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Research Article

Genetic divergence analysis in maintainer (B) and restorer (R) lines of pearl millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract

The present experiment was performed to analyze genetic diversity among 37 parental lines of pearl millet includes 10 maintainer and 27 restorer lines for grain yield and its attributes through Mahalanobis D^2 statistics. All the 37 genotypes were assigned into eight different clusters. Cluster II was the largest one composed of 12 genotypes subsequently cluster V with eight genotypes, cluster VII contains seven genotypes and cluster I had five genotypes. Whereas, clusters III, IV and VIII were solitary. High order of genetic divergence was noticed between cluster VI and VII (109.29), followed by cluster VII and VIII (72.27), cluster III and VII (66.65). Hybridization between genotypes from divergent clusters and with the best mean performance for productivity traits could be beneficial for developing promising hybrids. The trait 1000 grain weight contributed a maximum (25.53%) towards genetic diversity followed by days to 50% flowering (19.22%), panicle girth (13.21%), panicle length (8.71%), panicle weight (7.81%) and plant height (7.36%). Hence, major emphasis could be given to these traits for a better selection of divergent genotypes.

Key words: Cluster, diversity, Maintainer line, Pearl millet, Restorer line

INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) popularly known as 'cumbu' or 'bajra' is a small-grained diploid (2n=2x=14) C_4 crop with high dry matter production capability. It is originated in central tropical Africa and is cosmopolitan within the drier tropics of India and Africa. In India, pearl millet is grown about 7.54 million hectares with production and productivity of being 10.36 million tonnes and 1374 kg/ha, respectively (Anonymous, 2021). It is the fifth most important staple grain crop next to rice, wheat, maize and sorghum. It is an outstanding multipurpose coarse cereal crop for grain, fodder and stover across a wide range of environments around the world. It can adapt well to drought and adverse agro-ecological conditions, capable of rapid and vigorous growth, hence it is grown under marginal lands with low soil fertility and high temperature.

Pearl millet is rightly termed as 'poorman's food' and 'powerhouse of nutrition' as it is a rich source of carbohydrates (67.1%), proteins (11.6%), fat (5.2%), minerals (2.7%) and dietary fibre (1.2g/100g). It is having a low glycemic index, better fat digestibility and various vitamins (thiamine, riboflavin and niacin). It supports weight loss and helps in reducing cholesterol levels (Pearl Millet News, 2020).

The use of CGMS in pearl millet is possible only when potent and diverse maintainers and restorers are identified from the germplasm for developing component lines in the hybridization programme. Male sterile and isogenic counterparts (A lines) of maintainer lines or newly derived CMS lines from potential maintainers through backcross breeding can be used as female

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parents and subsequently restorer lines as male parents to generate experimental hybrids. To identify the best cross combinations, inbred lines could be evaluated to enable the selection of superior ones as parents for hybrid development programmes to enhance grain yield. Mahalanobis D^2 statistics is a multivariate analysis tool to estimate the magnitude of divergence in germplasm collections and to access the relative contribution of different characters towards genetic divergence.

The present investigation was conducted to (i) study genetic diversity patterns among maintainers and restorer lines to develop promising hybrids of pearl millet, (ii) categorize the parental lines into different clusters (iii) identify distant parents to achieve yield stability and heterozygosity.

MATERIALS AND METHODS

Experimental material comprised of 10 maintainers (B) lines and 27 restorers (R) lines procured from ICRISAT, Patancheru, Telangana were evaluated during *summer,* 2020 using RBD with three replications at ARS, Perumallapalle, Tirupati, Andhra Pradesh **(Table 1).**

Recommended agronomic practices were followed to raise a standard crop. Each entry was grown in a single row with a spacing of 45 × 15 cm. Observations were noted on a plot basis for days to 50% flowering and days to maturity. Data were tabulated from five randomly selected plants in the centre of row of each entry of each replication on yield, yield attributing and water use efficiency traits *viz.,* plant height, the number of productive tillers per plant, panicle length, panicle girth, green fodder yield per plant, dry fodder yield per plant, panicle weight, 1000 grain weight, threshing percentage, harvest index, grain yield per plant, SPAD chlorophyll meter reading at 45 DAS and specific leaf area at 45 DAS. The data analysis was executed with WINDOSTAT software version 9.2. Genetic divergence among the parental lines was worked out by employing Mahalanobis (1936) D^2 statistics. Genotypes are distributed into different clusters based upon Tocher's method by Rao (1952).

