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Genetic Divergence Studies in Sugarcane (*Saccharum* Spp. Hybrid Complex) under Waterlogged Conditions

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Abstract

Forty-four sugarcane clones/varieties were evaluated in a randomized complete block designwith two replications at PAU, RRS Kapurthala during cropping season 2019-20 under waterlogged conditions to study the genetic divergence among sugarcane clones/varieties. The waterlogging conditions were imposed at tillering, grand growth and maturation stages. The assessment of genetic diversity was based on the sevencane yield and its components traits i.e. germination (%) at 45 days, number of millable canes (000/ha), stalk length (cm), stalk diameter (cm), single cane weight (kg), cane yield (t/ha) and six juice quality traits i.e. brix (%), sucrose (%), CCS (%), purity (%), extraction (%) and CCS (t/ha). The results indicated that the clones/varieties differed significantly with respect to the traits studied and were grouped into seven clusters separately for cane yield components and quality traits based on the squared euclidian distance using Ward's method. Highest intra cluster distance was found in cluster VII for cane yield and its components traits, while in cluster II for quality traits. Maximum inter cluster distances were observed between clusters VI and VII for cane yield and its components traits, whereas between clusters II and VII for quality traits, which indicates that clones/varieties belonging to these clusters can be used in breeding programme because of presence of maximum diversity. The analysis of cluster means revealed that clusters III and VII presented higher index for cane yield and its components traits, while Cluster IV and VII presented better quality traits. It is suggested that clones/varieties within these clusters could show greater potentiality for breeding purpose by virtue of their desirable traits and these should be used for future breeding programme.

Key words: Cluster distance, diversity, sugarcane, waterlogged.

Introduction

Sugarcane (Saccharum spp. complex) is an important agro-industrial crop cultivated in both tropical and subtropical regions of the world for the production of sucrose (sugar or jaggery), ethanol, chemicals, bio-manure, paper board factories and cogeneration plants. Being a long duration crop, it is affected by various biotic and abiotic stresses that have resulted due to climatic changes in the near past. Waterlogging is one among the important abiotic stresses which influences the overall productivity of crop and recovery of sugar and the sugarcane growing in low lying, high rainfall areas and areas along the rivers are highly affected by waterlogging. Sugarcane varietal response to waterlogging varies greatly depending on inherent genetic characteristics, crop age and other growing conditions (1). For abiotic stress tolerance, there is no alternative except to develop varieties with genetic tolerance for them. But the narrow genetic base of modern sugarcane varieties is the major bottle neck of sugarcane improvement for biotic and abiotic stress tolerance which further complicated by highly heterozygous, complex polyploidy (10-12 copies of the genome), aneuploidy hybrids (100-120 chromosomes) and often with four species of Saccharum in their ancestry. The success of sugarcane breeding programme is highly dependent on the selection of rich and genetically diverse parents and the parental lines are selected on the basis of agronomic characters, pedigree records, bi-parental crosses and polycrosses between elite genotypes in sugarcane breeding programmes. The seed germination varies with the change in growing type (2). The lack of genealogy data and the improper identification of some genotypes may impair estimation of the genetic diversity among sugarcane accessions. Furthermore, continuous selection for the same traits such as sucrose content in breeding programmes has resulted in a decline in genetic diversity, limiting further progress in sugarcane breeding (3). Thus, it is more necessary to broaden the genetic base by crossing genetically diverse parents which may result in more transgressive segregants and better heterotic expressions. To further increase the yield potential should be given to traits which were having high heritability (%) combining with high genetic advance (4). Knowledge on genetic divergence is thus essential for identifying and organizing the available genetic resources with the goal of producing promising cultivars (5). A number of multivariate techniques have been used to determine genetic divergence in a variety of crops (6) but cluster analysis is one of the most commonly used

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Table-1: Mean squares of cane yield, its components and quality traits based on analysis of variances in sugarcane.

