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RESEARCH ARTICLE

Low cost high throughput image based root phenotyping pipeline for evaluation of sugarcane root system architecture under drought stress

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Abstract
Root System Architecture (RSA) plays an important role in the agronomic performance of a crop. Incorporation of these root traits in breeding program is hampered by the complexity in accessing the roots and its phenotyping. Lack of high throughput root phenotyping platforms for sugarcane is one of the major constraints in sugarcane root studies. In the present study an attempt was made to develop high throughput sugarcane root phenotyping pipeline comprising of a low cost plant cultivation platform and customized root image acquisition platform and image analyses using already available automated software. PVC tube system of specified dimension were used for plant growth and customized optical correction tank were used for imaging RSA. The acquired root images were fed into automated software GIAroots and about twentyquantitative root phenotype data were extracted and analysed. The working of the whole pipeline from plant growth to image analyses is demonstrated through comparative root phenotyping under drought using five genotypes of sugarcane wild relative *Erianthus arundinaceus* and three commercial sugarcane varieties.The relationships between the different root variables and genotypes in PCA biplots indicated high correlation among the different root traits. The study shows the low cost high throughput image based root phenotyping pipeline can be used to extract quantifiable root traits and analysed within a short span of time.

Keywords : Sugarcane; Root Phenotyping; Drought

Introduction

Root system significantly contributes to plants growth and development, physiological functioning and agronomic performance. They play a crucial role in nutrient and water uptake, translocation of fixed carbon from soil, imparting resistance to soil borne pests and diseases (Ana et al. 2015; René et al. 2015).

Root System Architecture (RSA) comprising of a set of root morphological parameters such as root length, root biomass and root diameter is of great agronomic importance. Among the RSA, long and prolific root system with adaptive plasticity are some of the tolerant root traits selected in a crop breeding program to develop drought tolerant crops (Prince et al. 2017; Lynch 2011, Lynch, 2014). Deeper and prolific root system can leverage upon more underground resources for supplying to the shoot whereas shallow root system can limit the shoot efficiency under drought by supplying limited resources (Narayanan et al. 2014). Incorporation of these root traits in breeding program is hampered by the complexity in root phenotyping.

Sugarcane is an economically important crop for sugar and possesses huge above ground biomass. Most of the commercial sugarcane varieties are characterized by shallow roots which makes them

not only susceptible to below ground constraints also makes unfit for mechanical harvesting (Pierre et al. 2019). Moisture stress is a major constraint faced by the sugarcane root system (Misra et al. 2020; Garcia et al., 2020; Valarmathi et al. 2018a). Sturdy and deep-rooted sugarcane varieties can not only withstand the below ground constraints, they are also most suitable for mechanical harvesting. Therefore, development of climate resilient sugarcane varieties will significantly help the farming community for sustainable sugarcane production. Sugarcane RSA and its important biological function under extreme environmental conditions is still not fully explored. Majority of the research on root system architecture in monocots were focused on crops such as rice and maize (Meister et al. 2014; Smith and De Smet 2012; Richards, 2008).

Due to the ease in phenotyping and accessibility of above ground parts, so far crop improvement program majorly focused on improving above ground traits under extreme climatic conditions for sustainable crop production.The ICAR-Sugarcane Breeding Institute, Coimbatore, India constantly focusses on the development of sugarcane varieties through introgression breeding suitable for various agro-climatic conditions of the country since 1912 (Hemaprabha et al. 2013; Nair 2011; Hemaprabha et al. 2006). Further emphasis on understanding the sugarcane RSA and linking the root traits with shoot traits will help in developing climate resilient sugarcane varieties. However the extensive breeding program on improving root traits suffers from the phenotyping difficulties associated with studying root traits (René et al. 2015). Lack of high throughput root phenotyping platforms for sugarcane is one of the major constraints in sugarcane root studies.

