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Full-Length Research Paper

Hierarchical cluster analysis of sugarcane germplasm based upon response to flowering stages

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ABSTRACT: Two experiments were conducted at El-Sabahia Research Station (31° 12' 54″ N 29° 58 ' 23″ E), Alexandria, Egypt, during 2018/2019/2020 season (plant cane crop) and 2020/2021 season (first ratoon crop) to investigate the flowering performance of sugarcane germplasm to facilitate crossing between synchronized germplasm at full flowering time. Eighty-seven sugarcane germplasm from various origins were used in this study. In the middle of August, 2018 three–budded/cuttings of each genotype were planted. The experimental design used was randomized complete blocks with two replications. After flowering season, all plots of 2018 plant-cane were cut on March, 2020 and allowed to grow the ratoon on March, 2021. The eighty- seven sugar cane germplasm under study were categorized into four groups. The first group included thirty-one sugarcane genotypes that flowered in both two seasons were grouped into four clusters. The second group consisted of twenty-seven genotypes that flowered only in plant cane's season were clustered into five clusters. The third group included six genotypes that flowered only in first ratoon season and they were 86E409, EH77/31-56, EH85/3-35, Mex 58-1866, CI 47-83 and G 2008-52. The fourth group included twenty-three genotypes that did not show any flowering response neither in plant cane nor first ratoon.

Keywords: Sugarcane, plant cane, first ratoon, germplasm

INTRODUCTION

Flowering is of considerable practical importance in new varieties sugarcane development. In breeding programs, control of flowering is necessary in the production of new varieties. So, knowledge of the factors which control flowering is valuable for plant breeder, who must be able to control the timing of flowering with precision. The improved sugarcane varieties resulted from controlled crosses has been greatly extended and accelerated during recent decades, where the majority of the present commercial varieties were originated in this manner. Induce flowering in Egypt is of great interest to sugarcane breeders because of strong argue to establish a successful long term breeding program to produce improved varieties. An improved knowledge of sugarcane breeding and selection highlights the need for making controlled crosses for special characters like high early sucrose content formation or resistance to diseases and pests, for the commercial sector. This work necessitates inducing sugarcane to flower. According to Rao *et al.* (1973), under the natural conditions of Alexandria, Egypt partially succeeded to produce little number of sugarcane varieties resulted from the open pollination among the available parents. However, those varieties did not meet the needs of both cane growers and manufacturers. Actually, their limited success could be referred to the variability of flowering time of cane varieties, known in nature as early, intermediate to late flowering. Accordingly, any defined parental genotypes cannot be simply crossed. Therefore, flowering dates must be modified by different treatments as controlled photoperiod (James, 1972) aiming at a simultaneous flowering for successful crossing and seed setting, required for breeding program. Allam (1999) mentioned that flowering represents constrain for having a sustainable local

breeding program. The development of new varieties of sugarcane (*Saccharum* spp.) from controlled crossing has been greatly extended and established successful long term breeding program to induce improved varieties (Mehareb *et al.,* 2016). The objective of this work was to investigate the flowering performance of sugarcane germplasm to facilitate crossing between synchronized germplasm at full flowering time.

MATERIALS AND METHODS

Two experiments were conducted at El-Sabahia Research Station (31° 12'54″ N 29° 58 ' 23″ E), Alexandria, Egypt, during 2018/2019/2020 season (plant cane crop) and 2020/2021 season (first ratoon crop).

The experimental procedures

Eighty-seven sugarcane germplasm from various origins were used in this study (Tables 1 and 2). In the middle of August, 2018 three–budded/cuttings of each genotype were planted in 3 ridges plots. Each ridge was 5 m long and 1 m apart. Thus, the plot area was 15 m². The experimental design used was randomized complete blocks with two replications. After flowering period, all plots of 2018 plant-cane were cut on March, 2020 and allowed to grow the ratoon on March 2021. The following parameters were recorded:

Number of genotypes flowered under natural flowering in four groups; only plant cane, only first ratoon, both plant cane and first ratoon and non-flowered genotypes.

