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Assessment of Genetic Diversity in Mid-late Maturing Sugarcane Clones under Waterlogging Condition in Lower Indo-gangetic Plains

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A B S T R A C T

The lower Indo-gangetic plains of India comprising of eastern Uttar Pradesh, Bihar and West Bengal are frequently flooded by tributaries of Ganga during monsoon and there is a need to provide waterlog stress resilient sugarcane clones to farmers for cultivation. Assessment of genetic diversity is important in any breeding endeavour to achieve success. Sixteen sugarcane clones of mid-late maturity groups were evaluated for twenty three different morphological and physiological characters in the year 2017-18. The experiment was carried out at Paddy Block of Research Farm, R.P.C.A.U.,Pusa, Samastipur, Bihar which is situated alongside the bank of river BurhiGandak, a tributary of river Ganga. The field remained waterlogged during monsoon with maximum depth of water being 140cm.Mahalnobis D^2 distances were computed and clusters were formed using tochers method which grouped genotypes in 6 clusters with cluster 5 and 6 having single genotypes. The lowest intra-cluster distance value of zero was observed for cluster 5 and 6 as they contained dingle genotype and highest value of intra-cluster distance was observed for cluster 2. The largest inter-cluster distance was observed between cluster 2 and 5 and lowest between cluster 1 and 3. Characters such as chlorophyll b content, chlorophyll a content and total chlorophyll content showed largest contribution towards divergence. Cluster 5 containing a single genotype CoP2061 had highest mean value for cane and sugar yield followed by cluster 1 and 3. Thus, genotypes in these clusters can be used in further breeding programmes. Similarly other genotypes in other clusters may well be used for targeting a particular trait in a particular environment in ideotype breeding programmes.

Introduction

Sugarcane is the world's leading crop in terms of harvested tonnage and a widely grown crop in tropical and subtropical areas of the world. Sugarcane provides sugar and many more products such as gur, khandsari, molasses, pressmud and Bagasse. Ethanol extracted from molasses can be used as fuel for transport system. Pressmud can be used as bio-compost to enrich soils and bagasse can be used for generation of electricity. India

produced about 376.9 million tonnes of sugarcane in an area of 4.73 million hectares in the year 2017-18 (Directorate of Economics and Statistics, Government of India). The sugarcane production in Bihar was around 165.11 lakh tonnes with the productivity around 67.9 tonnes per hectare in 2017.

A large part of India consisting of Assam, Bihar, West Bengal, Eastern U.P, coastal regions of Andhra Pradesh, Tamil Nadu, Kerala and Karnataka are exposed to stagnant water for two or more months during rainy season. India is one of the centres of origin of sugarcane and thus, gene pool from this region may have vital genes for waterlogging tolerance. Genomics has made rapid advances and sequencing of sugarcane genome would identify alleles in this crop that may be beneficial in crop improvement. Identification of genes and alleles conferring waterlogging stress tolerance would require identification of genotypes that perform better under waterlog stress conditions and its consequential use in creation of mapping populations or genome sequencing. Zhang *et al.,* (2018) has reported sequencing of haploid *Saccharum spontaneum* line AP85-441.

Sugarcane is a major crop in North West Alluvial agro-climatic zone of Bihar and all the districts except Vaishali and Begusarai in this zone, were affected by floods in the year 2017.The estimated crop damage in monetary value was pegged at 6858.7 million (95.96 million USD) due to flood affected cropped area of 87.3 million hectare in flood report 2017 of Bihar. "The rivers namely Ghaghra, Gandak, BurhiGandak, Bagmati, Kamla, Adhwara group of rivers, Kosi and Mahanada have Himalayan origin and have considerable portion of their catchment in the galcial region falling in Nepal and Tibet, and are therefore positioned to receive very copious rainfall during monsoon when discharge of these rivers is 50 to 90 times larger than fair weather flow. This causes frequent and large scale flooding of North Bihar. As such, 73.63 percent of the geographical area of North Bihar is considered to be prone to floods (The State Disaster Management Plan, Perspective)". The districts in North Bihar, the major sugarcane producing area of the state, are regularly affected by floods.

