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STUDY ON GENETIC DIVERSITY IN FORAGE PEARL MILLET (PENNISETUM GLAUCUM (L.) R. BR.) GENOTYPES UNDER NORTH GUJARAT CONDITION

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ABSTRACT A study on genetic diversity by basis statistics and Mahalanobis $D²$ cluster analysis of 13 fodder related traits in a set of 30 genotypes of forage pearl millet was conducted during *kharif* season (July-October) 2023 in a Randomized Block Design (RBD) with three replications Centre for Forage Research Station, S. D. Agricultural University, Sardarkrushinagar to identify potential parents for producing high-yielding hybrids. The thirty genotypes of forage pearl millet were grouped into eight cluster using Tocher method. Among them, three clusters are poly-genotypic. Cluster V was the largest cluster which included 10 genotypes followed by cluster III which included 6 genotypes. Cluster II and VII included 5 genotypes each while cluster I, IV, VI and VIII are mono-genotypic and comprised one genotypes each. Considering the magnitude of genetic distance, the per cent contribution of traits towards divergence (crude protein content, plant height, number of tillers per plant) along with the highest cluster means of these traits reflected by the genotypes belonging to clusters I and VI or I and VII or IV and VII through cluster analysis. Selecting genotypes with desirable characteristics for a breeding program would be made possible by a broad range of variety for the majority of the traits evaluated. *Keywords*: forage pearl millet, mahalanobis D^2 cluster analysis, genetic diversity

Introduction

 Pearl millet (*Pennisetum glaucum* (L.) R. Br.) feeds millions of impoverished households and their animals with food, fuel, fodder, and feed. It originated in Africa 4900 years ago. Its evolution under difficult conditions has allowed it to endure extreme climatic conditions in which other main cereal crops fail to flourish (Muimba-Kankolongo, 2021). It is a significant millet crop in India, cultivated over approximately 7.41 million hectares, with about 0.9 Mha of area dedicated to fodder (Satyavathi *et al.,* 2021). During the *summer* and *kharif* seasons, it is produced largely as a fodder crop and as a grain crop under rainfed conditions. In the hottest and driest areas of Southern India, pearl millet is also cultivated during the Rabi season. It has enormous potential and makes an ideal fodder crop. Its fodder is high in protein, calcium, phosphorus and other minerals, while being low in undesirable components such as hydrocyanic acid and oxalic acid (Gupta, 1975). As a fodder crop, it is leafy, nutritious and palatable. It can be fed to animals at any crop stage and is primarily present in two plant morphologies: dwarf bushy type (used for grazing) and tall type (used for green fodder, silage and hay) (Hancock *et al*. 2009). It provides crude protein and total digestible nutrients to cattle at 33% and 66%

less cost, respectively, as compared to the concentrated feed (Gorti *et al*., 2012), making it a valuable feed source for dairy farmers. In India, around 10 million farmers are engaged in the dairy sector, and for many, it serves as their sole source of income (Anonymous, 2022).

In the most recent census, 535.70 million livestock population is recorded, reflecting a 4.6% increase from the previous one (Anonymous, 2019). Thus, providing food for this expanding population will be a significant challenge. Lack of fodder drives up the price of concentrated feed and fodder, which impacts marginal and landless dairy producers and ultimately drives up the price of dairy products. Moreover, according to UNDESA's 2017 forecast, if the current pace of population growth continues, the world's population will reach 9.8 billion by 2050, which will result in a decline in the amount of cultivable land available for food production. This could result in similar feeding challenges for both animals and humans. Proactive steps should be taken to solve this issue given the predicted increase in fodder shortages in the future. One approach to address this issue is to increase crop production per unit area by developing high-yielding and quality fodder pearl millet genotypes. This can be achieved by leveraging the available genetic potential in the pearl millet gene pool along with implementing good cultivation practices.

 In order to utilize the germplasm in breeding activities, we need to understand the genetic

variability. The study of the level and patterns of genetic divergence in the forage pearl millet genotypes provide knowledge and idea of the genetic variability (Bhati *et al*., 2015). Information on genetic diversity facilitates parental selection from a huge number of germplasms. By creating an appropriate breeding plan, the data on genetic divergence and distance between parent genotypes can be used to evaluate a possible heterotic combination before trying crosses, saving time and money (Acquaah, 2009). Mahalanobis *D* 2 statistics is an effective tool in quantifying the degree of genetic divergence at the genotypic level based on generalized distance (Mahalanobis, 2018) Multivariate analysis, utilizing Mahalanobis D^2 statistics, has been found to be a potent biometrical tool in quantifying the degree of divergence in a germplasm collection of various crop plants (Rao, 1952) Mahalanobis *D* 2 statistics is very useful in selection of the parents in hybridization.