Duration of pre flag leaf stage

This stage was calculated as number of days from optimum days for flowering (September 5, day length = from 12.41 to 12.15 h) up to discontinuing formation of new leaves and beginning of the flag leaf formation and emergence (Mehareb *et al.,* 2021).

Duration of flag leaf stage

This stage was calculated as number of days from the beginning of flag leaf formation to as soon as the emergence of the inflorescence form flag leaf sheath occurred.

Duration of emergence stage

This stage was calculated from the starting of emergence of the inflorescence from flag leaf up to its full extension completed.

Minimum days to flower

The number of days from the beginning of photoperiod treatment until flowering of the first stalk per pot appeared.

Maximum days to flower

The number of days from the beginning of photoperiod treatment until flowering of last stalk per pot was appeared.

Duration of flowering period: Maximum days to flower - minimum days to flower + 1 (Mehareb *et al* 2021).

Percent of total flowered plants: number of flowered plants/number of plants per plot × 100.

Statistical analysis

The duration of pre flag leaf stage, duration of flag leaf stage, duration of emergence stage and the percentage values for total flowered stalks, were transformed to the corresponding angle values in degrees ARC-Sin according to Evwin *et al.,* (1966).Hierarchical cluster analysis was performed on the standardized data using a measure of Euclidean distance and Ward minimum variance method as outlined by Ward (1963).

RESULTS AND DISCUSSION

The eighty- seven sugar cane germplasm under study were categorized into four groups. The first group included thirty-one sugarcane genotypes that flowered in both two seasons. The second group consisted of twenty-seven genotypes that flowered only in plant cane season. The third group included six genotypes that flowered only in first ratoon season and they were 86E409, EH77/31-56, EH85/3-35, Mex 58-1866, CI 47-83 and G 2008-52. The fourth group included twenty-three genotypes that did not show any flowering response neither in plant cane nor first ratoon. Therefore, the eighty-seven studied sugarcane genotypes various considerably among themselves in their response to flowering under both two seasons (plant cane (PC) and first ratoon (FR)). Flowering for genotypes in PC was higher than FR season because percentage of daily humidity for plant cane were higher than first ratoon) during flowering stages and the number of days for flowering under the optimum temperature (18-31°C) during three month.

Cluster analysis (CA)

Cluster analysis is a tool for classifying substances into groups. The cluster analysis was used as an efficient

Table 1: Source country of sugarcane genotypes studied.

