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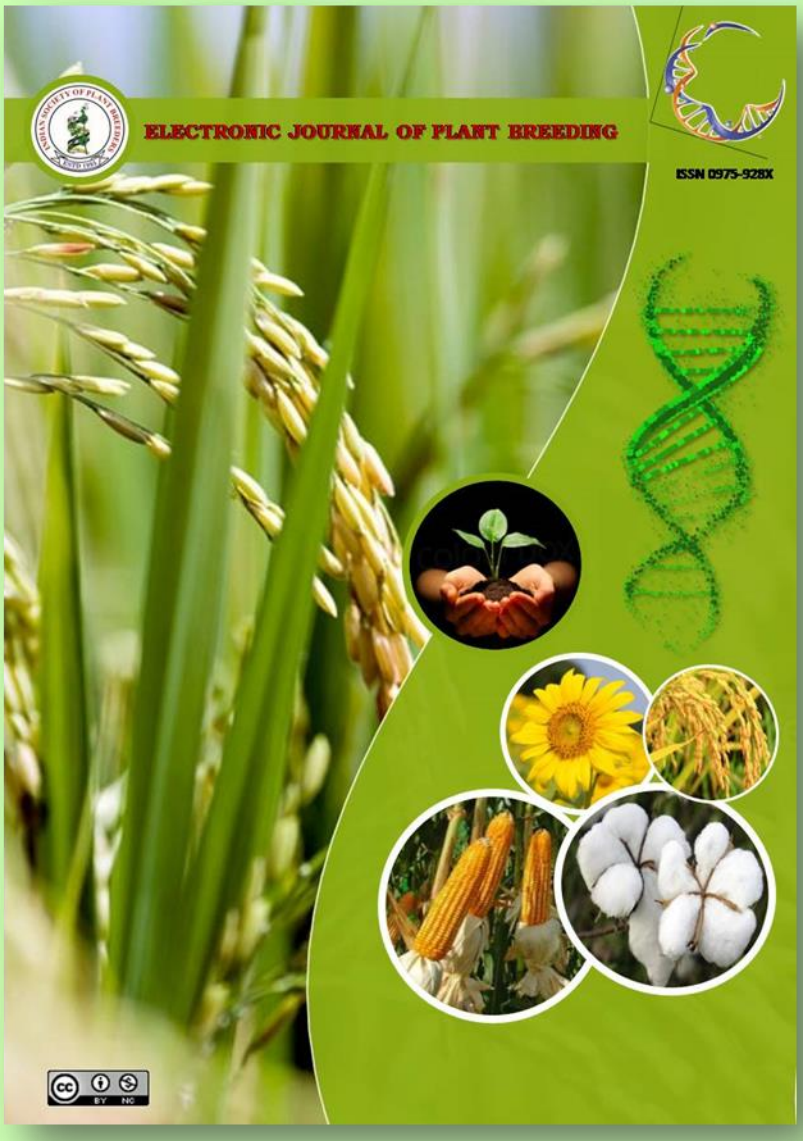

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Research Article

Genetic divergence among mutant genotypes of greengram [*Vigna radiata* (L.) Wilczek]

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Abstract
The nature and magnitude of genetic divergence was estimated among forty five mutant lines of greengram variety OBGG-52, developed by single and combination treatments with gamma rays, Ethyl Methane Sulphonate (EMS), N-methyl-N′-nitrosoguanidine (NG) and maleic hydrazide (MH) using multivariate analysis. These mutant genotypes were grouped in to 12 clusters based on D^2 values using Tocher's method. A large proportion of mutant lines showed divergence from the parent variety and also among themselves. No definite relationship of mutagenic origin and clustering of mutant lines were observed. The mutant lines developed from the same mutagenic treatments often grouped into different clusters indicating that each mutagenic treatment was effective in inducing diverse types of changes in the nine traits studied. Sixteen genotypes were grouped with their parents in cluster-I, while rest of 28 genotypes grouped in to another eleven divergent clusters. Cluster-IV had maximum intra-cluster distance (3.95), while inter-cluster distance was highest (8.27) between cluster-XI and cluster-XII. Genotype of Cluster-III was superior for yield per plant, seeds/pod where as Cluster-VIII was superior for 100-seed weight and pods per plant. Thus hybridization of genotype belonging to cluster-III with genotype in cluster-VIII is suggested for development of superior genotypes.