The present study aims to determine the relation among individuals, to estimate the relative contribution of various traits, and to guide the selection of the parents to develop transgressive segregation.

Material and Method

Seed materials

The experimental material consisted of 30 (thirty) diverse genotypes selected based on diverse performance of various traits (Table 1). The seeds are obtained from Main Forage Research Station, Anand Agriculture University, Anand, India.

Table 1: List of forage pearl millet genotypes used for the research

Sr. No.	Genotype	Sr. No.	Genotype
G ₁	$GAF-1$	G16	ICMO-1604
G2	$AFB-13$	G17	RAJ BAJRA
G ₃	$AFB-14$	G18	BAJRA BAWAL
G4	$AFB-15$	G19	$RBB-1$
G ₅	$AFB-16$	G20	$JMP-18-7$
G6	$AFB-4$	G21	$AFB-42$
G7	$AFB-17$	G22	$AFB-43$
G8	$AFB-18$	G23	$AFB-44$
G ₉	$AFB-19$	G ₂₄	$AFB-3$
G10	$AFB-20$	G ₂₅	$AFB-23$
G11	ICMU-1616	G ₂₆	$AFB-24$
G12	BAIF	G27	$AFB-25$
G13	$HC-20$	G28	$AFB-66$
G14	$AFB-21$	G29	$AFB-67$
G15	$AFB-22$	G30	JAINT BAJARA

Experiment details

The experiment was conducted during *kharif* season (July-October) 2023. A set of 30 forage pearl millet were sown in a Randomized Block Design (RBD) with three replications at Centre for Forage Research Station, S. D. Agricultural University, Sardarkrushinagar- 385506 (24-19 N, 72-19 E, 154.52 masl). The plots consisted of three rows of each genotype with a spacing of 15cm between plants and 30 cm between rows. We followed to suggested agronomic and plant protection measures.

Recording of data:

Observations were collected on 5 arbitrarily selected forage pearl millet plants from each line and means were calculated for all the traits excluding days to flowering and days to maturity which were documented on plot basis. The detailed description of the characters studied are presented in Table 2.

Statistical analysis

Cluster analysis was performed using Tocher's method as proposed by Vasconcelos *et al.* 2007 called modified Tocher from biotools packages.

Character	Abbreviation	Procedure
Days to flowering	DF	Number of days taken from the date of sowing to the date when the stigma in the main shoot panicle emerged
Days to maturity	DM	Number of days taken from the date of sowing to the date on which the plant attains physiological maturity
Plant height (cm)	PH	Measured from the ground level to the tip of the spike of main tiller
Number of tiller per plant	NTP	The number of ears bearing tillers including main tillers were counted at the time of maturity
Stem thickness (cm)	ST	Tagged plants at fifth internode from the top was measured using Vernier calliper
Number of leaf per plant	NLP	Number of leaves per plant were recorded by counting the leaves from individual tagged plant
Leaf length (cm)	LL	Leaf length was measured from the fourth middle leaf
Leaf width (mm)	LW	Leaf width was measured from middle leaf
Leaf: Stem ratio	LSR	Fresh weight of all leaves (g) Leaf: stem ratio $=$ Fresh weight of stem (g)
Dry fodder content $(\%)$	DFC	Dry matter content (%) = $\frac{Dry}{Fresh \ weight (g)} \times 100$
Dry fodder yield per plant (g)	DFYP	Dry matter yield per plant was calculated by multiplying the green forage yield with dry matter per cent.
Crude protein content $(\%)$	CPC	Estimated from an oven dried sample following nitrogen estimation by Kjeldahl method (Jackson, 1958). was recorded by weighing the five plants immediately after harvest at flowering stage and mean value was recorded
Green forage yield per plant (g)	GFYP	Recorded by weighing the five plants immediately after harvest at flowering stage and mean value was recorded