Sugarcane root system is reported to be highly divergent comprising of highly branched sett roots (roots originating from the sett), shoot roots (main roots originating directly from the shoot) and deep rope roots formed by the agglomeration of shoot roots (Lynch, 2014; Valarmathi et al. 2020). The reported root system architecture is poorly explored in sugarcane and how far these root systems are present in the modern day cultivars is also poorly known. Therefore, understanding the sugarcane root architecture is essential to develop genotypes with better absorption and soil-plant continuum thereby resulting in improved yield under adverse conditions.

On the other hand the natural variation existing in plasticity of root system functions in the wild germplasm is necessary to understand and incorporate them in the varietal improvement program. ICAR-Sugarcane Breeding Institute, Coimbatore, India endowed with large collection of sugarcane germplasm is recognised as ‘World Collection’ by the International Society of Sugarcane Technologists (ISSCT). These wild germplasm are known for high biomass, deep rooting, and various biotic and abiotic stress tolerance. Our own study on the large scale drought screening of *Erianthus* germplasm belonging to allied genera of sugarcane led to the identification of drought tolerant genotypes (Valarmathi et al. 2018a). Utilization of these germplasm root traits requires extensive phenotyping data.

With this background the present study was undertaken to develop highthroughput root phenotyping pipeline for automated data generation of sugarcane RSA. The study involves establishment of low cost plant growing platform and customized root image acquisition platform. The acquired root images were fed into semi-automated software and quantitative traits on root architecture were extracted and analysed. To demonstrate the working of the root phenotyping pipeline a comparative root phenotyping under drought and control condition was carried out using five genotypes of wild relative *Erianthus arundinaceus* and three genotypes of commercial sugarcane variety. This is the

first report on the establishment and validation of highthroughput low cost image based root phenotyping pipeline for sugarcane RSA.

Materials and Methods

Establishment of plant cultivation system and customized root imaging platform

The PVC tubes were used for the establishment of low cost plant cultivation platforms. PVC tubes with dimension of 45 cm tall and 7 cm diameterwere prepared and filled with soil and sand in 2:1 ratio. The base of the PVC tubes were sealed with autoclavable covers and the tubes were placed vertically in uniform height with the support of bricks (Fig. 1). The arrangement was kept under a rainout shelter in order to enable drought screening. Uniform settlings in three replications were planted and irrigated with measured and equal quantity of water.

For root imaging the plants were placed in a cylinder of 60 cm tall x 10 cm diameter and the set

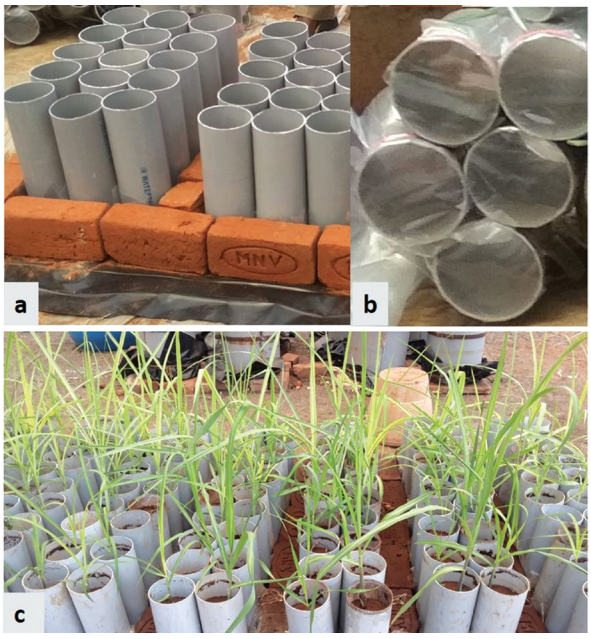

Figure. 1 PVC tubes used for plant cultivation, a. Set up showing the empty PVC tubes, b. PVC tubes covered at the base, c. Tubes after 45 days of sugarcane settling planting

up containing test tube and plant was kept in an optical correction tank (Fig. 2).The customized rectangular optical correction tank with dimension 80 x 20cm was filled with water and introduced into the imaging set up to correct optical refraction of the curved surface of the cylinder. The optical correction tank with a movable lid and the imaging side was made using extra polish glass plate. All other sides of the imaging tank was made with normal glass and covered with black background to avoid reflection.