RESULTS AND DISCUSSION

Based on the analysis of all the characters, the mean sum of squares was significant therefore, further analysis was done to compute D^2 values. The experimental material was distributed into eight definite and non-overlapping clusters on the basis of Mahalanobis squared Euclidean distance matrix, inferring the presence of diversity for all the characters studied (**Table 2 and Fig. 1**). Cluster II possessed a maximum of about 12 entries (3 'B' lines and 9 'R' lines) followed by cluster V comprising of eight genotypes ('R' lines), cluster VII had seven genotypes (1 'B' line and 6 'R' lines), cluster I contained five genotypes (3 'B' lines and 2 'R' lines) and the similar strong association was observed between ICMB 96555 and ICMB 98111 ('B' lines) in cluster VI. Whereas, clusters III, IV and VIII were solitary and had a single genotype each (ICMB 11999, ICMR 07888 and ICMR 08777, respectively). A study of diversity patterns with respect to the pedigree of genotypes revealed that genotypes with common parentage fell in either the same cluster or in different clusters with low inter cluster distances which indicated the presence of parallelism between genetic divergence and pedigree. Similarly, the grouping of 'B' and 'R' lines of pearl millet on basis of quantitative data was earlier obtained by Sumathi *et al*. (2016), Ramya *et al*. (2017), Kaushik *et al*. (2018), Sangwan *et al.* (2019) and Sharma *et al.* (2020) to identify potential parents for heterotic expression of yield components.

Inter and intra-cluster D^2 values for eight clusters are provided in **Table 3 and Fig. 2**. Intra-cluster distance varied between 0.00 and 40.57. Intra-cluster distance was maximum within cluster VII (40.57) then by cluster V (23.95) and cluster II (15.08) indicating presence of divergence among the genotypes of the same cluster. While inter-cluster distances exhibited a range of 16.36 to 109.29. A maximum inter cluster distance of 109.29 was noticed between cluster VI and VII consequently cluster VII and VIII (72.27) and between cluster III and VII (66.65). Whereas inter-cluster distance was registered minimum (16.36) between cluster I and IV succeeded by cluster II and IV (19.05) and between cluster I and III (19.71) which indicated the presence of the maximum number of gene complexes and narrow genetic diversity among parental lines of these clusters.

Based on inter-cluster distances the cluster combinations *viz.,* VI × VII, VII × VIII, III × VII, III × VIII and V × VI were found to be more divergent along with high mean performance for several characters. Hence, the genotypes of these clusters may have a better response to heterosis and crossing among them would be proposed to create a large spectrum of variation in hybrids and selection could be effective for improving yield and its component characters.

The cluster means for 15 characters are furnished in **Table 4**. Variation among the means of all the attributes indicated the divergent nature of clusters formed. All the mean values are ranked across the clusters for all fifteen traits. The first rank was given to the highest cluster mean and the clusters possessing next higher means were scored second, third and like up to the eighth rank for all characters except days to 50% flowering, days to maturity and SLA at 45 DAS to which the first rank was given to least mean. If the mean values of two clusters are similar, then the same rank was given for both the clusters. Accordingly, cluster VIII (ICMR 08777) secured the first rank with an overall score of 33 among the eight clusters followed by cluster VI (ICMB 96555, ICMB 98111) and cluster IV (ICMR 07888) indicating the

Table 1. List and pedigree details of 37 (10 B lines and 27 R lines) inbreds of pearl millet used in the study