Table 2: Characters studied along with the abbreviation and procedure

Result and Discussion

Basic statistics

The estimation of descriptive statistics *viz*., maximum, minimum, mean, standard error of mean (SEM) and coefficient of variation (CV) for the measured 13 traits indicated the existence of diversity among the genotypes (Table 3, Figure 1 and Figure 2). Among all the traits investigated, leaf width dry fodder content, dry fodder yield per plant and green forage yield per plant recorded variation in mean, range,

standard error of mean and coefficient of variation. The lowest variation in mean, range, standard error of mean and coefficient of variation was found in days to maturity, days to flowering and crude protein content. Results of the study revealed that there is a large quantity of variability for fodder related traits in forage pearl millet genotypes. Thus, genetic diversity analysis aids parent selection, maintains and utilizes desirable variations, and enhances insights into crop evolutionary pattern in breeding programme (Bollinedi *et al* 2020).

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	Table 5. Estimates of basic statistics for 15 trans in 50 forage pearl limite genotypes													
Sr. No.	Genotype	DF	DM	PH	NTP	ST	NLP	LL	LW		LSR DFC	DFYP	CPC	GFYP
G1	GAF-1	59	28	94.10	5.17	0.97	26.87	77.07	24.33	0.65	28.60	32.27	9.28	112.60
G2	$AFB-13$	62	31	112.93	2.73	0.73	26.87	76.20	23.53	0.74	29.13	33.83	11.36	108.63
G ₃	$AFB-14$	56	30	89.17	3.30	0.78	24.27	83.00 25.67		0.81	28.41	35.50	9.12	131.60
$\overline{G4}$	$AFB-15$	$\overline{56}$	30	98.07	3.17	1.18	18.47	79.53 30.87		0.78	27.94	30.27	9.54	142.27
G5	$AFB-16$	65	32	104.50	2.40	0.59	19.27	69.60 19.97		0.74	19.61	22.35	10.24	85.67
$\overline{G6}$	AFB-4	64	31	66.23	2.67	0.67	22.40	73.73	21.40	0.78	27.22	26.30	7.78	98.07
$\overline{G7}$	$AFB-17$	$\overline{62}$	$\overline{32}$	79.73	2.43	0.61	19.20	73.80 19.70		0.97	27.56	23.73	7.45	87.97
G8	$AFB-18$	59	30	67.70	2.83	0.72	24.33	75.53 24.13		0.73	25.87	28.43	6.70	103.93
G9	$AFB-19$	61	31	83.20	3.03	0.72	19.87	78.13	23.63	0.94	29.52	29.57	8.19	108.37
G10	$AFB-20$	61	30	89.20	2.90	0.70	22.47	77.07	23.93	0.57	31.10	31.84	9.45	107.20
G11	ICMU-1616	61	30	84.63	2.73	0.70	21.53	78.07 23.97		0.67	35.45	29.47	7.46	106.13
$\overline{G12}$	BAIF	$\overline{56}$	30	85.37	3.17	0.87	20.07	83.53	26.07	1.00	27.86	38.63	8.49	174.60
G13	\overline{HC} -20	$\overline{55}$	32	$\overline{5}5.17$	2.27	0.52	21.00	60.33 12.07		0.63	24.76	20.18	10.82	57.20
G14	$AFB-21$	$\overline{63}$	32	64.83	2.30	0.62	19.80	68.80	20.33	0.77	21.42	22.90	8.14	81.93
G15	$AFB-22$	$\overline{63}$	31	80.80	2.60	0.67	24.67	76.73	20.47	0.80	26.19	26.90	7.14	102.23
G16	ICMO-1604	64	31	80.30	$2.\overline{63}$	0.63	24.53	73.60	23.30	0.80	27.17	22.72	9.71	99.33
G17	RAJ BAJRA	62	32	77.23	2.43	0.61	25.40	74.80	20.20	0.89	27.61	22.39	8.28	87.57
G18	BAJRA BAWAL	$\overline{62}$	32	71.37	2.33	0.62	17.47	73.40 19.40		0.63	21.25	22.60	7.83	82.57
$\overline{G19}$	$RBB-1$	43	$\overline{33}$	57.13	2.07	0.59	20.67	65.73 19.37		0.69	22.08	16.70	7.97	81.70
G20	JMP-18-7	$\overline{50}$	33	63.73	2.20	0.56	18.87	62.80 19.33		0.72	23.51	16.17	9.92	76.70
G21	$AFB-42$	64	31	59.27	2.63	0.68	25.87		61.60 23.30	0.69	26.97	29.07	10.36	100.10
G22	AFB-43	60	30	82.80	2.83	0.71	17.20	76.27	24.13	0.49	25.32	28.57	7.66	104.20
G23	AFB-44	$\overline{62}$	32	65.00	2.37	0.60	19.00	76.93	20.00	0.75	20.58	19.86	9.29	84.03
$\overline{G24}$	AFB-3	46	$\overline{33}$	61.70	2.20	0.57	19.13	61.73	19.33	0.79	22.81	19.43	8.13	76.83
$\overline{G25}$	$AFB-23$	54	$\overline{32}$	51.87	2.23	0.53	24.07	80.20 14.73		0.45	23.68	17.37	8.51	73.77
G26	$AFB-24$	57	29	94.60	3.43	0.90	28.53	53.20 24.57		0.83	28.58	32.53	8.93	114.47
G27	$AFB-25$	53	32	52.80	2.23		0.56 18.07	47.00 17.27		0.50	$\overline{23.51}$	17.57	7.98	75.50
G28	AFB-66	64	31	78.97	2.70	0.68	25.87	73.73	21.93	0.49	26.64	25.03	7.95	101.47
G29	$AFB-67$	63	31	80.77	2.60	0.66	20.47	75.60	20.40	0.83	27.44	27.53	9.21	96.73
$\overline{G30}$	JAINT BAJARA	$\overline{56}$	$\overline{30}$	100.87	3.23	0.75	19.33	83.80	25.43	0.63	28.21	30.20	8.58	154.27
	MEAN	$\overline{59}$	31	77.80	2.73	0.69	21.85	72.38	21.76	0.73	26.20	26.00	8.71	100.59
	MIN	43	28	51.87	2.07	0.52	17.20	47.00	12.07	0.45	19.61	16.17	6.70	57.20
	MAX	65	33	112.93	5.17	1.18	28.53	83.80	30.87	1.00	35.45	38.63	11.36	174.60
	$S.Em. \pm$	1.21	1.2	2.73	0.11	0.03	1.14	3.66	1.17	0.04	1.55	1.53	0.22	6.44
	$C.V.$ %	3.57	2.23	6.08		6.91 8.72	9.04	8.75	9.29		9.05 10.24	10.21	4.42	$\overline{11.09}$