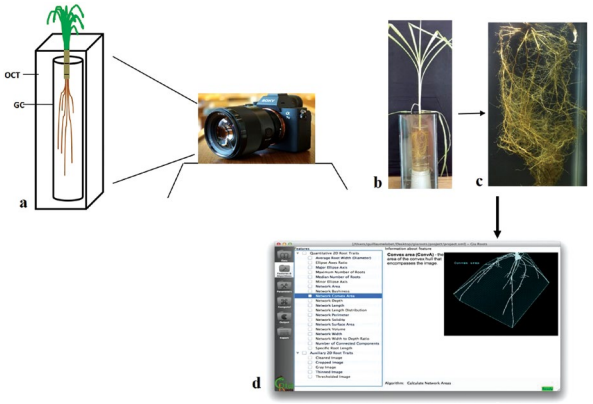

Figure. 2 Root imaging platform used for phenotyping root system architecture. a. Schematic diagram showing the imaging platform including glass cylinder (GC), optical correction tank (OCT) and camera aligned transversely at I m distance, b. The actual imaging platform set up, c. the 2D root images downloaded from the camera, d. Software screen showing extraction of quatitative root traits using the image

Imaging, data acquisition and analysis

For root imaging the tank was filled with water and the root samples kept in the cylinder were placed in the middle of the tank with help of holder. A destructive root sampling was done and the root samples were subjected to imaging using a fixed Nikon Digital SLR camera D5600 AF-P DX NIKKOR 18-55mm f/3.5-5.6G VR lens. The camera was aligned transverse to the custom-developed rectangular optical correction tank at a distance of 1m and the root samples were imaged. The captured multiple 2D images in three replica-

tion for each plant were processed identically. The images were identically cropped and converted to a resolution of 300 mm per pixel and fed into the semi-automated root image analysing software GiA Roots (General Image Analysis of Roots) (Galkovskyi et al. 2012). Using the 2D images twenty quantitative data on root system architecture were extracted from the software GiA Roots (Table.1).

Genetic material and drought exposure

Five genotypes of *Erianthus arundinaceus* (SES 288, IND 01-1091, IND 01-1099, IND 04-1335 and IND 04-1338) along with three commercial sugarcane varieties (Co 775, Co 86032 and Co 0212) were used in the study. Healthy single budded setts of *E. arundinaceus* and commercial variety were planted in portrays. Two weeks after germination of setts five uniform settlings were transplanted in PVC tubes (One settling per tube) per replication. Forty five days after planting the plants were exposed to drought stress for 15 days by withholding irrigation. A set of corresponding controls were maintained with regular watering. On 16th day destructive sampling was done and the plant roots were imaged.

Morphological and physiological data collection and monitoring of drought

Morphological drought symptoms were monitored through visual scoring of leaf drying. Leaf drying was measured manually and scored based on the percentage of dried leaf under drought stress. Physiological data on the leaf relative water content (RWC), canopy temperature and chlorophyll fluorescence were recorded during drought stress and compared with that of control plants. For measuring RWC fresh weight (FW) of leaf bits were recorded and then the leaf bits were saturated in distil water for 5hr to record the turgid weight (TW). Subsequently leaf bits were dried in oven at 65˚C for 48 hr and dry weight (DW) was recorded.The RWC was calculated from the equation of Schonfeld et al. (1988). Canopy temperature and chlorophyll fluorescence was measured using the chlorophyll fluorometer OS1P^{+}(OPTI-SCIENCES, USA).

Statistical Analysis

The recorded data were analysed statistically, central tendency and distribution of the variables were worked out. Principle component Analysis (PCA) was carried out individually for drought and normal conditions, using R packages (https://www.r-project.org/).