S. No.	Inbred line	Pedigree	B/R line
1	842B	Selection from KSU line BKM 2221	B line
2	ICMB 11999	(DMR 133 x HTBC 48-B-1-1-1-5)-9-1-B-B-1	B line
3	ICMB 12222	(ICMB 95444 x ICMB 92111)-4-B-4-3-B-B	B line
4	ARD 132B	[(81B x SRL 53-1) x 843B]-30-2-B	B line
5	ICMB 94444	(843B x 405B)-4-B	B line
6	ICMB 96555	(SPF3/S91-5 x ARD early bulk)-2-3-3	B line
7	ICMB 97333	(ICMB 89111 x ICMB 88004)-9-2-6-3-3-2-B	B line
8	ICMB 98111	HTBC HS-27-1-1-1	B line
9	ICMB 98444	(BSECBPT/91-40 x SPF3/S91-529)-12-1-1-5	B line
10	ICMB 98777	[(F4FC 1498-1-1-3 x J 104)-11-2-1-1]-7-3-1-B	B line
11	ICMR 06111	MC 94 C2-S1-3-1-3-3-2-2-B	R line
12	ICMR 06222	SDMV 90031-S1-3-3-2-1-3-2-2-1-B	R line
13	ICMR 06333	SDMV 90031-S1-93-3-1-1-3-2-2-1-1-B	R line
14	ICMR 06444	[((MC 94 S1-34-1-B x HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-37-1-1-1-2-B	R line
15	ICMR 06555	AIMP 92901 S1-296-2-1-1-3-B-1-B-B	R line
16	ICMR 06666	MRC HS-91-2-3-3-B-B-B-B-B	R line
17	ICMR 06777	MC 94 C2-S1-47-1-1-3-B-1-B-B	R line
18	ICMR 06888	MRC HS-219-2-1-2-B-B-B-B	R line
19	ICMR 06999	MRC S1-4-1-3-B-B-B-B	R line
20	ICMR 07111	MRC HS-41-2-2-3-B-B-P1-B-B-B	R line
21	ICMR 07222	MRC HS-130-6-1-1-B-B-B-B-B-B	R line
22	ICMR 07333	JBV 3 S1-95-3-1-2-B	R line
23	ICMR 07444	ICMV 91059 S1-20-1-2-1-4-1-B-B	R line
24	ICMR 07555	ICMS 8511 S1-17-2-1-1-4-1-B-3-2-2-B	R line
25	ICMR 07666	ICMS 7704-S1-126-5-2-1-3-2-2-2-B-3	R line
26	ICMR 07777	Jakhrana × ESRC II S2-81-3-2-2-2	R line
27	ICMR 07888	(RCB-2-S1-138-1-3 × MRC)-B-2-1-2-B	R line
28	ICMR 07999	(ICMV-IS 94206-7 × (SRC II C3 S1-1-1-2 x HHVBC)-1-3-3))-B-10-1-2-2	R line
29	ICMR 08111	(ICMS 7704-S1-127-5-1 × RCB-2 Tall)-B-19-3-4-5-3	R line
30	ICMR 08222	ICMR 312 S1-8-1-1-1-1-B-B-B-1-B	R line
31	ICMR 08333	RCB-2 S1-19-2-2-1-2-3-2-1-B-B-B	R line
32	ICMR 08444	[(((ICMV-IS 94206-15) × B-Lines)-B-6) × (MRC S1-156-2-1-B)]-B-13-1-3-3-2-B	R line
33	ICMR 08555	ICMV 91059 S1-4-2-3-2-1-1-4-B-1-5-B-B	R line
34	ICMR 08666	HHVBC tall (C1) S1-33-3-1-1-1-2-B-B-3-2	R line
35	ICMR 08777	[((MC 94 S1-34-1-B x HHVBC)-16-2-1) × (IP 19626-4-2-3)]-B-18-2-2-4-1-B	R line
36	ICMR 08888	ICMS 7704-S1-52-3-1-2-1-2-1-6-B-B	R line
37	ICMR 08999	JBV 3 S1-18-2-2-1-3-2	R line

Table 2. Distribution pattern of 37 pearl millet inbred lines (B & R) into eight clusters

S. No.	Cluster	Number of Genotypes	'B' lines	'R' lines
1	I	5	ARD 132B, ICMB 97333, ICMB 98444,	ICMR 06555, ICMR 07555
2	II	12	842B, ICMB 12222, ICMB 98777	ICMR 06888, ICMR 06999, ICMR 07222, ICMR 07333, ICMR 07666, ICMR 07777, ICMR 08333, ICMR 08444, ICMR 08555
3	III	1	ICMB 11999	-----------------
4	IV	1	------------------	ICMR 07888
5	V	8	------------------	ICMR 06111, ICMR 06222, ICMR 06333, ICMR 06777, ICMR 07111, ICMR 07999, ICMR 08222, ICMR 08999
6	VI	2	ICMB 96555, ICMB 98111	------------------
7	VII	7	ICMB 94444	ICMR 06444, ICMR 06666, ICMR 07444, ICMR 08111, ICMR 08666, ICMR 08888
8	VIII	1	------------------	ICMR 08777

