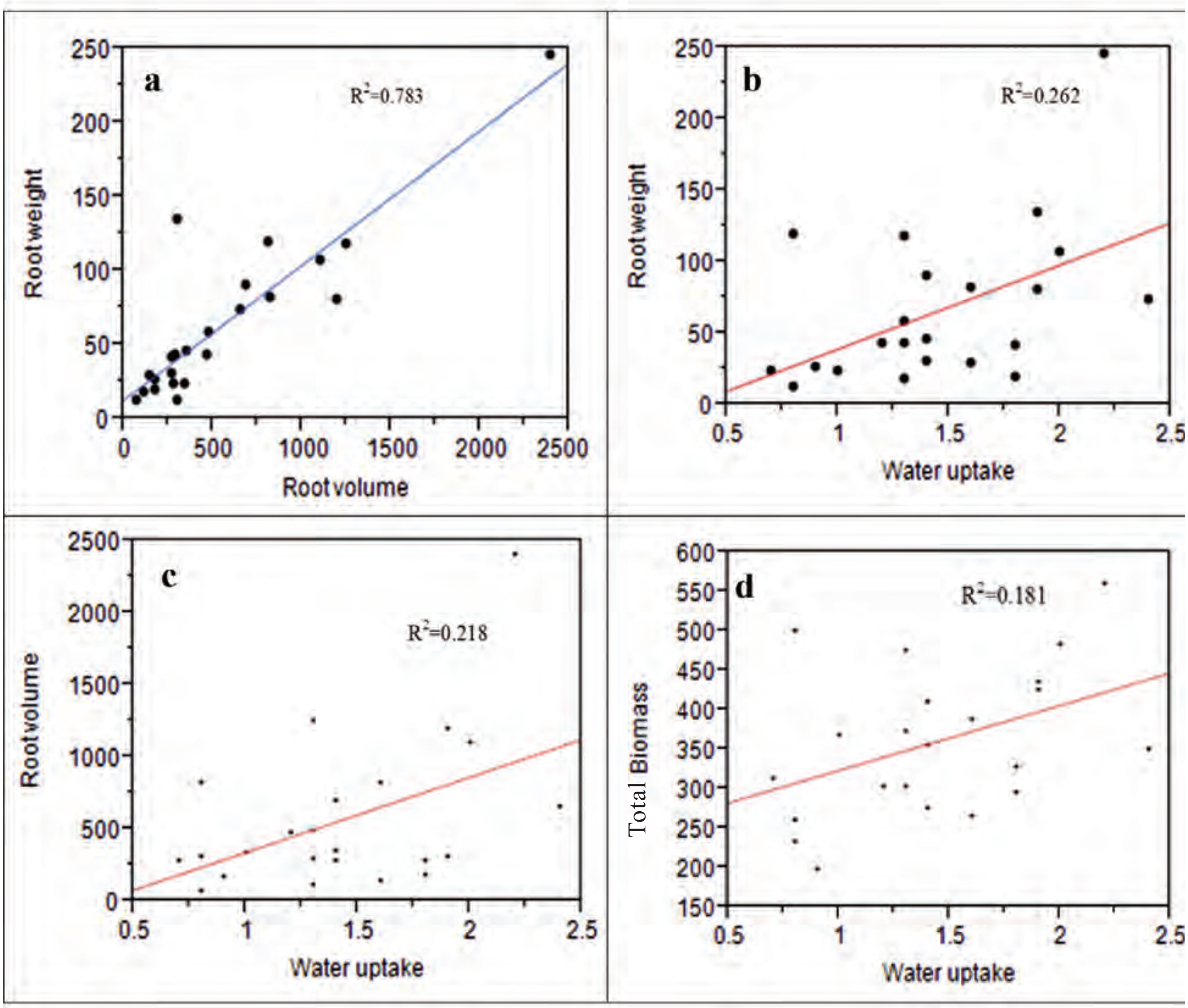

Fig. 8. Relationship among root traits and water uptake by commercial varieties. a) Root weight vs root volume (R^2=0.783) b) Root weight vs water uptake (R^2= 0.262) c) root volume vs. water uptake (R^2=0.218) d) Total biomass vs. water uptake (R^2=0.181)