Table 3: Estimates of basic statistics for 13 traits in 30 forage pearl millet genotypes

Fig. 1: Mean, minimum and maximum values of thirty forage pearl millet genotypes for thirteen traits

Fig. 2: Coefficient of variation of thirty forage pearl millet genotypes for thirteen traits

Cluster Analysis by Mahalanobis Distance

Clustering pattern

Mahalanobis generalized distance (*D* 2) was used to measure the genetic divergence among the genotypes and their grouping was done by Tocher's method given by Vasconcelos *et al*., 2007. The genotypes were grouped into eight clusters as listed in Table 4. Among them, three clusters are polygenotypic. Cluster V was the largest cluster which included 10 genotypes followed by cluster III which included 6 genotypes. Cluster II and VII included 5 genotypes each while cluster I, IV, VI and VIII are mono-genotypic and comprised one genotypes each. The explanation for this genetic diversity must be either intense natural and human selection for diverse and adaptable gene complexes, or the establishment of unique, solitary clusters maybe because their ancestors' geographic barriers impeded the gene flow Tiwari *et*

al. (2022). The least divergent genotypes were those that belonged to a similar cluster. A cross between genotypes from the same cluster is not likely to produce transgressive segregants. Therefore, a suitable transgressive segregant might be generated by using the parents from the several clusters with extreme divergence. This grouping pattern of genotypes suggested no parallelism between genetic divergence and the geographical distribution of genotypes.

Similar findings of Basavaraj *et al.* (2017), Kaushik *et al.* (2018), Kumar *et al.* (2020), Rasitha *et al*. (2020), Swaminathan *et al.* (2020), Sayed *et al.* (2022), Rajpoot *et al*. (2023) and Kavita *et al.* (2024) reported that the distribution of genotypes from different eco-geographical regions into clusters was at random, indicating geographical distribution does not necessarily exhibit genetic divergence.