A successful breeding programme needs to identify genotypes that may be used in breeding programmes and classificatory analysis such as D^2statistics clusters genotypes and enables to group similar genotypes so as to understand the extent of similarity or dissimilarity to make informed decision on the crosses to be made for achieving a particular objective in plant breeding, in this case breeding tolerance to waterlogging conditions. Diversity analysis has been commonly used by breeders across species under different environments however few studies have been done prior to this study in lower gangetic plains though the area is greatly affected by floods on an yearly basis. Furthermore, the study becomes significant as the crop was under severe waterlogging stress as a result of severe flooding in the year 2017.

Materials and Methods

Experimental layout and materials

The experiment was laid out in Randomized complete block design with three replications. The description of genotypes is given in table 1. The recommended package of practices for agro-climatic region of Bihar, were followed.

Experimental site topography and climate

The experiment was conducted at Paddy Block of Research Farm of R.P.C.A.U. Pusa, Samastipur, Bihar situated between 25.97^0 N latitude and 85.66^0 E longitudes at 51.8 m

above mean sea level. The plot which was well levelled remained waterlogged during monsoon as it was a lowland area alongside the river BurhiGandak. The maximum depth of water was about 140 cm for 30 days in August-September.

Observations recorded

Twenty three different morphological, biochemical and juice quality parameters *viz.* germination percentage at 45 DAP, number of shoots at 120 DAP, plant height at harvest (cm), leaf area per plant before and after waterlogging, number of nodes with aerial roots, cane diameter at harvest (cm), number of shoots at 240 DAP (000/ha), number of millable canes at harvest (000/ha), single cane weight (kg), Brix, Pol and Purity at 10&12 months stage (%), cane yield (t/ ha), CCS % at 10 and 12 months stage, sugar yield (CCS t/ha) at harvest, chlorophyll "a" content, chlorophyll "b" content and total chlorophyll content were studied and observations were recorded. Leaf area per plant was obtained as the product of breadth at the broadest part of leaf and length of the leaf with the factor 0.6274 (Bathla and Sharma, 1978). The leaf area per plant was obtained by summation of leaf area of all the leaves.

A sample consisting of five randomly selected cane stalks were crushed in a cane crusher and the juice obtained was poured in graduated measuring cylinders of 500 ml and brix hydrometer was suspended in this cylinder. The Brix reading was recorded when the brix hydrometer stopped oscillating in the cylinder. To obtain Pol reading, 100 ml juice of each sample was taken in a beaker and about 1-1.5g of basic anhydrous lead acetate was added to it. The mixture was then stirred and kept for some time so as to precipitate non soluble substance. The precipitated impurities were filtered off and clear filtrate juice was collected. The clear filtered juice was filled in 20 cm long polarimeter tube. This tube was placed in the body of polarimeter and pol reading was recorded. Schmitz table (Spencer and Meade, 1955), was used to note the sucrose percent in juice using corresponding values of the brix and Pol reading.

The percentage of sugar in total solid is called purity percentage. The juice purity percentage was calculated by using the formula; Purity Percent (%) = $\frac{Pol\ in\ juice}{Juice\ Brix} \times 100$. The cane yield was taken at the time of harvesting. Cane yield (t/ha) was recorded by harvesting and weighing the all canes in a plot and the values then converted into tonnes/ha. CCS %was estimated from sucrose in juice and brix reading using the formula; CCS percent = [S-(B-S) × 0.4] × 0.73, where S is Sucrose percent in juice (Pol %) and B isBrix percent in juice. Chlorophyll content measurement was done using DMSO as described by Hiscox and Israelstam, 1979. Three to five plants were sampled for observations.

Statistical Analysis

The divergence among sugarcane clones was estimated through the use of Mahalanobis Generalized Distance (Mahalnobis, 1928) as a measure of genetic dissimilarity and the genotypes were clustered using Tocher method as suggested by Rao (1952).The contribution of characters towards divergence was computed as given by Singh and Choudhary.