Results and Discussion

The current agricultural production system demands genotypes which are highly productive through efficient resource capture from the soil under extreme climatic conditions. Roots play an important role in capturing the underground resources. Recent studies have focused on understanding the root system architecture of the plant, plasticity of root growth and development and natural variation existing in RSA to identify beneficial/tolerant root traits which can be utilized to improve plant productivity (Lynch and Wojciechowski 2015; Ana et al. 2015; Valarmathi et al., 2018b; Valarmathi et al. 2020). It is also realized that improving RSA will result in better improvement in plant productivity (Lynch 1995; Grossman and Rice 2012; Kano et al. 2011). The major constraints with all the root studies were the difficulty in accessing the rhizosphere and phenotyping (Lynch 2014; Sato et al. 2014).

There are many challenges to understand the genetic and developmental basis of RSA in any crop. In order to address these challenges in understanding basic root development or high throughput superior root trait selection for breeding, different combinations of lab based, green house based and

Table 1. Twenty quantitative root traits extracted from the 2D images using GiA Roots

Traits		Co 775	Co 86032	Co 0212	SES 288	IND 1091	IND1099	IND 04 1335	IND 04 1338	Mean	SEM	MSS	MSS Int	C.D.	CD. int
Number of Connected Components	Control	124	211	302	601	427	499	687	456	413.375	0.408	5874.188**	9165.188**	1.181	3.341
	Drought	120	216	228	783	486	447	717	487	435.5	0.816	270527.3**		2.363	
Maximum Number of Roots	Control	24	33	27	74	66	60	72	68	52.667	0.635	374.083**	70.179**	1.837	5.194
	Drought	20	28	28	89	74	65	82	72	58.25	1.269	3546.75**		3.673	
Median Number of Roots	Control	21	24	27	56	17	40	35	42	32.75	0.527	212.521**	193.045**	1.525	4.314
	Drought	20	17	18	66	43	37	37	39	36.958	1.054	1120.688**		3.05	
Network Convex Area	Control	22.29	78.34	76.73	203.59	121.12	202.94	170.86	234.91	140.611	0.446	1416.253**	9272.107**	1.29	3.65
	Drought	30.43	52.33	27.21	254.48	242.44	214.37	258.86	109.72	151.475	0.892	40421.47**		2.581	
Network Surface Area	Control	30.54	35.18	44.8	89.45	72.56	69.78	92	79.48	64.224	0.416	41.218**	13.429**	1.205	3.409
	Drought	31.96	33.5	43.54	94.59	78.94	71.45	95.68	78.94	66.075	0.833	3791.675**		2.41	
Network Perimeter	Control	1227.03	1321.6	1676.99	3525.91	1336.57	3179.3	3123.83	3531.81	2365.376	1.037	97015.17**	394540.6**	3.001	8.488
	Drought	1253.89	1376.21	1671.34	3628.92	1986.54	2328.05	3979.14	3418.24	2455.291	2.074	6440226**		6.002	

Table 1. Twenty quantitative root traits extracted from the 2D images using GiA Roots (Cont...)

