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Assessing genetic diversity and correlation among 150 elite sugarcane clones based on morphological characterization

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Abstract
The genetic diversity of 150 elite sugarcane clones including 6 checks representing early and mid-late maturing group were assessed in the current study at the experimental farm of Punjab Agricultural University, Regional Research Station, Gurdaspur. The findings of the study emphasized that ample genetic diversity was existing in the clones for all the traits based on genetic diversity parameters. High heritability together with high genetic advance as percentage of mean was detected for no. of shoots, NMC, stalk length, single cane weight and cane yield which indicates that selection for these traits could be more effective because of genes showing additive behaviour for these traits. The correlation results suggest that to improve the cane yield in sugarcane traits such as number of shoots, number of millable canes, stalk length and single cane weight should be given more weightage. The extremity was found between cluster one and five (5.1), followed by cluster three and five (4.63), cluster five and six (4.45). The cluster 2 having clones showed more stalk length, cluster 3 having clones representing maximum no. of shoots and no. of millable canes, cluster 5 having clones showed more cane yield and cluster 7 representing clones having maximum stalk diameter, single cane weight and brix content. The more emphasis was given to the cluster having maximum genetic divergence for the basis of selection and selecting the parents for crossing. Hence, clusters with highest mean value for the required traits shall be take into consideration for selection as parents for crossing or for other population advancement methods in future sugarcane development programmes.

Keywords: Sugarcane, diversity, heritability, genetic advance and cluster

Introduction
Sugarcane (*Saccharum* spp. hybrid) is a valuable cash crop in India contributing in economic growth and food security in sub-tropical and tropical regions. Tropical region accounts for 45 percent area and 55 percent of the total sugarcane production in the country. Henceforth, sub-tropical region procures 55 percent area and 45 percent of total production. The average sugarcane yield in the India is about 69.4 t/ha. In 2018/2019, worldwide production of sugar was approximately 179.99 million metric tons (www.statista.com).

The presence of C4 photosynthetic mechanism in sugarcane makes it more effective in solar energy conversion and increase in biomass yield (Henry 2010 and Byrt *et al.,* 2011) [1, 2]. The emphasis on the crop is due to the fact that Bioethanol is a non-renewable resource that can meet the increasing global demand for energy while reducing GHG emissions (Goldemberg *et al.*, 2008) [3]. In addition, it is also used for alcoholic beverages, feed for animal, and as a fertilizer (trash) in crop production across the world (Moore *et al.,* 2013) [4].

The crop contributes 1.1 per cent to the GDP of the country, which is significant considering that it is grown on less than 2.57 per cent of the total agricultural land in India. The methodology of crop breeding has been seriously challenged by the growing demand for sugar as well as green-fuels as a result of population growth and current living standards (Hofsetz and Silva, 2012) [5]. The rising demand for sugar and ethanol has become manifold in the past 50 years. The attribution to production gain is due to the genetic enhancement of varieties that can adjust to a choosen environment. The vital role in increasing the productivity of sugarcane has been attributed to improvements in management techniques, fertilization and irrigation (Morais *et al.*, 2015) [6].

The key role of genetic diversity is irreplaceable in the success of any breeding program.

The immense understanding of genetic variability contributes at a large in improvement, boosting the systematic use of genetic variations in breeding programs by assisting proper selection of cross combination between large sets of parental genotypes.

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The study focused on the qualitative traits which were used for the estimation of levels of variation between the sugarcane germplasm.

The prime objective of the research was to deal with the amount of morphological variability among sugarcane germplasm based on qualitative traits. The deciding factor is the choice of parents for hybridization programme that will further contributes in the improvement of crop.

In plant breeding to produce the desirable recombinants for the selection of improved genotypes is the result of selection of parents with high genetic diversity. Keeping this in view, 150 sugarcane genotypes were subjected to examine the variability, heritability, genetic advance, trait associations and genetic divergence between the genotypes to impart a basis for selection of parents for further sugarcane improvement programme.