	Cane yield and its component traits								Quality traits					
	Germin ation (%)	No. of millable canes (000/ha)	Stalk length (cm)	Stalk diameter (cm)	Single cane weight (kg)	No. of intern odes	Cane yield (t/ha)	Brix (%)	Sucrose (%)	Purity (%)	CCS (%)	Extrac- tion (%)	CCS (t/ha)	
Grand Mean	44.75	72.12	222.78	2.72	1.14	17.36	57.93	17.15	15.29	89.20	10.62	45.43	6.16	
Treat MSS	243.33	445.48	3632.09	0.07	0.16	9.11	81.85	7.37	5.72	36.34	3.06	45.48	4.78	
Err MSS	35.80	41.83	56.09	0.07	0.04	4.45	47.98	0.48	0.41	1.58	0.22	1.70	0.41	
F Ratio	6.80	10.65	64.76	1.02	4.15	2.05	1.71	15.23	13.99	23.08	14.13	26.83	11.71	

Table-2 : Grouping of forty-four sugarcane clones/varieties in different clusters based on cane yield, its components and quality traits.

	Cane yield a	nd its component traits	Quality traits						
Cluster No.	No. of clones/ varieties	Clones/Varieties	Cluster No.	No. of clones/ varieties	Clones/Varieties				
I	6	Co 118, K-33/1, K2013-4, K-57/3, CoPb 18213, CoJ 85	I	9	Co 118, CoPb 92, Co 238, CoPb 14212, M 80, K2013-1, K-33/1, K-38/1, K-71/1				
II	6	CoPb 17212, K2013-2, Bo 153, K-72/33, K-38/1, K-9/5	II	2	CoPb 91, CoPb 14211				
III	6	Co 238, CoPb 14211, CoPb 16211, K2013-3, CoPb 18211, CoPb 93	III	14	CoJ 64, K-38/7, CoP 2061, Bo 154, K2013-4, K-53/3, K-57/3, K-58/2, K-9/5, CoPb 17212, Bo 91, Bo 153, K2013-2, CoP 9437				
IV	10	CoJ 64, K-2/4, CoPb 14212, K-66/1, Bo 91, CoPb 14181, CoP 2061, K-53/3, CoPb 94, K2013-5	IV	10	CoPb 93, K2013-3, CoPb 16211, CoPb 18213, CoPb 14181, K-72/3, CoPb 16212, CoPb 18211, CoJ 85, K2013-5				
V	2	CoJ 88, CoPb 14183	V	3	CoJ 88, K-71/3, K-2/4				
VI	7	CoPb 17211, M 80, K2013-1, K-31/10, Bo 154, CoP 9437, K-71/3	VI	5	CoPb 94, CoPb 17211, K-31/10, K-66/1, K-50/3				
VII	7	CoPb 92, K-38/7, CoPb 91, K-50/3, K-71/1, K-58/2, CoPb 16212	VII	1	CoPb 14183				

technique for this purpose. Thus, the current study was formulated with the aim to select better clones/varieties suitable for waterlogged conditions for future breeding programmes using the Ward's cluster analysis based on cane yield, its components and quality traits.

Materials and Methods

The experimental plant material consisted of forty-four diverse sugarcane clones/varieties comprising 39 clones/varieties (Co 118, CoPb 92, CoJ 85, CoJ 64, CoJ 88, CoPb 91, CoPb 93, CoPb 94, Co 238, CoPb 16211, CoPb 16212, CoPb 17211, CoPb 17212, CoPb 14211, CoPb 14212, CoPb 14181, CoPb 14183, K-38/7, K2013-1, K2013-2,K2013-3, K2013-4, K2013-5, M 80, K-31/10, K-66/1, K-71/3, K-57/3, K-71/1, K-9/5, K-50/3, K-72/3, K-38/1, K-33/1, K-2/4, K-58/2, K-53/3, CoPb 18213 and CoPb 18211) from local source and 5 varieties (Bo 154, CoP 9437, Bo 153, CoP 2061 and Bo 91) from Dr Rajendra Prasad Central Agricultural University, Pusa (Samastipur), Bihar. The plant materials were planted in a randomized complete block design with two replications