Table 2. Root traits of commercial sugarcane varieties in hydro culture

Sl. No.	Varieties	Formative phase (4 months of crop age)				Maturity phase (10 months of crop age)			
		Root. No.	Root length (cm)	Root vol. (ml)	Root wt. (g)	Root No.	Root length (cm)	Root vol. (ml)	Root wt. (g)
1	Co 99004	20.7	71	60	14.0	62	71.6	1200	80.0
2	Co 0403	9.0	64	34	4.0	45	64.3	275	41.5
3	Co 740	7.0	66	113	7.0	213	70.2	114	18.0
4	Co 94008	69.0	54	84	9.5	138	64.7	300	13.0
5	CoLk8102	39.8	88	40	10.5	159	81.7	1250	118.0
6	Co 97010	23.0	46	50	7.0	69	80.0	813	120.0
7	Co 87009	83.0	68	75	10.0	166	117.6	284	23.5
8	Bo 91	8.0	65	170	21.0	236	55.5	2400	153
9	Co 8371	36.0	38	35	12.0	272	29.4	70	13.0
10	Co99006	08.0	58	220	20.0	217	43.0	180	20.0
11	Co98014	24.0	78	140	17.5	249	76.0	354	45.5
12	Co0238	82.0	71	49	9.0	165	79.2	690	90.0
13	Co85019	92.5	52	255	15.0	185	55.8	172	27.0
14	Co62175	119.0	68	24	20.0	238	75.3	821	81.5
15	Co86032	91.0	54	110	10.5	182	73.2	289	43.0
16	Co6806	132.0	70	106	14.5	265	85.0	480	58.5
17	CoJ 64	87.5	85	142	16.0	175	70.8	1100	107
18	CoC 671	49.0	66	126	12.6	147	76.0	145	29.0
19	Co 8338	45.5	32	35	8.0	91	-	-	-
20	Co 95020	60.6	58	97	11.3	364	55.6	653	74.0
21	Co 89003	78.6	82	149	14.0	236	66.3	275	31.0
22	Co 06022	56.2	69	27	22.5	225	72.6	300	135.0
23	Co 92008	72.5	56	63	13.5	145	56.5	340	23.5
24	Co2001-13	64.5	84	160	15.5	129	81.6	470	43.0
25	Co 86010	34	65	160	20.0	164	64.2	520	62.0
	SED:		6.3126	4.914	0.839		2.621	29.25	2.680
	CD:		12.69	9.88	1.688		5.271	58.83	5.402
			(**)	(**)	(**)		(**)	(**)	(**)

both root length and branching (density) and depth of rooting compared to rainfed sugarcane. On the other hand mechanical injury induced root branching varied with the cultivar as observed in the present study. McCully (1995) and Malamy (2005) are of the view that the roots of homorhizic system can branch through lateral roots. Further, the lateral root development showed high

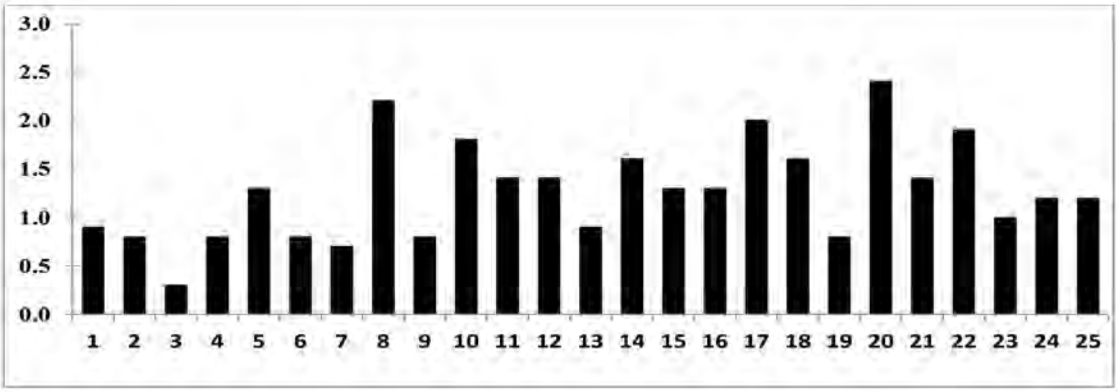

Fig. 9. Water uptake (liter day^{-1}) by commercial varieties in hydroponics. (No 1- 25 represent varieties as per the sequence presented in table)