Materials and Methods
150 sugarcane clones along with commercial checks (Co 0118, CoJ 85, CoJ 64, CoPb 91, Co 0238 and CoJ 88) representing early and mid-late maturing group were sown in Alpha lattice design with two replications having plot size of 2 rows x 2 meters x 0.75 meters at Punjab Agricultural University, Regional Research Station, Gurdaspur during 2016-17. Three budded setts of each clone at the rate of 12 buds/metre were planted. The recommended packages of practices were followed to raise the ideal crop growth as suggested by Punjab Agricultural University. The soil of experimental plot was clay loam with neutral pH and normal EC, medium in organic carbon and phosphorus and low in potassium.

Evaluated traits
Observations were recorded on number of shoots (000/ha), number of millable canes (NMC, 000/ha), stalk length (cm), stalk diameter (cm), single cane weight (SCW, kg), cane yield (t/ha), and Brix (%). The number of shoots, number of millable canes, cane yield (kg/plot) were calculated on plot basis; while stalk length and diameter were recorded on cane basis (five randomly selected canes). Stalk height (cm) was recorded at harvesting of the crop i.e. from the base to the point where tops are easily removable. Stalk diameter (cm) was measured for five stalks with the use of Vernier Calliper (Insize Digital Caliper 1112-125). NMC was the total number of matured canes excluding the tillers which were not developed into mature stalks.

Statistical Analyses
The observations recorded for various traits were statistically analyzed for ANOVA as per Panse and Sukhatme (1984) [7]; genotypic and phenotypic coefficients of variation were calculated as per Burton and De Vane (1953) [8]. Heritability in broad sense (h2bs) and genetic advance as per cent of mean were calculated as per Johnson et al. (1955) [9]. When doing cluster analysis and correlations, the FactoMineR (Sebastien Le et al., 2008) [10], Cluster (Machler et al., 2020) [11], and ggplot2 (Wickham, 2016) [12] packages were used using the modified means values of all the quantitative features. The ideal number of clusters was calculated by Elbow approach from the Cluster program.

Results and Discussion
Genetic diversity is crucial for plant breeders in order to produce new kinds and hybrids that are even better. The early genetic advantages in sugarcane are also maintained through a wider genetic base (Heinz 1987) [13]. According to Narain (2000) [14], the ability of populations and species to endure over evolutionary time in the face of changing environments depends on genetic diversity. For this reason, a precise assessment of genetic variability is required. The analysis of variance revealed that the clones had sufficient genetic diversity (table 1).

Table 1: ANOVA for different quantitative traits of sugarcane using alpha lattice design

Traits		Df	Sum Sq.	Mean Sq.	F value
No. of Shoot	Rep.	1	28	28.21.	2.902
	Trt.	149	46017	308.84***	31.764
	Rep:Blk	14	570	40.73***	4.189
	Residual	135	1313	9.72	
No. of Millable canes	Rep.	1	16	16.33	1.883
	Trt.	149	37944	254.66***	29.353
	Rep:Blk	14	507	36.25***	4.178
	Residual	135	1171	8.68	
Stalk length	Rep.	1	10431	10431***	722.253
	Trt.	149	407080	2732***	189.168
	Rep:Blk	14	340	24.	1.679
	Residual	135	1950	14	
Stalk Diameter	Rep.	1	1.014	1.0138***	327.658
	Trt.	149	11.19	0.0751***	24.271
	Rep:Blk	14	0.139	0.0099***	3.214
	Residual	135	0.418	0.0031	
Single cane weight	Rep.	1	0.448	0.4478***	229.54
	Trt.	149	19.339	0.1298***	66.54
	Rep:Blk	14	0.128	0.0091***	4.67
	Residual	135	0.263	0.002	
Cane yield	Rep.	1	421	420.9***	61.546
	Trt.	149	29906	200.7***	29.35
	Rep:Blk	14	645	46.1***	6.734
	Residual	135	923	6.8	
Brix (%)	Rep.	1	1.7	1.748.	3.04
	Trt.	149	559.7	3.756***	6.533
	Rep:Blk	14	18.8	1.344**	2.338
	Residual	135	77.6	0.575	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 2: Descriptive statistics for seven quantitative traits of 150 sugarcane clones