Traits		Co 775	Co 86032	Co 0212	SES 288	IND 1091	IND1099	IND 04 1335	IND 04 1338	Mean	SEM	MSS	MSS Int	C.D.	CD. int
Network Length	Control	1135.41	1260.14	1238.64	3258.09	2865.74	3120.71	3655.86	2953.16	2435.969	0.268	148789.7**	49492.77**	0.775	2.193
	Drought	1183.03	1237.26	1228.6	3666.49	3173.39	2990.05	3743.92	3155.65	2547.299	0.536	7044099**		1.551	
Specific Root Length	Control	1256.67	1497.42	1919.04	4253.33	3200.99	2838.56	4291.08	3668.26	2865.668	1.027	701079.9**	225893.5**	2.971	8.403
	Drought	1045.99	1609.95	1671.3	4651.03	3679.14	3787.47	4438.89	3974.93	3107.337	2.053	10198512**		5.942	
Average Root Width (Diameter)	Control	0.007	0.012	0.013	0.023	0.028	0.024	0.027	0.024	0.02	0.001	0.001**	0.0001ns	0.004	-
	Drought	0.006	0.011	0.011	0.025	0.023	0.024	0.026	0.023	0.012	0.003	0.001**		0.008	
Ellipse Axes Ratio	Control	0.24	0.31	0.27	0.59	0.5	0.52	0.58	0.52	0.458	0.008	0.011**	0.001	0.023	-
	Drought	0.21	0.3	0.28	0.64	0.53	0.51	0.61	0.57	0.489	0.016	0.144**		0.045	
Network Length Distribution	Control	0.57	0.61	0.47	0.95	0.96	0.89	0.89	0.97	0.879	0.017	0.024ns	0.013 ns	-	-
	Drought	0.57	0.63	0.47	1.21	1.02	0.88	0.95	1.1	0.924	0.034	0.327**		0.1	
Network Volume	Control	0.16	0.19	0.24	0.76	0.65	0.48	0.71	0.54	0.49	0.004	0.001ns	0.003**	-	0.035
	Drought	0.12	0.18	0.23	0.75	0.64	0.59	0.74	0.52	0.495	0.008	0.369**		0.025	
Network Solidity	Control	0.18	0.2	0.23	0.42	0.34	0.36	0.48	0.46	0.334	0.007	0.008**	0.001**	0.019	-
	Drought	0.2	0.19	0.25	0.47	0.38	0.34	0.5	0.43	0.36	0.013	0.085**		0.038	

Table 1. Twenty quantitative root traits extracted from the 2D images using GiA Roots (Cont...)

Traits		Co 775	Co 86032	Co 0212	SES 288	IND 1091	IND1099	IND 04 1335	IND 04 1338	Mean	SEM	MSS	MSS Int	C.D.	CD. int
Network Width to Depth Ratio	Control	0.22	0.3	0.42	0.76	0.49	0.72	0.75	0.74	0.573	0.009	0.019**	0.006*	0.027	0.076
	Drought	0.21	0.3	0.34	0.8	0.52	0.66	0.87	0.78	0.613	0.019	0.334**		0.054	
Network Bush-iness	Control	1.43	1.38	1.47	3.32	2.29	2.48	2.45	2.36	2.514	0.086	0.165ns	0.07 ns	-	-
	Drought	1.25	1.38	1.47	3.46	2.53	2.3	2.88	2.6	2.397	0.172	3.202**		0.499	
Network Width	Control	1.1	5.77	5.92	11.51	10.67	10.89	12.25	10.17	8.762	0.047	29.265**	7.179**	0.137	0.387
	Drought	1.04	5.47	5.69	16.63	12.36	11.78	17.15	12.2	10.323	0.094	135.345**		0.273	
Minor Ellipse Axis	Control	3.73	5.63	5.98	13.44	12.27	10.74	16.83	9.54	10.137	0.171	17.327**	2.592**	0.494	1.397
	Drought	3.11	5.13	5.03	16.38	13.39	10.2	17.77	10.47	11.338	0.341	146.117**		0.988	
Network Depth	Control	4.41	5.81	6.47	22.74	16.46	22.59	24.86	26.99	17.671	0.319	43.281**	8.539**	0.922	2.607
	Drought	4.21	5.28	6.51	27.26	21.2	21.66	28.46	27.61	19.57	0.637	596.336**		1.844	
Major Ellipse Axis	Control	8.16	13.97	14.42	30.29	21.75	19.08	26.77	29.47	20.549	0.402	89.876**	8.537ns	1.163	-
	Drought	8.06	13.71	15.23	31.51	23.55	17.01	32.62	28.54	23.285	0.804	432.505**		2.325	
Network Area	Control	3.41	5.42	9.94	26.8	22.81	32.89	29.9	28.09	21.738	0.337	1.329ns	22.566**	-	2.755
	Drought	5.7	6.78	7.34	27.72	26.69	24.35	31.42	27.16	21.405	0.673	755.793**		1.948	