during spring 2019-20 in the second week of March under waterlogged condition. Each clone was represented by a plot of 4 rows of 4m length maintaining inter row spacing of 90cm and seed rate in both the environments was kept 12 healthy buds per running 1 metre row. The standard cultural practices were carried as per recommendations to get ideal crop stand except irrigation. Artificial irrigation was imposed at tillering, formative/grand growth and maturation stages to create waterlogged conditions. Data were recorded on seven cane yield and its components traits i.e. germination (%) at 45 days of planting, number of millable canes (NMC) (000/ha), stalk length (cm), stalk diameter (cm), single cane weight (kg), number of internodes, cane yield (t/ha) and six juice quality traits at harvest i.e. brix (%), sucrose (%), CCS (%), purity (%), extraction (%) and CCS (t/ha) for each clone in each replication under following standard procedures and protocols. Cane yield (t/ha) was recorded from final harvested crop, number of millable canes were counted at maturity of crop per plot and converted in to number of millable canes per hectare (000/ha). Other cane yield

Ш Ш ΙV V ۷I Cluster VII 582.69 1001.90 676.04 1201.22 864.69 3075.68 Ι 424.52 Ш 172.22 766.65 658.92 2113.73 1752.48 1566.49 Ш 435.78 743.19 1944.95 2587.45 1702.53 IV 321.88 926.47 1514.66 2339.25 ٧ 208.84 1027.42 5240.12 VΙ 5815.44 279.18 VII 639.90

Table-3: Mean inter- and intra- (bold) cluster distances between clusters formed for cane yield and its component traits.

Table-4: Mean inter- and intra- (bold) cluster distances between clusters formed for quality traits.

Cluster	I	II	III	IV	V	VI	VII
I	10.44	52.13	21.32	38.56	22.12	44.74	126.71
II		20.08	93.40	91.58	44.65	56.98	157.13
III			8.44	22.15	26.79	40.23	82.40
IV				6.60	20.82	44.84	52.91
V					8.26	27.74	73.11
VI						18.68	53.53
VII							0.00

Table-5: Mean values of seven clusters formed for cane yield, its component traits and quality traits.

Cane yield and its component traits								Quality traits						
Cluster No.	Germi nation (%)	No. of millable canes (000/ha)	Stalk length (cm)	Stalk diamet er (cm)	Single cane weight (kg)	No. of intern odes	Cane yield (t/ha)	Cluster No.	Brix (%)	Sucro se (%)	Purity (%)	ccs (%)	Extrac tion (%)	CCS (t/ha)
I	46.82	65.85	212.05	2.87	1.24	17.86	55.63	I	17.46	15.16	86.85	10.39	43.70	5.78
II	39.77	63.35	228.69	2.78	1.28	18.11	54.74	II	17.40	15.65	90.04	10.92	38.21	7.05
III	57.55	73.27	232.09	2.81	1.28	18.28	64.07	III	16.74	14.73	88.01	10.17	46.96	5.72
IV	43.39	81.53	220.61	2.59	1.01	16.76	60.35	IV	18.22	16.45	90.37	11.49	47.25	7.37
V	49.78	92.69	197.00	2.63	0.78	14.95	58.50	V	18.05	16.28	90.30	11.37	43.94	5.35
VI	39.94	66.63	189.40	2.69	1.00	15.76	53.93	VI	15.30	14.08	92.01	9.93	43.76	5.64
VII	41.57	70.19	262.77	2.73	1.26	18.64	57.72	VII	15.53	14.99	96.55	10.79	48.49	6.83

components traits were recorded as per standard procedures from five randomly selected canes taken from each clone in each replication to measure stalk length, stalk diameter, single cane weight and number of internodes. Juice quality traits were determined using sucrolyser as per the standard procedure given by (8).