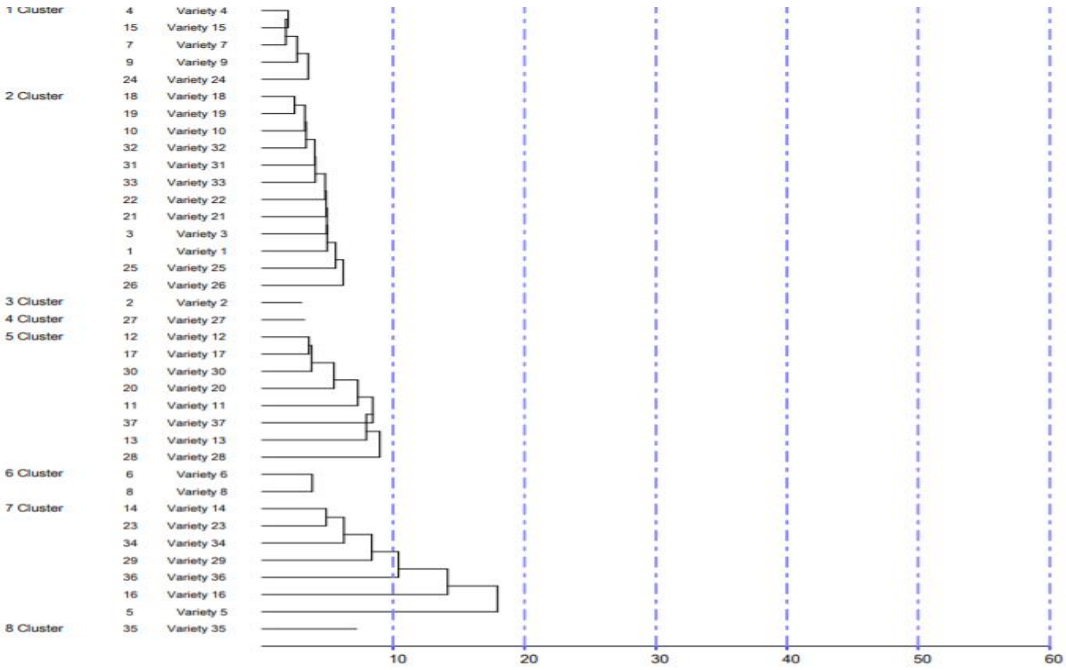

Fig. 1. Grouping of 37 inbred lines of pearl millet into eight clusters using Tocher's method

presence of superior lines in these clusters which can be widely used for the crop improvement programme. The least ranking was displayed by cluster VII next in order of I and III.

ICMB 11999 registered minimum values for days to 50% flowering (43.67) and maturity (78.33) which is desirable and also had the lowest cluster mean for panicle length (15.40 cm), plant height (107.07 cm) and dry fodder yield per plant (17.47 g). Among the maintainers evaluated, ICMB 11999 alone remained as single apart in cluster III stating that it is highly diverse and its counterpart (A line) can be used for the development of short duration hybrids. Similarly, monogenotypic cluster IV (ICMR 07888) recorded maximum values for the number of productive tillers per plant (2.53) and SPAD chlorophyll

Table 3. Inter (above diagonal) and Intra cluster (diagonal) D^2 and D values (in parenthesis) for eight clusters in 37 inbred lines of pearl millet