No.	Genotype	Source	No.	Genotype	Source	No.	Genotype	Source
1	86E409	Mauritius	30	EH 67-11	Unknown	59	G 2009-85	Egypt, Giza
2	62 D 509	Guyana, Demerara	31	EH 7311	Unknown	60	G 2009-98	Egypt, Giza
3	82 G 98	Egypt, Giza	32	EH77/31-56	Egypt, Hawamdeia	61	G 69-55	Egypt, Giza
4	84 C 130	Cuba, Central Jaranu	33	EH85/3-35	Egypt, Hawamdeia	62	G 70-112	Egypt, Giza
5	84 E 1	Mauritius	34	EH87/28-4	Egypt, Hawamdeia	63	G 73-189	Egypt, Giza
6	86 D 1	Guyana , Demerara	35	EH87/40-17	Egypt, Hawamdeia	64	G 73-36	Egypt, Giza
7	86 D 296	Guyana , Demerara	36	EH89/101-5	Egypt, Hawamdeia	65	G 74-99	Egypt, Giza
8	86 L 37	USA (Louisiana)	37	EI 2-44	Salvador	66	G 75-314	Egypt, Giza
9	B 34-104	Barbados	38	EI 264-2	Salvador	67	G 87-149	Egypt, Giza
10	B 36-21	Barbados	39	EI 266-2	Salvador	68	G 95-19	Egypt, Giza
11	B 37-61	Barbados	40	EI 37-10	Salvador	69	G 98-132	Egypt, Giza
12	B 52-268	Barbados	41	EL 18-1	Salvador	70	G 99-160	Egypt, Giza
13	BO 19	India, Bihar, Orissa	42	EL 18-4	Salvador	71	G200-5	Egypt, Giza
14	BO 21	India, Bihar, Orissa	43	EL 4-4	Salvador	72	G98-28	Egypt, Giza
15	BO 22	India, Bihar, Orissa	44	EN 1-33	Egypt- South Africa (Natal)	73	H 86-371	USA,Hawaii
16	BO 41-227	India, Bihar, Orissa	45	EN 3-3	Egypt- South Africa (Natal)	74	H 86-97	USA,Hawaii
17	BO 41-24	India, Bihar, Orissa	46	EN 3-7	Egypt- South Africa (Natal)	75	IK 76-79	Unknown
18	CB 61-58	Brizel, Campos, Rio de Janeira	47	EN 5-2	Egypt- South Africa (Natal)	76	IR 26-13	Iran
19	CI 47-83	USA, Florida, Clewiston	48	F 153	Taiwan	77	IS 76-183	Bangladesh, Ishurdi
20	CO 1157	India, Coimbatore	49	G 2007-111	Egypt, Giza	78	L 61-67	USA (Louisiana)
21	CO 214	India, Coimbatore	50	G 2007-28	Egypt, Giza	79	M 253-48	Mauritius
22	COK 30	India, Coimbatore	51	G 2008-52	Egypt, Giza	80	Mex 58-1866	Mexico
23	Crystalina	New Guinea	52	G 2009-2	Egypt, Giza	81	N 11	South Africa, Natal
24	EH 128-2	Unknown	53	G 2009-21	Egypt, Giza	82	Ph 10	Philippines
25	EH 1-5	Unknown	54	G 2009-31	Egypt, Giza	83	PS 59	Java, Pasuruan
26	EH 16-1	Unknown	55	G 2009-41	Egypt, Giza	84	PS 80-1007	Java, Pasuruan
27	EH 16-9	Unknown	56	G 2009-42	Egypt, Giza	85	PS 87-23004	Java, Pasuruan
28	EH 26-2	Unknown	57	G 2009-56	Egypt, Giza	86	SP 81-1763	Brazil, Sao Paulo
29	EH 26-3	Unknown	58	G 2009-84	Egypt, Giza	87	IAC 5120	Brazil, Campinas

Table 2: Distribution of the tested genotypes according to their flowering response under plant cane and first ratoon.

No	Flowering in both seasons	No	Flowering in only plant cane	No	Flowering in only the first ratoon	No	No flowering
1	EH 89/101-5	1	EI 37-10	1	86E409	1	86 L 37
2	EH87/28-4	2	86 D 1	2	EH 77/31-56	2	86 D 296
3	84 E 1	3	EI 2-44	3	EH 85/3-35	3	CO 214
4	62 D 509	4	EI 264-2	4	Mex 58-1866	4	84 C 130
5	EI 266-2	5	CO 1157	5	CI 47-83	5	IS 76-183
6	BO 19	6	EL 4-4	6	G 2008-52	6	G2000-5
7	EN 3-3	7	82 G 98			7	Crystalina
8	M 253-48	8	EH 87/40-17			8	IR 26-13
9	BO 22	9	IK 76-79			9	BO 21
10	BO 41-227	10	EN 3-7			10	G 95-19
11	EH 67-11	11	EN 1-33			11	H 86-97
12	EH 7311	12	G 75-314			12	PS 87-23004
13	EH 1-5	13	H 86-371			13	PS 59
14	EH 16-1	14	CB 61-58			14	PS 80-1007
15	EH 26-2	15	EH 16-9			15	EN 5-2
16	EH 128-2	16	EH 26-3			16	F 153
17	EL 18-1	17	L 61-67			17	TAC 5120
18	G 74-99	18	G 69-55			18	EL 18-4
19	G 73-189	19	G 73-36			19	G 87-149
20	N 11	20	G 70-112			20	G98-28
21	BO 41-24	21	B 52-268			21	SP 81-1763
22	B 36-21	22	B 34-104			22	Ph 10
23	G 99-160	23	G 2009-41			23	G 98-132
24	B 37-61	24	G 2007-28				
25	G 2009-2	25	G 2007-111				
26	G 2009-84	26	G 2009-42				
27	G 2009-31	27	G 2009-21				
28	G 2009-85						
29	G 2009-98						
30	G 2009-56						
31	COK 30						

Table 3: Distances between cluster centroids for genotypes were flowered in only plant cane.