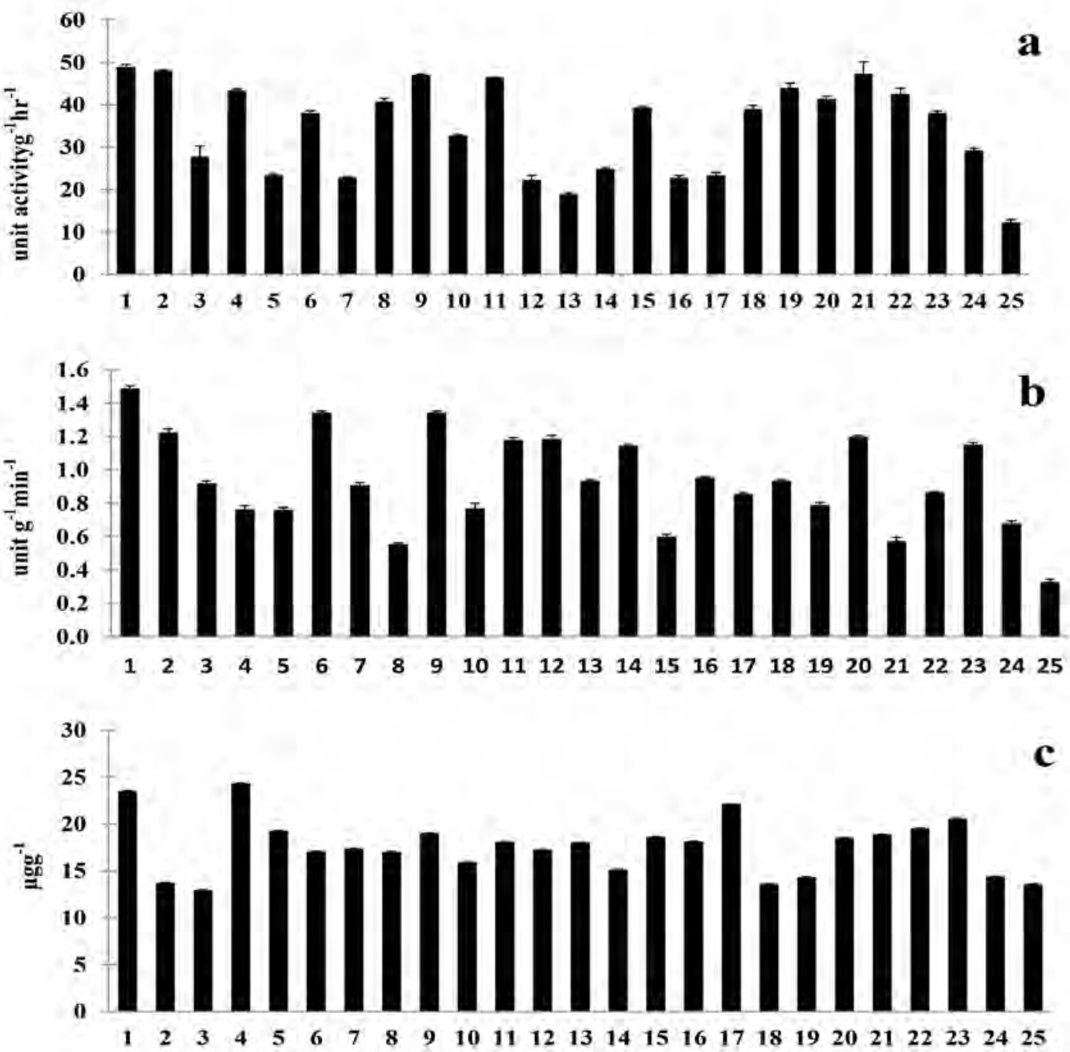

Fig 10. Biochemical characteristics of sugarcane root grown under hydroponic conditions a) Peroxidase activity in root, b) Superoxide Dismutase, c) Total Phenolics, Varietal difference is significant at $p \leq 0.05$. (No 1- 25 represent varieties as per the sequence presented in table)

Table 3. Biomass partitioning to shoot and root in sugarcane varieties in hydroculture