Key words
Greengram, Genetic divergence, Multivariate analysis, D^2 statistics, Micromutants

Introduction

Greengram (*Vigna radiata*) is one of the important food legumes in India whose genetic potential is yet to be fully exploited. For greengram, due to their autogamous nature and problem of flower drop, lack of synchronous maturity, susceptibility to disease like MYMV, improvement through hybridization and recombination becomes difficult. Since genetic variability is essential for crop improvement programme, induction of polygenic mutation by physical and chemical mutagens provide a powerful means of creating new and useful variability in greengram (Das *et al.* 2006). It would be of interest to as certain as to how the different micro mutant lines developed from the same parental variety differ among themselves and also from the parent. Such genetic diversity is generally considered as an important criterion for choosing diverse genotypes for the crop improvement. Greater the diversity in crop varieties better is the chance of evolving promising and desired types through hybridization technique. Multivariate analysis which takes into consideration of several quantitative traits simultaneously would be a dependable method in determining stable difference among the micro-mutants (Muduli and Das, 2014). The multivariate analysis based on D^2 technique has been found to be a powerful tool to estimates genetic divergence among the genotypes of a population and to classify the genotypes into relatively homogenous groups in such a way that within a cluster, diversity is minimized and between cluster diversity is maximized (Mahalanobis,1936). The respective genotypes from diverse clusters can be utilized in breeding programme depending upon breeding objectives. Genetic diversity analysis also helps in tagging and elimination of the duplicate accessions from genetic stock. It is generally assumed that the parents with more diversity involved in crossing programme give more heterosis than the closely related ones (Singh, 1991). Several researchers *viz.*, Katiyar *et al.* (2009); Abna *et al.* (2012); Patel and Patel (2012); Jayamani and Sathya (2013) also gave emphasis on need for high genetic diversity to create the high genetic variation and genetic gain under selection. The present investigation was under taken to study the genetic divergence among mutants developed by induced mutagenesis using multivariate analysis techniques.

Material and Methods

The material for present study comprised of 46 genotypes of greengram of which forty five are mutants developed by single and combined treatment with gamma rays, Ethyl Methane Sulphonate (EMS), N-methyl-N′-nitrosoguanidine (NG) and maleic hydrazide (MH) along with their

parents i.e OBGG-52. The single mutagenic treatments were 200, 400 and 600 Gy gamma rays (coded as G1, G2 and G3); 0.2%, 0.4% and 0.6% EMS (E1, E2 and E3) and 0.005%, 0.010% and 0.015% NG (N1, N2 and N3) and maleic hydrazide (MH) (0.01%, 0.02% and 0.03%). Three combined treatments of 400Gy gamma rays + 0.4% EMS, 400Gy gamma rays + 0.01% NG and 400Gy gamma rays + 0.02% MH were coded as GE2, GN2 and GM2, respectively. The source of gamma-rays was Gamma cell of Bhava Atomic Research Centre (BARC), Trombay, Mumbai. For treatment with EMS, NG and MH, the seeds (500 seeds for each code) were presoaked in distilled water for 6 hours, blotted dry and then treated with freshly prepared aqueous solution of above chemical mutagens for 6 hours, with intermittent shaking. For combination treatments, seeds were first irradiated with 400Gy gamma rays and then treated with 0.4% EMS or 0.01% NG or 0.02% MH solution in the same manner as described above. After treatment, the seeds were thoroughly washed with running water to bleach out the residual chemicals and then dried on blotting paper after treatment. A set of 500 healthy seeds was soaked in only distilled water which served as control. The M1 generation was grown with the utmost care self-pollination. Seeds from all the M1 plants were harvested separately and were advanced to M_2 generation. Individual selected plants from selected M_2 lines were harvested separately and grown as one line in M_3 generation. The selected M_3 progenies along with the parent variety were grown in M_4 generation. Within progeny selection was done for yield at 20 % selection intensity. In all 45 selected mutant progenies from fifteen mutagenic treatments (top three from each treatment) and the parent variety were carried forward next generation. The selected forty five mutant lines of M_4 generation along with the parent variety were grown M_5 generation in randomized block design with three replications during *rabi* season 2010. The experimental materials were sown in RBD with three rows of 3.0 meters length with a spacing of 25cm x 10cm at Odisha University of Agriculture and Technology, Bhubaneswar. Recommended doses of manures and fertilizers were applied and necessary plant protection measures were taken. Observation on days to flowering and days to maturity were recorded on plot basis where as other seven biometric characters i.e. plant height, clusters per plant, pods per plant, pod length, seeds per pod, 100-seed weight and yield per plant were recorded on ten random plants per plot in each replication. Genetic divergence with regard to nine characters were estimated by Mahalanobis D^2 statistics and genotypes were grouped into different clusters following Tocher's method (Rao, 1952).