field based root phenotyping platforms has been tried in several crops (Clark et al. 2011;Shashidhar et al. 2012; Kanbar et al., 2004). However high throughput root phenotyping for sugarcane is still unexplored and a large knowledge gap exists among understanding sugarcane RSA under irrigated as well as under extreme climatic conditions. The focus of this study was to establish a high throughput root phenotyping pipeline involving low cost cultivation system, root imaging platform, using the 2D images extraction of quantitative root traits through semi-automated software and analyses.

Plant cultivation in PVC tubes to study sugarcane RSA

As the first step, a low cost plant cultivation system using the PVC tubes were established for high throughput phenotyping of sugarcane RSA. The platform was successfully used to grow sugarcane settlings for a period of 60 days. Fifteen days old settlings were transplanted in PVC tubes, drought stress was imposed after 45 days by withholding irrigation and successfully root phenotyping was carried out after 15 days of drought exposure. This is the first study on high throughput phenotyping of sugarcane RSA using PVC tubes, whereas it is extensively used in rice root phenotyping to some extent in other crops such as sorghum and maize (Kanbar et al. 2002; Venuprasad et al. 2002; Kanbar et al. 2004; Shashidhar et al. 2012).

A PVC tube with a dimension of 1m length and 18-20cm diameter is used to screen rice from seedling to maturity phase (Shashidhar et al. 2012).

For sorghum and maize PVC tubes of 150cm in length and 25cm in diameter were used (Shashidhar et al., 2012).The desirable properties of a plant cultivation system should be useful in collecting data of agronomic relevance and the ability to grow plants at any developmental stage of the crop. PVC tubes serves as a useful plant cultivation system for high throughput data collection on various plant traits including root. The dimension of the PVC tubes used in the study can be modified depending upon the growth stage to be screened. The dimension of 45x 7cm was found to be appropriate for screening of settlings upto 60 days. The PVC tubes of 150cm long and 30 cm diameter were used for screening sugarcane at the tillering phase (upto 120 days) (Valarmathi et al. 2018a). The PVC tube experiment with soil as the medium can be modified according to the developmental stage of sugarcane. Phenotyping for trait of agronomic relevance soil is the preferred plant cultivation medium, in order to mimic the field conditions (Hargreaves et al., 2009; Clark et al. 2011).

Drought exposure, root imaging using customized platform and data extraction using automated software

Morphological leaf rolling and lower leaf drying was observed after 15 days of drought exposure in commercial sugarcane varieties Co 775, Co 0212 and Co 86032, while *Erianthus* germplasm showed only leaf rolling. Lower canopy leaf temperature (24°C -26°C), higher leaf (fully opened third leaf) relative water content (75- 80%) and high chlorophyll fluorescence (Fv/Fm, 0.6-0.7) were recorded in *Erianthus* whereas the commercial sugarcane genotypes recorded higher canopy leaf temperature of 28°C - 30°C, lower leaf relative water content of 60- 65% and low chlorophyll fluorescence (Fv/Fm) of 0.3-0.4 in stress exposed plants. The genotypes of *Erianthus* and commercial sugarcane genotypes under control recorded canopy leaf temperature of 22°C -24°C,

leaf relative water content of 80- 85% and chlorophyll fluorescence (Fv/Fm) in the range of 0.7-0.8. There was no difference in the physiological parameters among the clones of *Erianthus* and commercial sugarcane genotypes under fully irrigated conditions. Canopy leaf temperature, leaf relative water content and high chlorophyll fluorescence (Fv/Fm) are some of the important physiological traits which gives a clear reflection of drought responses of a genotype (Siddique et al. 2000; Anjum et al., 2011). Low canopy temperature, higher leaf relative water content and higher chlorophyll fluorescence (Fv/Fm) recorded in *Erianthus* genotypes indicates the drought tolerance mechanism of these genotypes with better regulation on photosynthetic rate as well as transpirational cooling under drought stress conditions.