Results and Discussion

The clustering of genotypes following tochers method grouped the genotypes in 6 clusters as shown in table 1. Genotypes CoP 09437, CoP 15439, CoP15440, B.O 156 and B.O 91 were present in cluster 1, clones CoP 12438, CoP 12439, CoP 11439 andCoP 14439 formed cluster 2, clones B.O 155, CoP 14438 and CoP 16439 became part of cluster 3, clones

B.O 154 and CoP16440 formed cluster 4. Cluster 5 and 6 contained one genotype each i.e CoP2061 and CoP15441 respectively.

Inter and Intra cluster distances has been shown in table 3. Intra-cluster distances present on diagonal of table depicts lowest intra-cluster distance value of zero for cluster 5 and 6 while highest value of intra-cluster distance was observed for cluster 2. The largest inter-cluster distance was observed between cluster 2 and 5 and lowest between cluster 1 and 3. All inter cluster distance was found to be between these two extremes.

Cluster means for all the characters studied along with population mean for the charcter has been presented in table 4. The abbreviation used for the characters studied has been presented in column 3 of table 4. Cluster 1 had larger mean value than population mean for NAR, S240, NMC, B10M, P10M, PU10M, CCS10, B12M, P12M, PU12M, CCS12M, CY and SY, whereas cluster 2 had lower mean values for most of the studied characters except G%, S120, S240, SCW, B12M and PH. Cluster 3 had higher mean than population mean for most of the characters studied except G%, NMC, B10M, P10M, B10M, P10M and PU10M. The genotypes in cluster 4 had higher average mean than population mean for G%, LAPPAW, NAR, CD, S240, SCW, PU12M, CHLa, CHLb and TCHL.. Cluster 5 containing a single genotype CoP2061 had higher mean values for almost all the characters except LAPPBW, NAR, PU12M and CHLb whereas cluster 6 having a single genotype had lower mean values for G%, S120, LAPPBW, LAPPAW, CD, S240, NMC, SCW, CCS12M, CY, SY, CHLb and PH than population mean among the characters studied. Cluster 5, 1 and 3 had higher higher mean value than population mean for cane yield and sugar yield in descending order.

The contribution of individual characters towards divergence is shown in table 5. It can be seen that chlorophyll b content, chlorophyll a content and total chlorophyll content had largest contribution towards divergence. Cane yield followed by purity at 12 months had small contribution towards divergence. Number of shoots at 240 DAP and number of millable canes (000/ha) had negligible contribution towards divergence.

The clustering of the studied genotypes based on Mahalnobis D^2 distances showed that genotypes can be grouped into 6 clusters as shown in table 2. Cluster 1 contains 5 genotypes followed by cluster 2, 3 and 4 with 4, 3 and 2 genotypes respectively.

Clusters 5 and 6 contains one genotype each suggesting that these genotypes were too different from other genotypes with respect to the characters studied to be clustered within any of them. Clone BO91 has been an elite sugarcane variety that has been used in breeding programmes world over. BO91 along with its half sib CoP09437 finds place in cluster 1 whereas CoP12438 and CoP 14438 are present in clusters 2 and 3 respectively.

The largest intra cluster distance was observed for cluster 2 suggesting that genotypes within this group had more variability compared to genotypes within other groups. The value zero is seen for intra-cluster distance of cluster 5 and 6 as these clusters contained a single genotype. The largest inter-cluster distance was observed between cluster 2 and 5 and lowest between cluster 1 and 3 suggesting that these clusters were highly divergent and most similar with respect to the studied characters respectively.