	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	2.41	2.10	5.49	6.78
Cluster 2		1.24	4.41	5.16
Cluster 3			4.16	6.40
Cluster 4				7.66

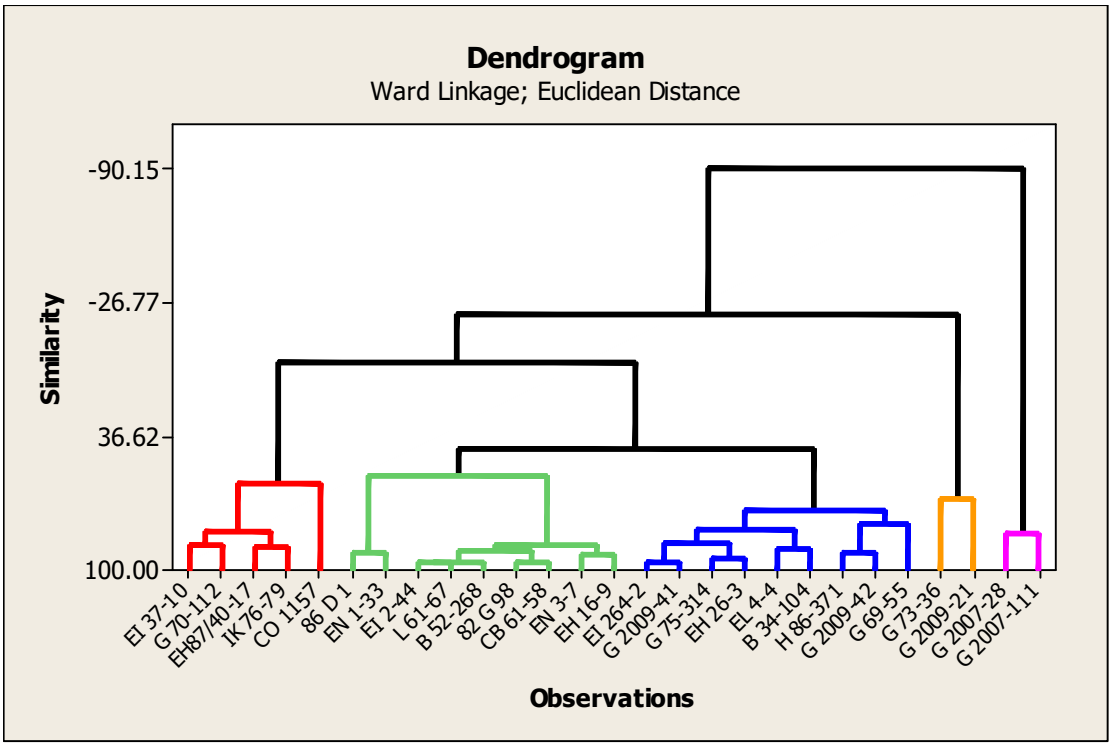

Figure 1. Dendrogram showing the distance among twenty- seven sugarcane genotypes wereflowered in only plant cane based on flowering stages and its related attributes.

procedure to emerge the structural relationships among studied genotypes and suggestions a hierarchical classification of them.