Germination (%) at 45 days after planting, purity (%), CCS (%), extraction (%) and CCS (t/ha) were calculated as :

Germination (%) =
$$\frac{\text{Number of buds germinated per plot}}{\text{Total number of buds planted per plot}}$$
 100

Purity (%) = $\frac{\text{Sucrose (\%)}}{\text{Brix (\%)}}$ 100

CCS (%) = $\frac{0.292 \text{ Sucrose(\%) { (0.0.35 \text{ Purity(\%)) - 1}}}{\text{Purity (\%)}}$ 100

Extraction (%) =
$$\frac{\text{Juice weight (kg)}}{\text{Cane weight (kg)}}$$
 100

CCS (t/ha) = Yield (t/ha)
$$\times$$
 CCS (%)

Statistical Analysis: The analysis of variance (ANOVA) was performed using CPCS1 software (9). Data were also subjected to Ward's method of Hierarchical cluster analysis using statistical software Indostat version 9.2 andby using squared Euclidean distance method (10). Dendrogram of the data was developed for visualizing and classifying the results.

Results and Discussion

Analysis of variance revealed the significant differences among the clones/varieties studied indicating the sufficient genetic variation among the clones/varieties for all the traits studied (Table-1). Coefficient of variability ranged from 3.69 to 16.20, which indicated the consistency of the experimental conditions.

Cluster pattern of clones/varieties : Forty-four sugarcane clones/varieties were divided into seven

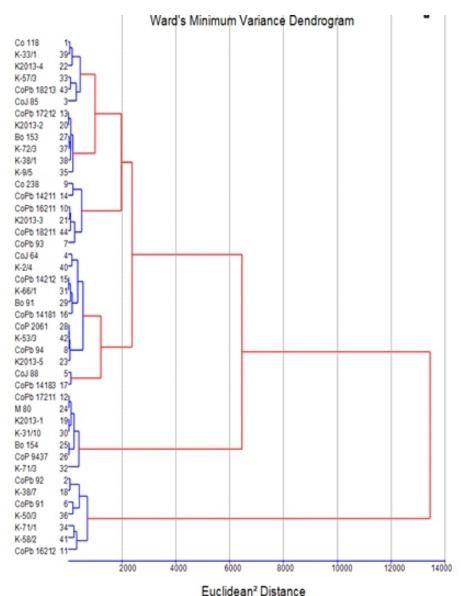


Fig.-1: Cluster pattern diagram for cane yield and its components traits.

clusters separately on the basis of cane yield components and quality traits (Table-2). The results indicated that clusters I, II, III, IV, V, VI and VII for cane yield and its components traits were categorized into 6 (Co 118, K-33/1, K2013-4, K-57/3, CoPb 18213 and CoJ 85), 6 (CoPb 17212, K2013-2, Bo 153, K-72/3, K-38/1 and K-9/5), 6 (Co 238, CoPb 14211, CoPb 16211, K2013-3, CoPb 18211 and CoPb 93), 10 (CoJ 64, K-2/4, CoPb 14212, K-66/1, Bo 91, CoPb 14181, CoP 2061, K-53/3, CoPb 94 and K2013-5), 2 (CoJ 88 and CoPb 14183), 7 (CoPb 17211, M 80, K2013-1, K-31/10, Bo 154, CoP 9437 and K-71/3) and 7 (CoPb 92, K-38/7, CoPb 91, K-50/3, K-71/1, K-58/2 and CoPb 16212) clones/varieties. respectively. Fig 1 represents the cluster pattern for clones/varieties for cane yield and its components traits. The clusters I, II, III, IV, V, VI and VIIfor quality traits were categorized into 9 (Co 118, CoPb 92, Co 238, CoPb 14212, M 80, K2013-1, K-33/1, K-38/1 and K-71/1), 2 (CoPb 91 and CoPb 14211), 14 (CoJ 64, K-38/7, CoP 2061, Bo 154, K2013-4, K-53/3, K-57/3, K-58/2, K-9/5, CoPb 17212, Bo 91, Bo 153, K2013-2 and CoP 9437), 10 (CoPb 93, K2013-3, CoPb 16211, CoPb 18213, CoPb 14181, K-72/3, CoPb 16212, CoPb 18211, CoJ 85 and K2013-5), 3 (CoJ 88, K-71/3 and K-2/4), 5 (CoPb 94, CoPb 17211, K-31/10, K-66/1 and K-50/3) and 1 (CoPb 14183) clones/varieties, respectively. Fig-2 represents the cluster pattern for clones/varieties for quality traits.