Clusters	I	II	III	IV	V	VI	VII	VIII
I	**9.51** **(3.08)**	21.21 (4.61)	19.71 (4.44)	16.36 (4.04)	25.78 (5.08)	32.95 (5.74)	51.75 (7.19)	46.74 (6.84)
II		**15.08** **(3.88)**	21.87 (4.68)	19.05 (4.36)	29.51 (5.43)	51.95 (7.21)	40.78 (6.39)	47.94 (6.92)
III			**0.00** **(0.00)**	23.84 (4.88)	45.59 (6.75)	49.13 (7.01)	66.65 (8.16)	66.09 (8.13)
IV				**0.00** **(0.00)**	31.69 (5.63)	36.88 (6.07)	48.43 (6.96)	28.81 (5.37)
V					**23.95** **(4.89)**	54.33 (7.37)	44.66 (6.68)	43.50 (6.60)
VI						**10.23** **(3.20)**	109.29 (10.45)	43.38 (6.59)
VII							**40.57** **(6.37)**	72.27 (8.50)
VIII								**0.00** **(0.00)**

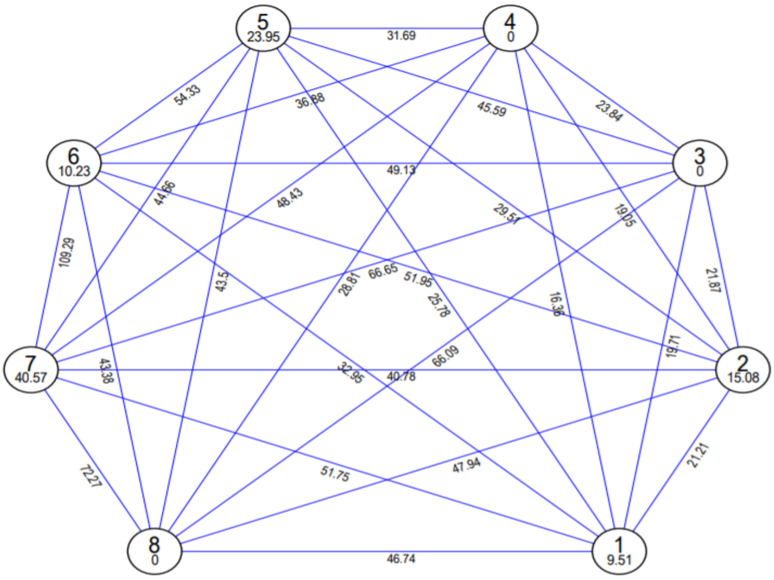

Fig. 2. Inter-cluster and Intra-cluster distances (D^2) among eight clusters of pearl millet

meter reading at 45 DAS (50.33). While cluster VI comprised of only 'B' lines with a high 1000 grain weight (13.74 g) and harvest index (42.27 %).

Furthermore, restorer line ICMR 08777 of cluster VIII was found to be highly diversified among 37 genotypes with the highest mean values for several characters *viz*., plant height (179.73 cm), panicle length (26.07 cm), panicle girth (2.95 cm), panicle weight (73.27 g), green fodder yield per plant (197.20 g), threshing percentage (69.65 %) and grain yield per plant (51.33 g) hence, it can be used as a potential pollinator for elite CMS lines to develop dual purpose hybrids with high grain and fodder yield potential. Contrastingly, genotypes of cluster VII were inferior for most of the characters. The number of times each character ranked first and their relevant contribution towards genetic divergence are depicted in **Table 5**. Thousand grain weight contributed maximum (25.53%) towards diversity followed by days to 50% flowering (19.22%), panicle girth (13.21%), panicle length

Table 4. Cluster mean analysis of yield and yield attributes with overall character wise score in 37 inbred lines of pearl millet