First group: flowering in only plant cane

Genetic diversity

Twenty-seven genotypes of sugarcane were clustered into five clusters based on D2 -statistics in such way that genotypes within a cluster had a low D2 values than those of in-between the traits. The composition of clusters has been depicted in (Table 3 and Figure 1). The distribution pattern of genotypes showed that cluster 1 and cluster 2, the both had maximum number of genotypes (9) followed by cluster 1 (5) genotypes. The inter-cluster distance differed from 7.66 to 1.24. The highest inter-cluster distance was observed in the cluster 4 and 5 (7.66). Conversely, minimum distance was noticed in cluster 2 and 3 (1.24); showing close relationship between these clusters would not provide good results. The existence of genetic divergence among the twenty-seven genotypes of sugarcane was examined by employing Mahalanobis's D2 statistic. The clustering pattern of these genotypes on the basis of D2 analysis has been presented in (Table 3). The sugarcane genotypes were grouped into five distinct clusters. In the present study, based on Euclidean distance, the studied varieties were estimated with flowering and its related traits and were discriminated as exposed in Figure 1. Mean values for pre-flag leaf, flag leaf, and emergence stages, Minimum and maximum flowering days, flowering period duration, and percentage of total flowered plants. Data in (Table 4) showed that the highest value of pre

Table 4: Summary of cluster analysis showed the twenty-seven sugarcane genotypes were flowered in only plant cane.

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Group	Genotype	Duration of pre flag leaf stage	Duration of Flag leaf stage	Duration of Emergence stage	Minimum days to flower	Maximum days to flower	Duration of flowering period
Cluster 1	EI 37-10, CO 1157 , H87/40-17, IK 76-79 G 70-112	155.2	31.4	8.0	186.6	194.6	9.0
Cluster 2	82G 98, 86D1 EI 2-44, EN 3-7 EN 1-33,CB 61-58, EH 16-9 L 61-67, B 52-268	228.4	14.5	12.3	242.9	255.2	13.3
Cluster 3	EI 264-2,EL 4-4,G 75-314, H 86-371, EH 26-3, B 34-104 G 2009-41,G 2009-42, G 69-55	157.2	14.9	13.1	172.1	185.2	14.1
Cluster 4	G 73-36,G 2009-21	179.0	14.0	35.0	193.0	228.0	36.0
Cluster 5	G 2007-28 , G 2007-111	140.5	11.0	10.5	151.5	162.0	11.5

flag stage (228.4 day) was recorded by Cliuster2, while Cliuster5 recorded the lowest value (140.5 day) of this trait. Data showed the superiority of Cliuster5 in pre flag stage over the other four Clusters, since it early 14.7, 16.7, 38.5 and 87.9 days of pre flag stage over those given by Cliuster1, liuster3, Cliuster4 and Cliuster5, respectively. Correspondingly result in (Table 4) cleared that the best value of flag stage (11.0 day) was obtained by Cluster 5, while the late value (31.4 day) was recorded by Cluster 1. Moreover, Cluster 1 superior the other four Clusters in Emergence stage. Cluster 1 recorded short time in Emergence stage (8.0 day), while Cluster 4 recorded (35.0 day). Also result in (Table 4) cleared that the highest value of Duration of flowering (36.0 day) was recorded by Cluster 4, while Cluster 1 recorded the lowest value (9.0 day) of this trait. As well as, it was noticed that the superiority of Cluster 4 in Duration mainly attributed to its superiority in Emergence stage. Cluster 5 gave the lowest value of Minimum days to flower and Maximum days to flower (151.5 – 162 day) due decrease in value of Pre flag stage and flag stage compared with other Clusters, while Cluster 2 recorded the highest value of minimum days to flower and maximum days to flower (242.9 – 255.2 day) due increase in value of Pre flag stage.