Cane yield and sugar yield are the most important characters from breeder's perspective as farmers and industry want

increased productivity. It can be seen that cluster 5 containing single genotype CoP2061 had highest values for cane and sugar yield followed by cluster 1 and 3. Thus genotypes in these clusters can be used in further breeding programmes. The standard practice in breeding to obtain high amount of variability in segregating generations is to cross most divergent plants and screen the segregating populations to obtain transgressive segregants. Thus it would be advisable to cross genotypes in cluster 2 with CoP2061 forming cluster 5 to obtain large variability for yield in segregating populations and screen for transgressive segregants for yield under waterlogged condition. Sugarcane is a highly heterozygous crop and genotypes being vegetatively propagated contains large reserves of potential variability. Thus, even selfing of clones can result in enough variability to breed new varieties. Genotypes of cluster 5, 2 and 3 can be intermated to breed for high yield under waterlogging condition as they probably contain high yield QTLs as shown by their high mean values for cane and sugar yield.

Table.1 Clones used in the study, their parentage and source

Sl. No.	Clones	Parent	Source
1.	BO 154	CoSe98235 X UP 9742	S.R.I. Pusa
2.	BO 155	BO 122 FC	S.R.I. Pusa
3.	BO 156	BO 150 (self)	S.R.I. Pusa
4.	BO 91	BO 55X BO43	S.R.I. Pusa
5.	CoP 09437	BO 91 GC	S.R.I. Pusa
6.	CoP 11439	CO 88039 GC	S.R.I. Pusa
7.	CoP 12438	BO 91 X CO 1158	S.R.I. Pusa
8.	CoP 12439	BO 97 XCoH 15	S.R.I. Pusa
9.	CoP 14438	BO 91 GC	S.R.I. Pusa
10.	CoP 14439	Co Pant 90223 GC	S.R.I. Pusa
11.	CoP 15439	CoSe 01268 GC	S.R.I. Pusa
12.	CoP 15440	CoSe 01268 GC	S.R.I. Pusa
13.	CoP 15441	CO 88216 GC	S.R.I. Pusa
14.	CoP 16439	CoSe 92423 X CoS 8436	S.R.I. Pusa
15.	CoP 16440	CoS 86216	S.R.I. Pusa
16.	CoP 2061	CoLk8102 X HR 83/65	S.R.I. Pusa

Table.2 Inter and Intra-cluster distances

Cluster	1	2	3	4	5	6
1	1209.178					
2	19456.74	2885.932				
3	6150.289	6792.781	752.8985			
4	37452.73	106242.5	69855.26	1366.228		
5	63967.15	149615.5	105528.4	5661.313	0	
6	6792.781	46130.92	23354.06	13560.34	30781.73	0

Table.3 Cluster and population means for different characters

S.No	Characters	Abbreviation used	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Population Mean
1	Germination percentage at 45 DAS	G%	41.38	44.92	41.93	43.44	46.00	35.11	42.52
2	Number of shoots at 120 DAP (000/ha)	S120	60.15	74.42	64.10	54.86	76.54	24.85	62.62
3	Leaf area per plant before waterlogging (cm^2)	LAPPBW	1460.73	1603.18	2175.58	1970.49	1384.60	1029.29	1662.37
4	Leaf area per plant after waterlogging (cm^2)	LAPPAW	1715.19	1711.17	2160.87	1947.27	2190.08	1249.36	1827.33
5	Number of nodes with aerial roots	NAR	7.30	6.73	7.39	7.25	5.75	7.83	7.10
6	Cane diameter at harvest(cm)	CD	2.25	2.20	2.40	2.44	2.48	2.30	2.31
7	Number of shoots at 240 DAP(000/ha)	S240	101.21	100.85	101.66	95.75	112.62	69.20	99.24
8	Number of millable canes (000/ha)	NMC	88.66	83.32	83.32	75.11	100.83	63.42	83.81
9	Single cane weight (Kg)	SCW	0.683	0.670	0.702	0.727	0.843	0.680	0.699
10	Brix at 10 months	B10M	17.3	17.2	17.1	17.1	17.9	17.9	17.3
11	Pol at 10 months	P10M	15.02	14.78	14.83	14.83	15.54	15.56	14.97
12	Purity at 10 months	PU10M	87.1	86.0	86.8	86.6	87.0	87.1	86.7
13	CCS% at 10 months	CCS10	10.30	10.09	10.17	10.16	10.66	10.68	10.25
14	Brix at 12 months	B12M	19.0	18.8	19.0	18.7	19.9	19.7	19.0
15	Pol at 12 months	P12M	16.76	16.42	16.77	16.45	17.41	17.58	16.73
16	Purity at 12 months	PU12M	88.3	87.6	88.4	88.1	87.7	89.2	88.1
17	CCS% at 12 months	CCS12M	11.58	11.30	11.60	11.36	12.00	12.21	11.55
18	Cane yield(tonnes/ha)	CY	59.66	54.69	59.61	55.73	84.96	43.12	58.46
19	Sugar yield at harvest (tonnes/ha)	SY	6.91	6.19	6.84	6.46	10.19	5.27	6.76
20	Chlorophyll a (mg per gram fresh weight)	CHLa	0.739	0.591	0.530	1.150	1.119	0.878	0.747
21	Chlorophyll b (mg per gram fresh weight)	CHLb	0.114	0.109	0.153	0.269	0.119	0.107	0.139
22	Total chlorophyll (mg per gram fresh weight)	TCHL	0.853	0.699	0.683	1.419	1.238	0.958	0.886
23	Plant height at harvest(in cm)	PH	228.33	242.15	231.19	217.58	250.58	188.50	229.88