Fifteen days drought stressed plants were subjected to destructive sampling for root phenotyping along with control plants. A desirable property of any imaging technology is clear resolution and should allow optimal data acquisition. Many of the newly developed high-throughput root phenotyping platforms in crops such as rice and maize use 2D images to extract large number of quantitative data (Le Marie et al. 2014;Clark et al. 2013; Burton et al. 2012). The major advantage of 2D imaging is high throughput phenotyping and the possibility of measuring several root parameters without manual intervention. A 2D imaging has been used with cameras or flatbed root scanners. In this study, root samples were imaged using high resolution Nikon Digital SLR camera aligned with an optical correction tank. The images were processed uniformly and fed into the semi-automated "GiARoots" software using default settings.

GiA Roots (General Image Analysis of Roots) is a semi-automated software used for high-throughput analysis of root system images. The software through user-assisted algorithms distinguish root from background noise and serves as a fully automated pipeline that extracts dozens of quantitative root system phenotypes. Quantitative information on each phenotype, along with intermediate steps is returned to the end-user for full reproducibility. Using this software twenty root traits were obtained from the output and stastically analysed. This phenotyping pipeline used to image can be used along with any automated software developed for high throughput imaged based anlyses of root traits.

Genetic variation measured among different root traits

The twenty root traits extracted from the software were statastically analysed. The various root traits among *Erinathus* genotypes and commercial sugarcane genotypes were significantly varied (Table.1). Majority of the root traits were found to be significantly superior in all the *Erianthus* genotypes compared to that of commercial sugarcane varieties. Among *Erianthus* genotypes SES 288 and IND 04-1335 showed better root traits under drought condition (Table 1). The PCA revealed the diversity among the genotypes studied for the RSA traits. Under drought, 95% of the total variability was explained by the first two PCs, similarly under normal condition 93 % of the total variability was explained by the first two PCs (Table 2 and Fig. 3).

The variables contributed almost equally for 1PC under both normal and drought conditions (Table. 2), while for the 2PC MeNR and NP are the major contributors under normal and the traits NP, NCA and MeNR contributed more under drought conditions. The relationships between the different variables and genotypes with respective principal components are illustrated by the principal

Table 2. Eigenvalues, and percent of variance explained by principal components

PCs	Control			Drought		
	Eigen value	Variance percent	Cumulative variance percent	Eeigen value	Variance percent	cumulative variance percent
Dim.1	17.61	88.04	88.04	18.60	93.00	93.00
Dim.2	1.18	5.88	93.92	0.54	2.72	95.72
Dim.3	0.44	2.22	96.14	0.37	1.87	97.59
Dim.4	0.35	1.75	97.89	0.28	1.38	98.97
Dim.5	0.22	1.12	99.01	0.09	0.46	99.42
Dim.6	0.15	0.74	99.75	0.08	0.38	99.80
Dim.7	0.05	0.25	100.00	0.04	0.20	100.00

component biplots (Fig. 3) for the stressed and normal conditions respectively. Smaller angles between dimension vectors in the same direction indicated high correlation of the traits in terms of discriminating genotypes. Genotypes excelling in a particular trait were plotted closer to the vector line and further in the direction of that particular vector. Under both normal and drought stress, the *Erianthus* genotypes were found to be scattered in the positive side of the first principal component, the dispersion or scattering of the genotypes were more in drought in comparison to normal condition with most of the RSA traits while commercial varieties grouped together (Fig.3 and Fig.4).

A similar grouping pattern was observed among the commercial genotypes. Even though the susceptible and tolerant genotypes grouped together, the dispersion of genotypes were more under drought conditions. The PCA analysis using the root phenotypic data were highly informative and the root phenotyping pipeline can be used as a rapid low cost high throughput screening platform.