Result and Discussion

The analysis of variance revealed significant differences among the mutant lines for all nine characters indicating that the mutagenic treatments were effective in inducing mutations in these polygenic traits and the treatments had wide diversity among themselves. All the mutant lines along with the parent variety were grouped into twelve genetic clusters by Tocher's method of grouping based on D^2 values (Table 1). Cluster II, the largest group, included the parent variety (OBGG-52) and sixteen mutant lines of which two were derived from gamma rays, two from EMS, four from MH and six from combination treatments. Cluster I comprised sixteen mutant lines of which five were from gamma rays, six from EMS, four from NG and one from combination treatments. Cluster III included three mutants while Cluster IV had two only. The rest clusters (V to XII) were represented by one mutant each which were produced from different mutagenic treatments. It is interesting to observe that the mutants developed from gamma ray treatments were distributed into four clusters (I, II, IX and XII) while those from EMS into three clusters (I, III and V) and also from NG into five different clusters (I, III, VI VII and VIII). Of the nine mutants from combination treatments, six were grouped in Cluster II (cluster with the parent) and one each in Cluster I, IV and IX. Clustering pattern of the mutants revealed that most clusters comprised mutants derived from different mutagenic treatments. Conversely, mutants derived from same mutagen also grouped into different clusters. So, no definite pattern of mutagenic treatment origin of the culture and their clustering was observed which corroborates the earlier studies (Mohapatra *et al*., 1987; Mishra, 1995. Sarma and Talukda, 1996; Momin *et al.*, 2006). Thus, from above it can be inferred that the different mutagenic treatments were effective in inducing diverse type's changes in different quantitative traits OBGG-52 though their magnitude and frequency varied.

The genetic divergence (D^2) among the forty five micro mutants and parent variety, based on nine traits ranged from 14.62 (between I and VI) to 68.43 (between XI and XII) and vast majority of the estimates were significant (Table 2) indicating that mutagenic treatment were effective in inducing enough genetic variability in different quantitative traits and in isolation of mutant lines with diverse changes in multivariate traits from the parents. The maximum inter-cluster distance was recorded 8.27 between cluster-XI and XII followed by 7.50 between cluster-IV & XII and 7.44 between cluster-IX & XI. The genotypes grouped in these clusters indicated them to be of diverse nature. The intra-cluster divergence was maximum in Cluster IV (15.60) followed by Cluster II (12.35). Cluster-