Intra and inter cluster distances: The intra cluster distances for cane yield and its component traits ranged from 172.22 to 639.90. Highest intra cluster distance (639.90) was reported in cluster VII followed by cluster III (435.78) and I (424.52), while lowest intra cluster distance

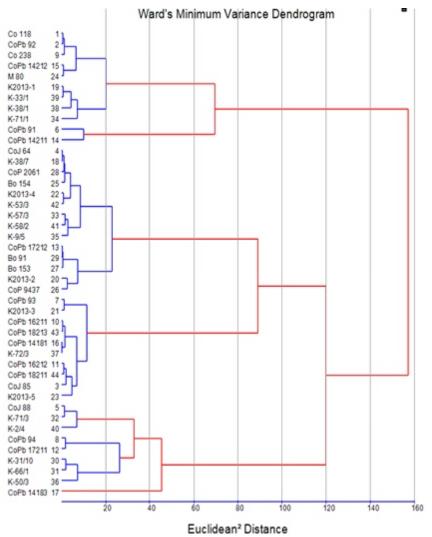
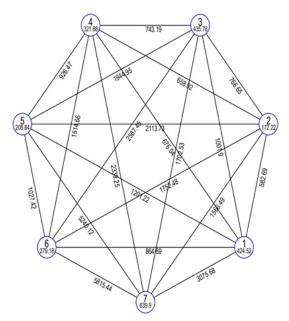


Fig-2: Cluster pattern diagram for quality traits.

(172.22) was reported in cluster II. The inter cluster distances ranged from 582.69 to 5815.44. Maximum inter cluster distance was found between clusters VI and VII (5815.44) followed by clusters V and VII (5240.12) and clusters I and VII (3075.68), whereas minimum inter cluster distance was observed between cluster I and cluster II (582.69). Fig-3 represents the configuration of clusters and their mutual relationship for cane yield and its components traits. The intra cluster distances for quality traits ranged from 0.00 to 20.08. Highest intra cluster distance (20.08) was reported in cluster II followed by cluster VI (18.68) and I (10.44), while lowest intra cluster distance (0.00) was reported in cluster VII. The inter cluster distances ranged from 20.82 to 157.13. Maximum inter cluster distance was observed between clusters II and VII (157.13) followed by clusters I and VII (126.71) and clusters II and III (93.40), whereas minimum inter cluster distance was observed between cluster IV and cluster V (20.82). Fig-4 represents the configuration of clusters and their mutual relationshipfor quality traits. Highest intra and inter cluster distances indicated that clones/varieties belonging to these clusters can be used in breeding programme because they are more diverse in contrast to the lower intra and inter cluster distances. Identical suggestions for selection of different clones under waterlogged conditions from maximum divergent clusters were also given by (11,12,13).