Table 4: Distribution of forage pearl millet genotypes evaluated for green forage yield into different clusters of forage pearl millet

Cluster	No. of Genotype	Name of Genotype
		$GAF-1$
		ICMO-1604, AFB-13, AFB-16, AFB-20, AFB-67
Ш		AFB-14, AFB-19, ICMU-1616, BAIF, AFB-24, JAINT BAJARA
IV		$AFB-15$
	10	RAJ BAJRA, BAJRA BAWAL, AFB-4, AFB-17, AFB-43, AFB-18, AFB-44, AFB-66,
		AFB-21, AFB-22
VI		$HC-20$
VII		RBB-1, JMP-18-7, AFB-3 AFB-23, AFB-25
VIII		$AFB-42$

Cluster mean of 30 genotypes

The cluster mean values showed a wide range of variation for all the traits under study **(Table 5).** Cluster VIII had highest mean for days to flowering. Cluster VII had highest mean for days to maturity. Cluster VI had highest mean for dry fodder content and crude protein content. Cluster IV had highest mean for plant height, stem thickness, leaf length and leaf width. The highest cluster mean value for leaf stem ratio, dry fodder yield per plant and green fodder yield per plant was recorded in cluster III. Cluster I recorded highest

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mean for number of tiller per plant and number of leaf per plant. So, the improvement for a particular character can be done by selecting a genotype giving

the best performance in a particular cluster for a hybridization programme.

Table 5: Cluster mean for 13 different characters in thirty (30) genotypes of forage pearl millet

Cluster	DF	DM	PН	NTP	ST	NLP	LL	. . LW	LSR	DFC	DFYP	CPC	GFYP
		(2)	(3)	$\left(4\right)$	(5)	(6)	(7)	(8)	$\left(9\right)$	(10)	(11)	(12)	(13)
	59.00	84.33	94.10	5.16	0.97	26.86	77.06	24.33	0.65	28.59	32.27	9.28	112.6
П	63.20	93.33	93.54	2.65	0.66	22.72	74.41	22.23	0.73	27.64	27.65	9.99	99.51
Ш	57.94	89.39	89.64	3.15	0.78	22.27	76.62	24.89	0.81	25.46	32.65	8.46	131.57
IV	56.00	90.00	98.07	3.17		18.47	79.53	30.86	0.77	21.42	30.27	9.54	142.27
v	62.20	94.00	73.47	2.55	0.65	21.53	74.37	21.17	0.73	26.62	24.67	7.82	93.39
VI	55.33	97.00	55.17	2.27	0.52	21.00	60.33	12.07	0.63	35.45	20.17	10.82	57.20
VII	49.20	98.33	57.45	2.19	0.56	20.16	63.49	18.01	0.63	22.83	17.45	8.50	76.90
VIII	63.66	93.33	59.27	2.63	0.67	25.87	61.60	23.30	0.68	29.13	29.07	10.36	100.10

Intra and inter cluster distance

The intracluster and intercluster distance (D^2) (Table 6 and Figure 3) indicated that the maximum intra cluster distance was observed for cluster III ($D^2 =$ 78.19) followed by cluster II ($D^2 = 75.26$). The least intra cluster distance ($D^2 = 0.00$) was observed for cluster I, IV and VI. The cluster with maximum no. of

genotypes (cluster V) showed intracluster distance of 50.42. The intracluster distance is due to the heterogeneous nature of the genotypes within a cluster. The low intracluster distance indicated that the genotypes in the clusters were closely related Sharma *et al*. 2020.

Table 6: Average intra and inter cluster D^2 value of thirty genotypes of forage pearl millet

		П	Ш	IV		VI	VП	VIII
	0.00	298.12	199.89	245.42	351.13	575.03	553.99	274.25
п		75.26	128.64	210.54	129.75	234.90	278.66	105.20
Ш			78.19	144.17	118.22	305.15	273.27	129.98
IV				0.00	286.25	371.44	418.46	231.29
v					50.42	247.33	156.02	101.93
VI						0.00	183.56	183.84
VII							65.27	188.15
VIII								0.00

Fig. $3:$ Cluster diagram showing average intra and inter cluster $D²$ value of thirty forage pearl millet genotypes