Table.4 Contribution of characters towards divergence

Characters	Times ranked first	Contribution in percentage
Germination percentage at 45 DAS	0	0
Number of shoots at 120 DAP (000/ha)	0	0
Leaf area per plant before waterlogging (cm^2)	0	0
Leaf area per plant after waterlogging (cm^2)	0	0
Number of nodes with aerial roots	0	0
Cane diameter at harvest(cm)	0	0
Number of shoots at 240 DAP(000/ha)	0	0
Number of millable canes (000/ha)	1	0.8333
Single cane weight (Kg)	1	0.8333
Brix at 10 months	0	0
Pol at 10 months	0	0
Purity at 10 months	0	0
CCS% at 10 months	0	0
Brix at 12 months	0	0
Pol at 12 months	0	0
Purity at 12 months	2	1.6667
CCS% at 12 months	0	0
Cane yield(tonnes/ha)	5	4.1667
Sugar yield at harvest (tonnes/ha)	0	0
Chlorophyll a (mg per gram fresh weight)	26	21.6667
Chlorophyll b (mg per gram fresh weight)	73	60.8333
Total chlorophyll (mg per gram fresh weight)	12	10
Plant height at harvest(in cm)	0	0

Ideotype breeding aims to develop genotypes for particular environment by targeting phenology of a crop and modifying it in a certain way. The study shows that genotypes in cluster 5 and cluster 4 have higher chlorophyll content even under waterlogged condition and thus breeding programmes targeting this trait may well utilise these genotypes. Similarly other genotypes can well be used for targeting a particular trait under different environments.

The characters that were the most important contributors towards divergence in the present study were chlorophyll content (chlorophyll a and b, total chlorophyll). The character cane yield followed by purity at 12 months had small contribution towards divergence whereas number of shoots at 240 DAP and number of millable canes at harvest (000/ha) had negligible contribution towards divergence. Single cane weight, cane yield and quality attributes like Brix percent and purity percent were observed to be contributors towards divergence by Punia e*t al*.(1983) while cane yield and purity percent were observed to be primary contributors towards divergence by Srivastava *et al*., (1999)and Sanghera *et al*., (2015) The genotypes were not grown under waterlogged condition by these workers. Bal Krishna *et al.*, reported CCS% at 12 months, Leaf area

Index 60 days after waterlogging, Leaf area Index 30 days after waterlogging and plant height at 150 days after planting to be contributors towards divergence in descending order under waterlogged condition however, they had not measured the chlorophyll content in studied genotypes. Chlorophyll content is a biochemical attribute which has been observed to be the most important character towards divergence under waterlogged condition in the present study however it has not been reported by earlier workers as most of the earlier workers did not use it as a character under study.

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