	Shoots	NMC	SL	SD	SCW	CY	BR
Mean	28.71	25.06	199.4	2.11	0.83	19.61	17.56
Range	11.50 – 83.00	8.50 – 72.00	117.50-300.00	1.45-2.85	0.29-1.45	5.00-55.50	13.60-21.00
GCV	42.39 (H)	44.02 (H)	18.48 (M)	8.97 (L)	30.47 (H)	49.73 (H)	7.10 (L)
PCV	44.16 (H)	46.01 (H)	18.58 (M)	9.43 (L)	31.10 (H)	52.41 (H)	8.45 (L)
H	92.14 (H)	91.53 (H)	98.88 (H)	90.52 (H)	96.04 (H)	90.04 (H)	70.60 (H)
GA	24.06 (H)	21.74 (H)	75.50 (H)	0.37 (L)	0.51 (L)	19.06 (M)	2.16 (L)
GAM	83.83 (H)	86.75 (H)	37.85 (H)	17.85 (M)	61.52 (H)	97.21 (H)	12.29 (M)
Skewness (lga [,3:9])	1.32	1.28	0.20	-0.09	0.37	0.98	-0.45
Kurtosis (lga [,3:9])	5.32	5.05	2.75	4.42	2.78	3.59	3.37

Variability, heritability and genetic advance

In order to determine the degree of selectivity, it is useful to take into account the type and degree of heritable genetic variation present in the population. The estimate of PCV and GCV were high for number of shoots, number of millable canes, single cane weight and cane yield and medium for stalk length and low for stalk diameter and brix percentage. The PCV was higher for all traits than the GCV, but the difference was really small, so environmental factors only had a small effect. Similar findings were reported by Chaudhary, 2001 [17] for single cane weight, stalk length, and number of millable canes and by Ranjan and Kumar, 2017 [15], Feylssa Tedessa *et al.*, 2014 [16], for single cane weight and cane yield.

Heritability is a calculation of the phenotypic variance due to genetic causes and has a predictive function in breeding plants and is high for all traits studied. The high heritability indicated that selection for these traits would be promising because they are unaffected by environmental influences. The genetic advance was observed high for no. of shoots, no. of millable canes, stalk length and medium for cane yield and lowest for stalk diameter, cane yield and brix percent whereas genetic advance as percentage of mean was recorded high for all the traits except stalk diameter and brix percent which shows moderate label. High heritability as a percentage of the mean associated with high genetic advance were observed for shoot number, NMC, stalk length, individual cane weight and cane yield, indicating that selection for these traits can be more effective due to the additive behavior of genes for these traits. When high heritability was observed for stalk diameter and brix content along with high genetic advance as percentage of the mean, indicates the existence of both additive and non-additive genetic effects for this trait and the results are consistent with the findings of Gowda *et al.*, 2016 [18]. Heritability in association with genetic advance would give a more reliable and meaningful selection (Johnson *et al.* 1955) [10]. High heritability in conjugation with high to moderate genetic advance indicates that the most likely heritability was the result of additive gene actions and selection might be productive for these traits. The skewness and kurtosis values for the cane yield and its contributing traits are given in the table 2. The traits SH, NMC, CY, SCW and SL showed positive skewness. Complementary gene interaction is associated with positive skewness while duplicate (additive x additive) a gene interaction is associated with negative skewness. Kurtosis is any measure of the "peak" of the probability distribution of a real valued random variable.

Kurtosis is positive in the presence of gene interactions and negative or close to zero in the absence of gene interaction (Pooni *et al.*, 1977; Choo and Reinbergs, 1982; Kotch *et al.*, 1992) [20-22]. A wide study on gene interaction is inevitable so as to increase the efficiency of our selection and breeding programme. All the traits studied shows the positive kurtosis suggested the presence of gene interaction.Under complementary relationship selection intensity could be higher in comparison to duplicate relationship. It has been suggested that gains in population efficiency may be greater with complementary gene interaction than duplicate gene interactions (Choo and Reinbergs, 1982) [21].