IV having highest average D^2, was genetically most heterogeneous group followed by cluster-II (seventeen mutants) and cluster-I (sixteen mutants). The Clusters I, III, V, VI, VII, VIII and X were relatively closer to the parental cluster (Cluster II), while Cluster IX and XII was far diverse from the parental cluster. The relative importance of the yield components towards divergence can be judged by comparing the cluster means of nine characters. The cluster means for different characters indicated considerable difference between the clusters for all characters (Table-3). Examination of cluster mean for different characters of different D^2 clusters showed that eight of the twelve clusters exhibited higher mean seed yield than Cluster II, which included the parent variety (2.38g/plant). Highest yield was recorded in Cluster III (3.29g/plant), represented by top three mutants (ON3-2, ON3-3 and OM1-3). These mutants showed higher number of clusters/plant, pods/plant, seeds/pod and longer pods. Cluster mean values for these characters of Cluster III are 2.88 clusters/plant, 10.2 pods/plant,, 10.03 seeds/pod and 6.38cm pod length, Cluster VIII had second highest yield and represented by the mutant ON1-3, while cluster V had third highest yield and represented by the mutant OE1-2. Both exhibited longer pods with more number of clusters/plant, pods/plant and seeds/pod. All the other clusters were intermediate in their mean character components. The comparison of cluster means revealed that each mutagenic treatment was effective in inducing diverse type of changes for all the quantitative traits, though their magnitude and frequency varied and cluster-III gave exceptionally high values for yield and other yield contributing traits. Thus hybridization of the divergent mutant lines from these clusters are expected to produce more transgressive segregants for yield. Production of transgressive variants by hybridization of mutant lines has earlier been reported (Micke, 1976; Maluszynski *et al*., 1991). Close examination of characters of Cluster III and Cluster VIII revealed that the hybridization of ON3-2 or ON3-3 or OM1-3 with ON3-1 is expected to produce high yielding segregants. High grain yield per plant, seeds/pod, pods/plant and 100-seed weight were recorded from Cluster III represented by three mutant lines ON 3-2, ON 3-3 and OM 1-3. The mean plant height and pods per plant were maximum for cluster-VIII. Moreover, the cluster means for different characters revealed the extent of diversity of groups of mutants from the parental cluster and also among themselves.

The major contributors to the genetic divergence among the micromutant lines found in this study were 100-seed weight , plant height, and pods per plant which indicating that the mutagenic treatments were effective in inducing more heritable variation for these characters (Table 4). Contributions of various characters towards genetic divergence was maximum by 100-seed weight (25.39 %) followed by plant height (13.78 %) and pods per plant (10.78%). Differential contribution of the traits to genetic divergence among the mutant cultures of greengram is in broad agreement with earlier reports (Mishra,1995; Momin *et al*., 2006; Mishra and Pradhan, 2006). Rank totals brought out same pattern of relative contribution of all the nine characters judged by the first criterion. Moreover, high contribution of a trait to genetic divergence among the mutant cultures of a variety indicates isolation of mutant lines with diverse genetic changes in the trait. Thus, differential order of contribution of traits to genetic divergence can be attributed to the genetic architecture of the parental varieties.

Multivariate analysis like Mahalanobis D^2 analysis, which takes into consideration of several quantitative traits simultaneously, would be a dependable method in determining stable differences among the micromutants. This analysis has been efficiently used for determining genetic diversity/ affinity among different micromutants in greengram (Sarma and Talukda, 1996; Momin *et al.,* 2006). In present study, most of these cultures not only showed lot of divergence in traits from the respective parents, but also exhibited divergence among them to be classified into different clusters. A good number of mutant cultures showed divergence from the parent and also among themselves. Thus, some of these mutants with reasonably good yield and showing divergence between them in different traits, more particularly in productive traits, may be of breeding value for use in hybridization programme. The character mean of different D^2 clusters revealed wide differences in different traits. It is assumed that maximum amount of heterosis will be manifested in cross combinations involving the parents belonging to most divergent clusters (Panigrahi *et al.,* 2014). Considering the inter-cluster distance and mean performance of the clusters, the crosses between parents from cluster-III with parents from cluster-VII having intermediate inter-cluster distance and better mean performance is expected to produce promising and desirable positive transgressive segregants for yield and yield components. However, for a practical plant breeder, the objective is not only high heterosis but also to achieve high level of production. To improve any particular trait donor for hybridization could be chosen from an appropriate cluster and that should be utilized in breeding Programme. Some reports suggested the importance of moderate genetic diversity and expected to throw heterotic hybrids (Parameshwarappa *et al.,* 2009). Thus, the

genotype(s) from cluster with more diversity as well as moderate diversity both can be included in breeding programme to isolate the good recombinants. Based on cluster mean, the promising donors for some important agro morphological traits may also be isolated for trait manipulation and/or recombination and/ or transgressive breeding.