Second group: flowering in both plant cane and first ratoon

Genetic diversity

Thirty- one sugarcane genotypes flowered in both plant cane and first ratoon were grouped into four clusters based on D2 -statistics in such a way that genotypes within a cluster had a low D2 values than those of in-between the characters. The composition of clusters has been depicted in (Table 5 and Figure 2). The distribution pattern of genotypes showed that cluster 1 and cluster 2, the both had maximum number of genotypes (9) followed by cluster 1 (5) genotypes. The inter-cluster distance differed from 10.43 to 1.57. The highest inter-cluster distance was observed in the cluster 1 and 3(10.43). Contrariwise, minimum distance was noticed in cluster 1 and 4 (1.57); display close relationship between these clusters would not provide good results. Data presented in Table (6) showed that the highest value of pre flag stage (156.91 day) was recorded by Cluster 3, while Cluster 1 recorded the lowest value (76.0 day) of this trait. By contrast, Results cleared that the Cluster 2 had the shortest time in Flag stage over the other three Clusters, while Cluster 3 recorded the long period (38.05 day). Also result in (Table6) cleared that the highest value of emergence stage (17.27 – 16.0 days) was obtained by Cluster 3 followed by Cluster 1 receptivity, while the lowest value (10.5 day) was recorded by Cliuster 2. Cluster 1 gave the lowest value of Minimum days to flower (99.0 day) due decrease in value of Pre flag stage compared with other Clusters, while Cluster 3 recorded the highest value of Minimum days to flower (194.95 day) due increase in value of Pre flag stage. Cluster 3 gave the highest value of Maximum days to flower (212.23 day) due increase in value of Pre flag stage, flag stag and emergence stage. As well Cluster 4 recorded the lowest value of Maximum days to flower (115.0 day) due decrease in value of Pre flag stage compared with other Clusters. Data in (Table 6) showed that Cluster 3 superior of the other four Clusters in Duration. This superiority of Cluster 3 in Duration mainly attributed to the difference between maximum days to flower and Minimum days to flower. The highest value of flowering% (43.56 and 39.83%) was obtained by Cluster 2 followed by Cluster 4 receptivity, meanwhile the lowest value (33.3 and 35.7%) recorded by Cluster 1 and Cluster 3 respectively.

Third group: genotype flowered only in first ratoon

Results given in (Table 7) showed that the genotypes

Table 5: Distances between cluster centroids

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	Cluster2	Cluster3	Cluster4
Cluster1	3.74	10.43	1.57
Cluster2		9.00	2.33
Cluster3			9.39

Table 6: Summary of cluster analysis showed the thirty -one sugarcane genotypes were flowered in both plant cane and first ratoon.

	Genotype	Pre flag stage	Flag stage	emergence stage	Minimum days to flower	Maximum days to flower	Duration of flowering period	Flowering %
Cluster 1	84E1	76.00	23.00	16.00	99.00	115.00	17.00	33.30
Cluster 2	EH87/28-4, EI 266-2, BO 19,G 2009-85, G 2009-56	103.40	13.60	10.50	117.00	127.50	11.50	43.56
Cluster 3	BO 22, 62D 509, EN 3-3, EH 73-11, EL 18-1, G 74-99, G 73-189 N 11, G 2009-84, B 36-21 G 99-160	156.91	38.05	17.27	194.95	212.23	18.27	35.70
Cluster 4	G 2009-98,EH89/101-5, COK 30, B 37-61, G 2009-2, BO 41-24, EH 1-5, EH 16-1, EH 26-2 EH 128-2, BO 41-227, EH 67-11 M 253-48,G 2009-31	120.68	14.32	13.36	135.00	148.36	14.36	39.83