Interrelationships between all traits were examined and 9 significantly positive associations were found, while 2 significantly negative associations were observed (Figure 1). The number of shoots has a significant positive association with the number of tillers, stalk length and cane yield. The no. of millable canes showed a significant positive association with stalk length and cane yield. The stalk length indicated a significant positive association with no. of shoots, NMC, single cane yield and cane yield. The stalk diameter indicated a significant positive correlation with single cane weight. Thus, correlation studies suggest that to increase the cane yield in sugarcane traits such as no. of shoots, no. of millable canes, stalk length and single cane weight should be given more weightage. Masri *et al.*, 2022 [23] and Patra *et al.* 2022 [24] also observed similar results for no. of millable canes, stalk length and single cane weight and by D Kumar *et al.* 2022 [25] for stalk height, no. of millable canes and stalk length.

Cluster Analysis

Cluster analysis groups a large number of genotypes into a fewer homogeneous clusters which in turn expedite the selection of diverse genotypes. In the present study 150 sugarcane clones are grouped into 7 clusters in which 118 sugarcane clones grouped in Cluster 1 followed by 17 in cluster 2, 5 in cluster 3, 4 in cluster 4, 4 in cluster 5, 1 in cluster 6 and 1 in Cluster 7 as shown in table 3 and fig 2. The distribution of accessions into different diversity classes are shown in table 4. Clusters consisting of individual members are considered to have closer relationships with each other compared to clusters that are significantly more distant. The greatest distance was found between clusters 1 and 5 (5.1), followed by clusters 3 and 5 (4.63), clusters 5 and 6 (4.45), suggesting that crosses containing parents belonging to these clusters would give greater variability in the segregating generations.
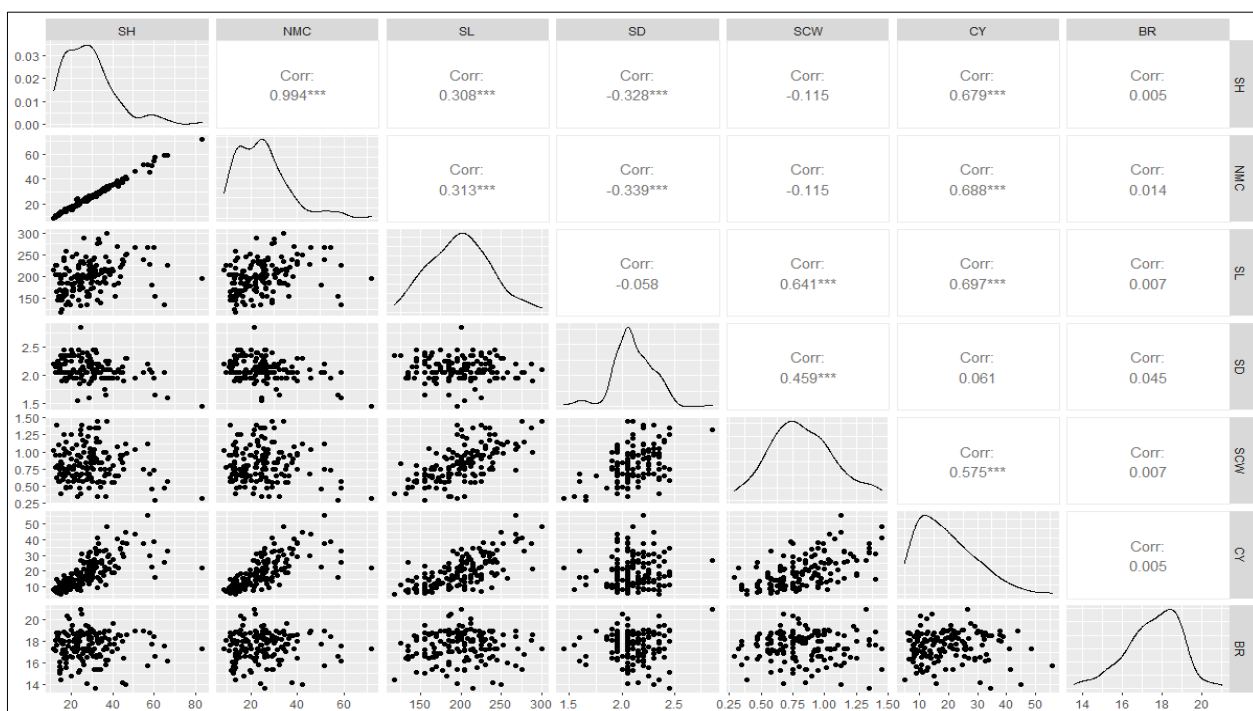
Fig 1: Pairwise genotypic correlations of different quantitative traits of sugarcane clones. The upper panel shows the numerical values of the correlation. Asterisk *, ** and *** indicate significance of correlation at 0.05, 0.01 and 0.001 probability levels. The bottom panel shows the scatterplots.
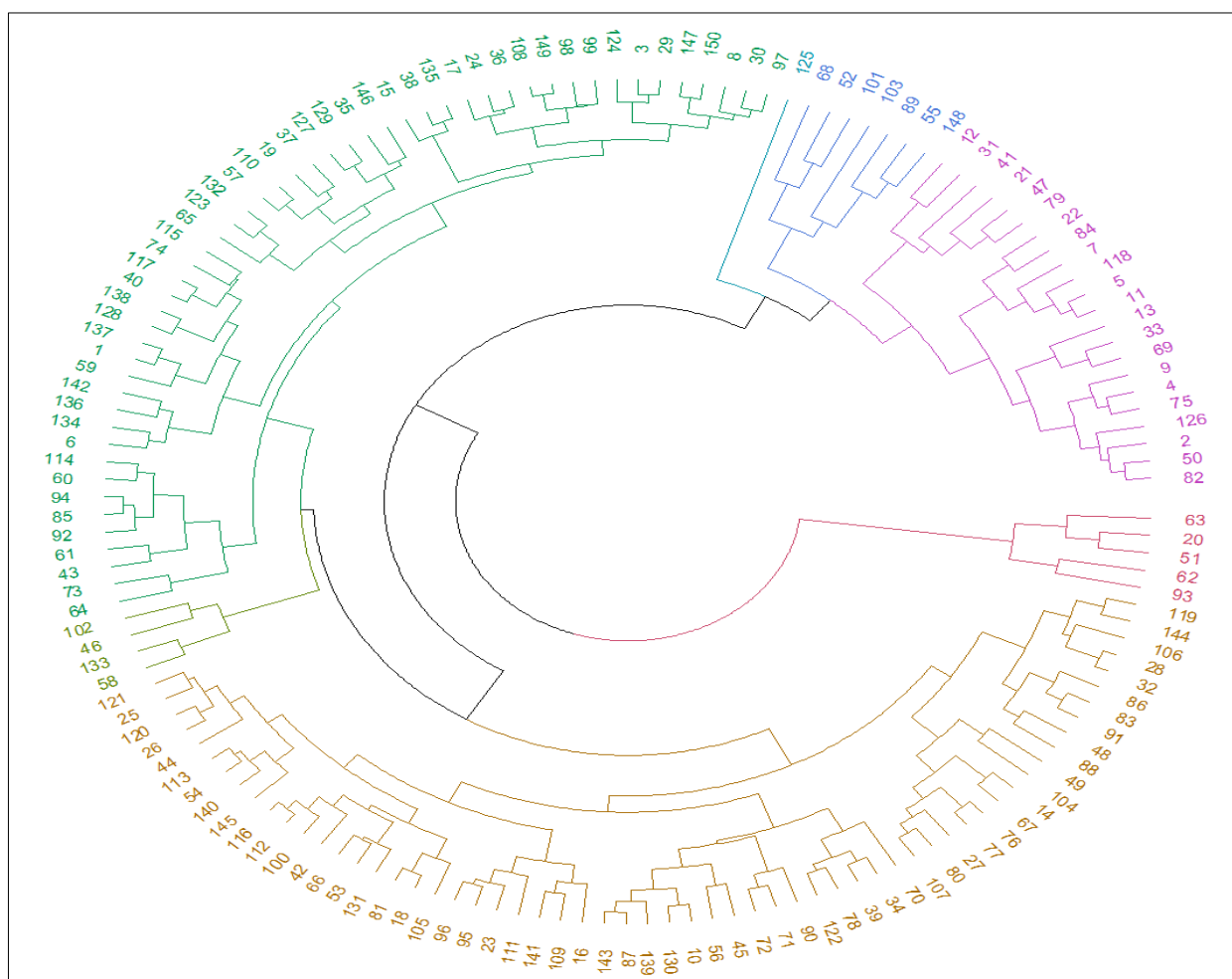