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Table 1. Grouping of mutant into different genetic clusters using Tocher's method

Cluster No.	Number of Mutants	Mutant number
I	16	OG1-1 (1), OG1-2 (2), OG1-3 (3), OG2-3 (6), OG3-2 (8), OE1-1 (10), OE1-3 (12), OE2-1 (13), OE2-2 (14), OE2-3 (15), OE3-1 (16), ON1-2 (20), ON2-1 (22), ON2-2 (23), ON2-3 (24), OGE2-1(37)
II	17	OG3-1 (7), OG3-3 (9), OE3-2 (17), OE3-3 (18), OM1-1 (28), OM2-1 (31) OM2-2 (32), OM3-1 (34), OM3-2 (35), OM3-3 (36), OGE2-2 (38), OGN2-1 (40) , OGN2-2 (41), OGM2-1 (43), OGM2-2 (44), OGM2-3 (45), OBGG-52 (Parent)(46)
III	3	ON3-2 (26), ON3-3(27),OM1-3 (30)
IV	2	OM1-2 (29), OGE2-3 (39)
V	1	OE1-2(11)
VI	1	ON1-1 (19)
VII	1	ON1-3 (21)
VIII	1	ON3-1 (25)
IX	1	OG2-2 (5)
X	1	OM2-3 (33)
XI	1	OGN2-3 (42)
XII	1	OG2-1 (4)

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Table 2. Average intra- and inter-cluster divergences (D^2) and distances (D) for different clusters of mutants.

Cl. No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	10.54	21.41	17.94	25.54	18.17	<u>14.62</u>	15.80	28.11	20.69	27.70	35.57	28.22
	(3.25)	(4.63)	(4.24)	(5.05)	(4.26)	(3.82)	(3.98)	(5.30)	(4.55)	(5.26)	(5.96)	(5.31)
II		12.35	24.64	26.50	19.54	22.11	24.89	29.40	49.14	23.73	22.16	46.37
		(3.52)	(4.96)	(5.15)	(4.42)	(4.70)	(4.99)	(5.42)	(7.01)	(4.87)	(4.71)	(6.81)
III			8.58	33.19	14.83	31.84	24.62	19.06	33.85	21.82	21.85	30.27
			(2.93)	(5.76)	(3.85)	(5.64)	(4.96)	(4.37)	(5.82)	(4.67)	(4.67)	(5.50)
IV				15.60	48.27	27.47	24.26	54.05	31.73	33.39	29.76	56.17
				(3.95)	(6.95)	(5.24)	(4.93)	(7.35)	(5.63)	(5.78)	(5.46)	(7.50)
V					0.00	24.31	31.46	18.51	50.74	21.62	35.14	31.73
					(0.00)	(4.93)	(5.61)	(4.30)	(7.12)	(4.65)	(5.93)	(5.63)
VI						0.00	18.90	48.30	38.23	31.62	54.31	35.16
						(0.00)	(4.35)	(6.95)	(6.18)	(5.62)	(7.37)	(5.93)
VII							0.00	24.56	23.16	26.94	36.21	52.46
							(0.00)	(4.96)	(4.81)	(5.19)	(6.02)	(7.24)
VIII								0.00	43.19	23.71	31.47	45.24
								(0.00)	(6.57)	(4.87)	(5.61)	(6.73)
IX									0.00	49.57	55.37	34.32
									(0.00)	(7.04)	(7.44)	(5.86)
X										0.00	39.80	39.95
										(0.00)	(6.31)	(6.32)
XI											0.00	**68.43**
											(0.00)	(8.27)
XII												0.00
												(0.00)

Note - Diagonal figures shows the intra-cluster values. Others are inter-cluster values.
Figures in parenthesis show the average intra- and inter-cluster distances.
Bold & Underline figure shows the highest & lowest inter-cluster values respectively.