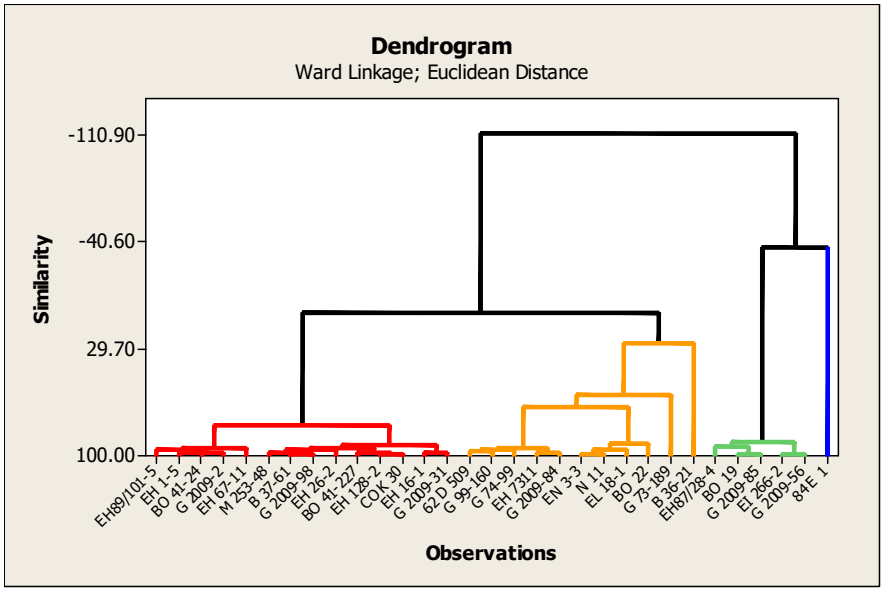

Figure 2: Dendrogram showing the distance among thirty- one sugarcane genotypes flowered in both plant cane and first ratoon based on flowering stages and its related attributes.

CI47-83 and G 2008-52 produced the highest value of Pre flag stage (227.0 day), followed by 85/3-35genotype (255 day).While 86E409 genotype had the lowest value of this trait (99.0 day). Furthermore, the present results revealed Sugarcane genotype CI 47-83 out flag stage 77/31-56 and G 2008-52 genotypes in flag stage by 2 and 3 day, while Mex 58-1866 genotype recorded the lowest value (3.5 day) in flag stage. Data disclosed that Mex 58-1866 genotype recorded the highest value of Emergence stage (70.0 day) due decrease in value of flag stage compared with other genotypes. While the lowest value (6.5 day) was recorded by G 2008-52

Table 7: Summary of the twenty-seven sugarcane genotypes were flowered only in first ratoon.

Genotype	Duration of pre flag leaf stage	Duration of Flag leaf stage	Duration of Emergence stage	Minimum days to flower	Maximum days to flower	Duration of flowering period	Flowering %
86E409	99	13.0	7.0	112.0	119.0	8.0	33.3
77/31-56	125	18.0	12.0	143.0	155.0	13.0	20.0
85/3-35	255	7.0	10.0	262.0	272.0	11.0	15.4
Mex 58-1866	108	3.5	70.0	111.5	181.5	71.0	14.3
CI 47-83	277	20.0	20.0	297.0	317.0	21.0	20.0
G 2008-52	277	17.0	6.5	294.0	300.5	7.5	18.2

genotype. In contrast,86E409 genotype gave the lowest value of Minimum days to flower (112.0 day) due decrease in value of Pre flag stage compared with other genotypes, while CI 47-83 genotype recorded the highest value of Minimum days to flower (297.0 day) due increase in value of Pre flag stage. CI 47-83 genotype gave the highest value of Maximum days to flower (317.0 day) due increase in value of Pre flag stage and flag stag. Also 86E409 genotype recorded the lowest value of Maximum days to flower (119.0 day) due decrease in value of Pre flag stage compared with other genotypes. Also result in (Table 7) cleared that the highest value of Duration (71.0 day) was recorded by Mex 58-1866 genotype, while G 2008-52 genotype recorded the lowest value (7.5 day) of this trait. As well as, it was noticed that the superiority of Mex 58-1866 genotype in Duration mainly attributed to its superiority in Emergence stage. The highest value of flowering% (33.3 %) was obtained by 86E409 genotype; meanwhile the lowest value (14.3 %) recorded by Mex 58-1866 genotypes.

Conclusion

The eighty-seven studied sugarcane genotypes various considerably among themselves in their response to flowering under both two seasons (plant cane (PC) and first ratoon (FR). Flowering for genotypes in PC was higher than FR season because daily humidity %for PC (2019/2020).Twenty-seven genotypes of sugarcane were clustered into five clusters based on D2 –statistics**.** Minimum distance was noticed in cluster 2 and 3 (1.24); showing close relationship between these clusters would not provide good results**.** Result cleared that the highest value of Duration of flowering (36.0 day) was recorded by Cliuster4, while Cliuster1 recorded the lowest value (9.0 day)**.** Cliuster4 recorded the lowest value of Maximum days to flower (115.0 day) due decrease in value of Pre flag stage compared with other Clusters. It was noticed that the superiority of Mex 58-1866 genotype in Duration mainly attributed to its superiority in Emergence stage.

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