Cluster mean values for different traits

Cane yield and its component traits: The highest cluster mean for germination (%) was found in cluster III (57.55%) followed by cluster V (49.78%) and cluster I (46.82%), while the lowest cluster mean was found in cluster II (39.77%). The highest cluster mean for number of millable canes (000/ha) was found in cluster V (92.69) followed by cluster IV (81.53) and cluster III (73.27), while the lowest cluster mean was found in cluster II (63.35). The highest cluster mean for stalk length (cm) was found in cluster VII (262.77 cm) followed by cluster III (232.09)



Euclidean² Distance (Not to the Scale)

Fig.-3: Configuration of clusters and their mutual relationship by Euclidean 2 distance method in sugarcane clones/varieties for cane yield and its component traits.

cm) and cluster II (228.69 cm), while the lowest cluster mean was found in cluster VI (189.40 cm). The highest cluster mean for stalk diameter (cm) was found in cluster I (2.87 cm) followed by cluster III (2.81 cm) and cluster II (2.78 cm), while the lowest cluster mean was found in cluster IV (2.59 cm). The highest cluster mean for single cane weight (kg) was found in cluster III, II (1.28 kg) followed by cluster VII (1.26 kg), while the lowest cluster mean was found in cluster V (0.78 kg). The highest cluster meanfor number of internodes was found in cluster VII (18.64) followed by cluster III (18.28) and cluster II (18.11), while the lowest cluster mean was found in cluster V (14.95). The highest cluster mean for cane yield (t/ha) was found in cluster III (64.07 t/ha) followed by cluster IV (60.35 t/ha) and cluster V (58.50 t/ha), while the lowest cluster mean was found in cluster VI (53.93 t/ha).

Juice quality traits: The highest cluster mean for brix (%) was found in cluster IV (18.22%) followed by cluster V (18.05%) and cluster I (17.46%), while the lowest cluster mean was found in cluster VI (15.30%). The highest cluster mean for sucrose (%) was found in cluster IV (16.45%) followed by cluster V (16.28%) and cluster II (15.65%), while the lowest cluster mean was found in cluster VI (14.08%). The highest cluster mean for CCS (%) was found in cluster IV (11.49%) followed by cluster V (11.37%) and cluster II (10.92%), while the lowest cluster mean was found in cluster VI (9.93%). The highest cluster mean for purity (%) was found in cluster VII (96.55%)

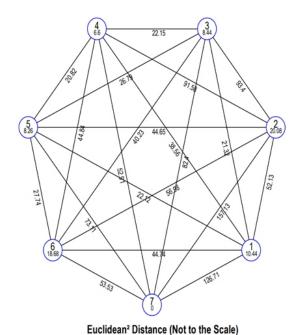


Fig.-4: Configuration of clusters and their mutual relationship by Euclidean² distance method in sugarcane clones/varieties for quality traits.

followed by cluster VI (92.01%) and cluster IV (90.37%), while the lowest cluster mean was found in cluster I (86.85%). The highest cluster mean for extraction (%) was found in cluster VII (48.49%) followed by cluster IV (47.25%) and cluster III (46.96%), while the lowest cluster mean was found in cluster II (38.21%). The highest cluster mean for CCS (t/ha) was found in cluster IV (7.37t/ha) followed by cluster II (7.05t/ha) and cluster VII (6.83t/ha), while the lowest cluster mean was found in cluster V (5.35t/ha).

Cluster III and VII reported to had higher index values for cane yield and its component traits germination (%), stalk length (cm), single cane weight (kg), number of internodes and cane yield (t/ha), while Cluster IV and VII had better quality in respect of brix%. sucrose%, purity%, CCS%, extraction% and CCS (t/ha). Therefore, clones/varieties belonging to these clusters can be used as breeding material for development of sugarcane varieties under waterlogged stress. (11,12,13) also suggested the selection of clones based on cluster mean and divergent cluster distance for the better exploitation of genetic potential under waterlogged conditions, which are in accordance with the current results.The clones/varieties belonging maximum genetic distance and higher cluster mean for particular traits could be intercrossed to produce a good number of sugarcane progenies with a better breeding potential.

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