Fig 2: Dendrogram showing grouping of genotypes in different clusters.

Table 3: Inter cluster distance between different cluster

Cluster Variables	1	2	3	4	5	6
2	4.197265					
3	1.298255	3.949788				
4	3.318217	2.035923	3.394279			
5	5.100058	1.78038	4.633543	3.087574		
6	1.238634	3.591834	1.652168	3.294352	4.454996	
7	4.239707	2.508278	3.676494	2.768393	1.654565	3.879234

Table 4: Cluster Means of seven quantitative traits of 150 Sugarcane genotypes

Cluster Variables	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7
SH	24.53	43.24	66.80	31.00	44.75	15.00	24.50
NMC	21.21	38.44	59.60	28.13	39.25	13.50	21.50
SL	192.25	248.24	178.00	208.13	243.13	117.50	200.00
SD	2.13	2.06	1.76	1.64	2.28	2.35	2.85
SCW	0.82	1.01	0.43	0.51	1.11	0.39	1.33
CY	16.52	36.52	23.73	13.79	41.44	5.00	26.75
BR	17.63	17.91	17.10	17.71	14.38	14.40	21.00

The study revealed that cluster mean values exhibit high variation for all the traits (Table 4). The cluster 2 having clones showed more stalk length, cluster 3 having clones representing maximum no. of shoots and no. of millable canes, cluster 5 having clones showed more cane yield and cluster 7 representing clones having maximum stalk diameter, single cane weight and brix content. More emphasis was placed on the cluster that contributed the most to the divergnece in order to determine the cluster type for selection and the parents to be selected for hybridization From now on, clusters with the highest average value for the desirable traits will be taken into account for hybridization, or in future for other population improvement programmes of sugarcane breeding.

Table 5: List of genotypes grouped in different clusters of sugarcane germplasm

Cluster Number	No. of Genotypes	Name of Genotypes									
cluster1	118	13	4	6	8	9	10	13	14	15	
		16	17	18	19	23	24	25	26	27	28
		29	30	32	33	34	35	36	37	38	39
		40	42	43	44	45	48	49	50	53	54
		56	57	59	60	61	65	66	67	69	70
		71	72	73	74	75	76	77	78	80	81
		82	83	85	86	87	88	90	91	92	94
		95	96	97	98	99	100	104	105	106	107
		108	109	110	111	112	113	114	115	116	117
		119	120	121	122	123	124	127	128	129	130
		131	132	134	135	136	137	138	139	140	
		141	142	143	144	145	146	147	149	150	
Cluster2	17	2	5	7	11	12	21	22	31	41	47
		55	79	84	89	118	126	148			
Cluster 3	5	20	51	62	63	93					
Cluster 4	4	46	58	102	133						
Cluster 5	4	52	68	101	103						
Cluster 6	1	64									
Cluster 7	1	125									

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