From Table 6 and Figure 3, the relative divergence of each from another cluster (i.e., inter cluster distance) indicated greater divergence between cluster I and VI ($D^2 = 575.03$), the former was characterized by higher number of tiller per plant and number of leaf per plant while the latter by dry fodder content and crude protein content. It was followed by cluster I and VII ($\overline{D}^2 = 553.99$) with cluster I having higher number of tiller per plant and number of leaf per plant and cluster VII having highest mean for days to maturity. Clusters IV and VII ($D^2 = 418.46$) were the next divergent clusters in which cluster IV recorded highest mean for plant height, stem thickness, leaf length and leaf width and cluster VII had highest mean for days to maturity. Moreover, Clusters IV and VI (D^2) = 371.44) were the fourth divergent cluster in the present findings. The larger distance between clusters implying greater genetic divergence between the genotypes of these clusters (Bekis *et al.,* 2021; Yadav, 2018). To attain maximal variability in the generational separation, the parents chosen for hybridisation should come from two clusters separated by greater distances (Sarker *et al*., 2013; Viana *et al*., 2015; Hoogerheide *et al*., 2017). However, the least inter cluster distance was observed between cluster V and VIII ($D^2 = 101.93$) and cluster VIII and II ($D^2 = 105.20$). Compared to genotypes grouped in other clusters, the genotypes that belonged to these clusters were comparatively closer to

one another. The crossing of genotypes from these clusters consequently results in reduced heterotic value in F1and a smaller range of variability in the segregating population (Ayenewet *et al*., 2020). The purpose of this analysis was to preserve a relatively wide genetic base and prevent the selection of parents from genetically homogeneous clusters (Sabesan *et a*l., 2009).

Percent contribution of 13 characters for divergence in forage pearl millet

The components of D^2 due to each trait variable were ranked in where rank I being allotted to the highest value. The total of these ranks over all conceivable combinations $[n (n-1)/2 = 435]$ would give indirect information about the trait's importance in terms of its percentage contribution to total divergence (Figure 4 and Table 7). Crude protein content (26.90 %) contributed the maximum towards genetic divergence followed by plant height (15.86 %), number of tiller per plant (11.26 %), leaf: stem ratio (10.80 %), dry fodder yield per plant (8.97 %), green forage yield per plant (8.51%) , days to flowering (8.05%) , stem thickness (4.83 %), leaf length (1.61 %), number of leaf per plant (1.38) , leaf width (1.15%) and dry fodder content (0.69%) while days to maturity contributed negligible towards the total genetic divergence.

Sr. No.	Characters	Time ranked first	Contribution $(\%)$
	Days to flowering	35	8.05
2	Days to maturity	0	0.00
3	Plant height	69	15.86
4	Number of tiller per plant	49	11.26
5	Stem thickness	21	4.83
6	Number of leaf per plant	6	1.38
7	Leaf length	7	1.61
8	Leaf width	5	1.15
9	Leaf: Stem ratio	47	10.80
10	Dry fodder content	3	0.69
11	Dry fodder yield per plant	39	8.97
12	Crude protein content	117	26.90
13	Green forage yield per plant	37	8.51

Table 7: Contribution of various traits towards total genetic divergence

Fig. 4: Contribution of various traits towards total genetic divergence

Conclusion

Genetic diversity is of major interest to plant breeders, more diverse the parents, greater are the chances of obtaining heterotic expression in F1 with possibility of broad spectrum of variability in segregating generations. Cluster analysis supported the results obtained by mahalanobis D^2 cluster analysis validated the diversity pattern in the pearl millet population. The wide genetic variability was found among thirty genotypes which were divided into eight clusters shows large variable cluster distance. Genotypes with high performance from clusters at a greater intercluster distance (clusters I and VI or I and VII or IV and VII) can be used in the breeding program to develop the superior hybrids by exploiting heterosis in segregating generation. Thus, the study will be highly beneficial to breeders for selection of the potential parental genotypes from the genotypes in the study.

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Authorship Contribution

This work was carried out in collaboration among all authors.

Design of work: Y. A. Viradiya, Ritu Sharma Data collection: Ritu Sharma, P. J. Patel

Data Analysis: Ritu Sharma, Disha R. Patel Drafting: Ritu Sharma, Y. A. Viradiya, Rajeshri G. Vekariya

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Conflicts of Interest

The authors declare that they have no competing interests related to this study

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