Clusters	Characters															Productivity Traits	
	DF	DM	SCMR	SLA	PH	NPT	PL	PG	GFY	DFY	PW	1000 GW	TH	HI	GY	Total score	Final Rank
I	49.20 (6)	84.67 (5)	45.75 (5)	210.11 (5)	118.20 (7)	1.29 (7)	16.91 (6)	2.25 (4)	60.93 (8)	17.60 (7)	19.64 (8)	10.65 (3)	59.24 (7)	31.12 (7)	11.82 (7)	92	7
II	46.86 (3)	82.81 (3)	46.06 (4)	204.45 (3)	134.39 (6)	1.73 (4)	19.92 (5)	1.94 (8)	96.21 (6)	23.71 (5)	27.87 (5)	8.11 (6)	60.53 (6)	32.62 (6)	17.16 (5)	75	5
III	43.67 (1)	78.33 (1)	45.30 (6)	232.21 (7)	107.07 (8)	2.27 (2)	15.40 (8)	2.13 (6)	71.73 (7)	17.47 (8)	21.40 (6)	7.86 (7)	64.51 (4)	35.03 (5)	13.76 (6)	82	6
IV	48.67 (5)	84.33 (4)	50.33 (1)	235.77 (8)	142.33 (5)	2.53 (1)	16.33 (7)	2.17 (5)	96.93 (4)	33.73 (2)	41.73 (3)	9.30 (5)	66.49 (3)	37.07 (3)	27.83 (3)	59	3
V	51.08 (7)	86.29 (6)	44.02 (7)	196.92 (2)	151.50 (3)	1.30 (6)	25.53 (2)	2.46 (3)	101.28 (3)	25.54 (4)	28.65 (4)	10.15 (4)	63.99 (5)	35.83 (4)	18.50 (4)	64	4
VI	43.83 (2)	79.33 (2)	50.28 (2)	208.27 (4)	159.73 (2)	1.73 (4)	21.70 (4)	2.63 (2)	109.93 (2)	29.73 (3)	46.53 (2)	13.74 (1)	69.19 (2)	42.27 (1)	32.26 (2)	35	2
VII	53.76 (8)	88.52 (7)	41.90 (8)	177.23 (1)	148.50 (4)	1.40 (5)	22.27 (3)	2.03 (7)	96.44 (5)	23.51 (6)	21.27 (7)	7.19 (8)	49.46 (8)	22.89 (8)	10.56 (8)	93	8
VIII	48.00 (4)	84.67 (5)	47.50 (3)	211.42 (6)	179.73 (1)	2.13 (3)	26.07 (1)	2.95 (1)	197.20 (1)	59.87 (1)	73.27 (1)	11.22 (2)	69.65 (1)	38.49 (2)	51.33 (1)	33	1

Note: Numbers in brackets indicate the given rank based on mean values. Total score is the aggregate of ranks for all traits and final rank is listed accordingly.

DF : Days to 50% flowering
DM : Days to maturity
SCMR : SPAD chlorophyll meter reading
SLA : Specific leaf area (cm^2g^{-1})
PH : Plant height (cm)
NPT : Number of productive tillers per plant
PL : Panicle length (cm)
PG : Panicle girth (cm)
GFY : Green fodder yield per plant (g)
DFY : Dry fodder yield per plant (g)
PW : Panicle weight (g)
1000 GW : 1000 grain weight (g)
TH : Threshing (%)
HI : Harvest index (%)
GY : Grain yield per plant (g)

Table 5. Number of times appearing first in ranking and per cent contribution of different characters towards genetic diversity in pearl millet

S. No.	Characters	Number of times appeared first	Contribution (%)
1	Days to 50 % flowering	128	19.22
2	Days to maturity	6	0.90
3	SPAD chlorophyll meter reading at 45 DAS	17	2.55
4	Specific leaf area at 45 DAS	4	0.60
5	Plant height	49	7.36
6	Number of productive tillers per plant	14	2.10
7	Panicle length	58	8.71
8	Panicle girth	88	13.21
9	Green fodder yield per plant	26	3.90
10	Dry fodder yield per plant	6	0.90
11	Panicle weight	52	7.81
12	1000 grain weight	170	25.53
13	Threshing	18	2.70
14	Harvest index	23	3.45
15	Grain yield per plant	7	1.05

(8.71%), panicle weight (7.81%), plant height (7.36%), green fodder yield per plant (3.90%) and harvest index (3.45%). In contrast, other traits attributed less role in cluster formation indicating narrow genetic diversity for those characters.

Conclusively, the present study grouped 37 inbred lines of pearl millet into eight distinct clusters. Based on genetic distances, crossing among maintainers of cluster VI (ICMB 96555 and ICMB 98111), cluster VII (ICMB 94444) and cluster III (ICMB 11999) and restorers of cluster VII (ICMR 06444, ICMR 06666, ICMR 07444, ICMR 08111, ICMR 08666 and ICMR 08888) and cluster VIII (ICMR 08777) is recommended as it may give elite hybrids of pearl millet. The heterotic pool of B and R lines can be developed from the identified superior parental lines.

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