Table 3. Mean of nine characters in different clusters of OBGG-52 mutants

Cluster No.	Days to 50 % flowering	Days to maturity	Plant height (cm)	Clusters/ plant	Pods/ plant	Pod length (cm)	Seeds/ pod	100-seed weight (g)	Yield/ plant (g)
I	37.6	57.6	26.8	3.08	8.9	6.16	9.07	3.16	2.54
II	37.2	57.2	24.4	2.78	8.1	5.79	8.81	3.49	2.38
III	38.6	58.2	25.5	2.88	10.2	6.38	10.03	3.35	3.29
IV	37.8	58.2	21.2	2.82	7.2	5.70	8.75	3.19	2.24
V	37.3	56.7	28.6	3.17	10.0	6.33	9.53	3.44	3.19
VI	37.0	57.0	26.7	2.70	7.0	5.77	8.87	3.06	1.90
VII	37.3	58.3	26.4	2.87	7.3	6.50	9.50	3.33	2.23
VIII	38.3	58.7	30.0	3.30	10.3	6.37	9.83	3.68	3.21
IX	38.7	59.0	27.0	3.40	9.5	6.33	8.53	2.96	2.47
X	39.3	59.0	26.8	2.90	7.6	6.07	8.63	3.63	3.00
XI	37.3	57.7	20.3	2.67	9.8	6.13	9.67	3.61	2.99
XII	40.7	58.7	27.6	3.03	10.0	5.83	8.60	3.10	2.51

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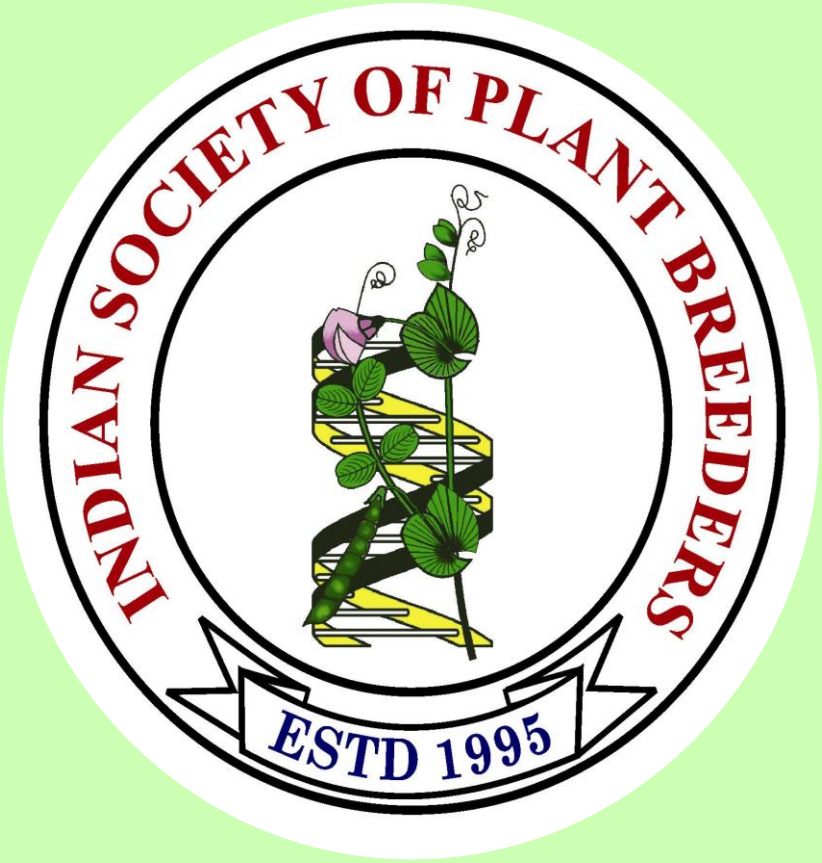
Table 4. Relative contribution of different characters to genetic divergence in mutants of OBGG-52

Sl. No.	Characters	Rank total	Rank average	Average D^2	Percent of total D^2
1.	Days to flowering	5691	5.499 (8)	1.992	9.31 (6)
2.	Days to maturity	5158	4.984 (5)	2.018	9.43 (5)
3.	Plant height (cm)	4868	4.703 (2)	2.948	13.78 (2)
4.	Clusters/plant	5419	5.236 (6)	1.848	8.64 (7)
5.	Pods/plant	5040	4.870 (3)	2.307	10.78 (3)
6.	Pod length (cm)	5127	4.954 (4)	2.034	9.51 (4)
7.	Seeds/pod	5885	5.686 (9)	1.259	5.88 (9)
8.	100-seed weight (g)	3691	3.566 (1)	5.430	25.39 (1)
9.	Yield/plant (g)	5666	5.474 (7)	1.556	7.28 (8)

Note : Numbers in parentheses indicate order of contribution (descending) to genetic divergence.



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