Sl. No.	Varieties	Formative phase			Maturity phase		
		Shoot wt. (g)	Root wt. (g)	R/S ratio	Shoot wt. (g)	Root wt. (g)	R/S ratio
1	Co 99004	69.3	14.0	0.20	345	80.0	0.23
2	Co 0403	41.0	4.0	0.09	285	41.5	0.14
3	Co 740	43.6	7.0	0.16	355	18.0	0.05
4	Co 94008	55.5	9.5	0.17	246	13.0	0.05
5	CoLk8102	64.3	10.5	0.16	356	118.0	0.33
6	Co 97010	34.6	7.0	0.20	379	120.0	0.31
7	Co 87009	60.5	10.0	0.16	290	23.5	0.08
8	Bo 91	93.0	21.0	0.22	408	153	0.37
9	Co 8371	57.5	12.0	0.21	220	13.0	0.06
10	Co99006	156.0	20.0	0.13	275	20.0	0.07
11	Co98014	142.5	17.5	0.12	310	45.5	0.14
12	Co0238	57.0	9.0	0.16	320	90.0	0.28
13	Co85019	102.5	15.0	0.15	170	27.0	0.16
14	Co62175	154.5	20.0	0.13	305	81.5	0.26
15	Co86032	81.0	10.5	0.13	260	43.0	0.16
16	Co6806	94.0	14.5	0.15	315	58.5	0.18
17	CoJ 64	125.0	16.0	0.13	375	107.0	0.28
18	CoC 671	100.6	12.6	0.13	235	29.0	0.12
19	Co 8338	49.5	8.0	0.16	-	-	-
20	Co 95020	102.5	11.3	0.11	275	74.0	0.26
21	Co 89003	97.0	14.0	0.14	245	31.0	0.12
22	Co 06022	85.3	22.5	0.26	299	135	0.45
23	Co 92008	106.5	13.5	0.13	344	23.5	0.07
24	Co2001-13	87.5	15.5	0.18	260	43.0	0.16
25	Co86010	122.0	28.4	0.23	320	68.4	0.21
SEd:		315.85	0.8396	0.006	11.17	2.680	0.008
CD:(.05)		635.1	1.6881	0.022	22.46	5.402	0.060
		(NS)	(**)	(**)	(**)	(**)	(**)

genotypic plasticity in response to both biotic and environmental changes. Mouchel et. al. (2004) reported intraspecific variability in root branching and development in *Arabidopsis*.

Biometric traits of sugarcane root system

Root length varied significantly among varieties at 4th and 10th months of crop age (Table 2). Root volume varied from 35ml to 255ml at 4th month while at 10th month it varied from 70ml to 2400ml indicating the root mass accumulation from 4th to 10th month (5 months of growth). The variety (BO 91) with high root volume (2400 ml) also registered higher root weight of 153g (Table 2) and the R^2 value for the root weight/root volume was high (0.783). Water uptake of few varieties showed good relations with root weight and volume. Root traits *viz.*, volume and dry mass seems to have direct relation with water uptake in the varieties studied (Fig. 8).Water uptake ranged from 0.7 litre day^{-1} to 2.4 l day^{-1} among varieties (Fig. 9) at five months of age . The influence of root anatomy on water movement has been studied by comparing the anatomical characteristics of sugarcane cultivars that differ in hydraulic properties (Saliendra and Meinzer, 1992; Rae et al., 2014). In shoot roots of these cultivars, the area of the stele and the ratio between area of stele and area of the cortex vary. The average radius of the metaxylem elements and total area occupied by the meta xylem was found to be related to hydraulic conductance. The innate nature of the varieties for efficient use of water when water is unlimiting, indicates the undisturbed growth of the plants as seen in this study i.e., variety BO 91 with high water uptake also registered high root biomass (Table 3). Root volume increased several fold at 10th month in several Co canes (Table 2). The vigorous root growth and higher biomass partitioning to root as reflected by root/shoot ratio indirectly indicates the efficiency of the varieties to sustain greater above ground structure. Several of the varieties identified as tolerant to drought, registered high water consumption on daily basis (Co 99004, BO 91, CoJ 64, Co 95020 & Co 06022) in stress free condition indicating their efficiency of utilizing available water in both stressed and stress free environments. Variation for root length among cultivars was reported by Spaull (1980), wherein branching frequency did not vary significantly.

Root enzymes

Peroxidase activity ranged from 12.2 to 48.9 units $g^{-1} h^{-1}$ in the varieties and SOD activity ranged from 0.33 units g^{-1} to 1.3 units mg^{-1} protein. The varietal differences were statistically significant (Fig.10). A similar trend was observed with respect to total phenolics level. Higher peroxidase and SOD activities are common observation upon stress imposition. However, in the stress free environment the variations do signify the intrinsic potential to detoxify the system. Wound induced increase in activities of ROS enzymes have been reviewed by several workers. An intense accumulation of phenolic compounds was observed in root tissues grown in soil with addition of copper. The accumulation of the phenolics in plants subjected to heavy metal stress is a defense strategy against oxidation stress caused by reactive oxygen species (Michalak, 2005). The higher ROS enzyme activity and total phenolics, in normal situation might indicate the metabolic activity as well as preparedness for stress situations or it may be adjustment for its growth and survival in hydro culture.

The roots of sugarcane commercial varieties differ significantly for biometric traits i.e., length, number, volume, weight and R/S ratio. The variation among the Co canes suggest the existence of large variability which can improve the cane selection for prolific root system to sustain both plant and succeeding ratoon crops. The mechanical injury caused to roots demonstrated varietal variation for responding to wound and senescence behaviour of injured roots. Further, indepth studies would reveal the rhizosphere characteristics of sugarcane system for future exploitation.

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