Conclusion

The working of a low cost root phenotyping pipeline for sugarcane from plant growth to image analyses has been successfully demonstrated by a comparative root phenotyping study under drought

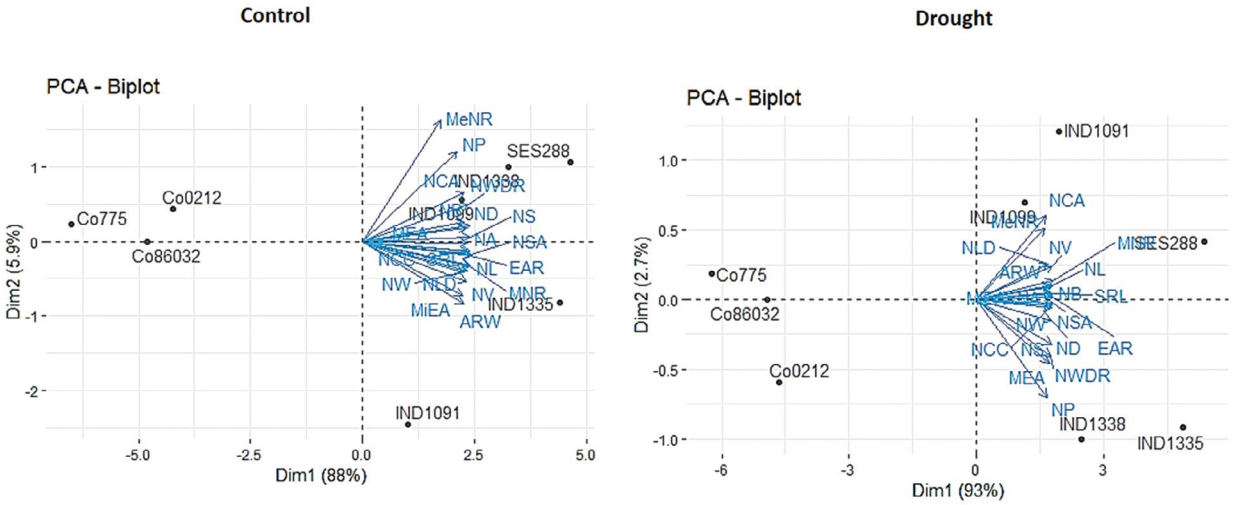

Figure. 3 PVC biplot showing the behavior of genotypes and variables under drought and control conditions

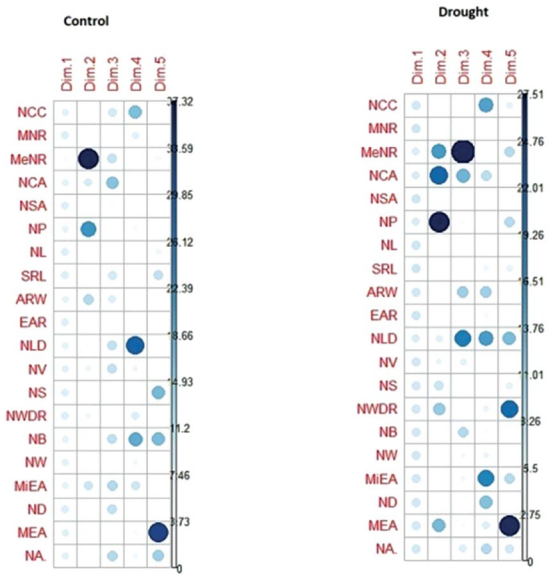

Figure. 4 Contribution of different Variable to principal components under drought and control conditions

with sugarcane wild relative *Erianthus arundinaceus* and genotypes of commercial sugarcane variety. The relationships between the different root variables and genotypes with respective principal components by the principal component biplots indicated high correlation of the traits in terms of discriminating genotypes.The root phenotyping pipeline can be used as a rapid low cost high throughput root screening platform in sugarcane.

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