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Induced polygenic variability in M_4 generation of cumin (*Cuminum cyminum* L.)

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Abstract
Using only chemical and gamma ray treatments, ethyl methane sulphonate and gamma rays, the induction of mutation in cumin was conducted to cause mutational alterations in two cumin kinds. For the M_4 offspring of the cumin varieties, highly significant mean squares were seen. There was substantial diversity for all traits under study, as evidenced by significant treatment differences at the genotypic and phenotypic levels. For seed yield per plant, high heritability and high genetic advance were noted. Seed yield per plant was significant and positively correlated with umbels per plant, test weight, branches per plant, umbellets per umbel, and plant height at the genotypic level, according to an association analysis between seed yield per plant and the other nine quantitative characters. By looking at factors such seeds per umbel, test weight, branches per plant, and plant height, path coefficient analysis revealed a positive direct effect on seed yield per plant.

Keywords: Mutation, cumin, M_4 generation, heritability, genotypic and phenotypic correlations and path co-efficient

Introduction
Cumin (*Cuminum cyminum* L.) is a herbaceous, annual spice and herb. This plant has a basic chromosome number of 2n = 14, which places it in the Apiaceae family. The Mediterranean region is where the cumin crop grows the greatest, followed by Asia and, most likely, the Mediterranean region. The hermaphrodite blooms of the cumin crop are dicotyledonous and allogamous (Sastry and Anandaraj, 2013) [26]. Due to its long history of use as a spice and medicine plant, cumin seeds have significant industrial value (Rathore *et al.*, 2013) [25]. India is the world's greatest producer, consumer, and exporter of cumin, the second-most popular spice crop after black pepper (Hashemian *et al.*, 2013) [11]. Cumin is a highly valuable crop and growing area is mostly centred in Gujarat and Rajasthan.

With a semi-erect to erect growth habit, cumin is a fragrant small tropical annual bushy herb that grows to a height of 15 to 60 cm. It has a cylindrical stem that is smooth, angular, and occasionally hairy, and it branches out from the base into many branches that form a corymbase shape. These branches bear long, finely divided, deep green linear leaves with sheething bases. On compound umbels, little, delicate white or pink flowers appear after 60 to 90 days. The fruit's (schizocarp) elongated, tapering, greenish (yellow-brown) seed is on average 6 mm long, compressed laterally, and tapered toward both ends. The fruit is an achene with two mericarps and a single seed that is lateral, fusiform or oval in shape and 4-5 mm long (Sastry and Anandaraj, 2013) [26].

Cumin seeds have a 17.7% protein, a 23.8% fat, a 35.5% carbohydrate, and a 7.7% mineral content (Peter, 1996) [23]. The crucial elements are calcium (6452.3 ± 53.3 mg/kg), potassium (5412.2 ± 12.2 mg/kg), iron (294.4 ± 7 mg/kg), zinc (39.3 ± 0.1 mg/kg), manganese (34.7 ± 0.3 mg/kg), phosphorus, and sodium (Chien and Potty, 1996). Along with the aforementioned components, the seeds also contain vitamins, including B_1, B_2, A, and C (Pruthi, 1989) [24].

It was planted on 8.42 lakh hectares in India, where 5.47 lakh tonnes were produced each year (Anon., 2020) [3]. Ninety to ninety-five percent of the nation's total area planted in cumin is in Rajasthan and Gujarat, and these two states also produce the majority of the nation's cumin. It was farmed in Gujarat over an area of 3.37 lakh hectares, producing 3.32 lakh tonnes (Anon., 2020) [3].

With the primary purpose of creating cultivars of strategically vital crops, mutation breeding has replaced traditional breeding over the past three decades. Only spontaneous mutations are used by conventional plant breeders. Even after accounting for the internal and environmental factors that contribute to them, the frequency of spontaneous mutations is too low to serve as

the basis for systematic breeding. Comparing the speed of breeding to the traditional way of creating novelties, a rise in mutation rate caused by mutagenic agents improves the likelihood of occurrence of the desired mutant.

For plant breeders, creating genetic diversity is a constant problem. For this, a number of strategies are available. Due to cumin's tiny bloom size and the lack of relevant variety in the germplasm for economic characteristics, hybridization, the conventionally most used approach, has a very limited application in this crop. Induced mutation has been shown to be an effective method for creating genetic diversity for breeding superior varieties using conventional methods.

With increasing dose, the mutation rate rises, but non-genetic primary damage also does, resulting in a decline in germination, an increase in growth inhibition, and other morphological and physiological problems as the plant grows. The frequency of chromosomal mutations with noticeable morphological and physiological alterations typically increases with dosage.

Given the lack of useful variation in cumin's germplasm, the crop's very small floral structure, and the promiscuity of mutation breeding, it was thought plausible to approach mutation breeding in this crop, leading to the conception of the current study with the goal of applying physical and chemical mutagens to increase genetic variability in cumin.

Material and Methods

The research project under consideration, "Induction of mutation in cumin (Cuminum cyminum L.)," was carried out at the Agronomy Instructional Farm, Sardarkrushinagar Dantiwada Agricultural University, Dantiwada, during rabi 2017-18 and 2018-19, and at the Plasticulture Development Farm, Centre for Natural Resource Management, Sardarkrushinagar Dantiwada Agricultural University, Dantiwada during *rabi* 2019-20 and 2020-21.

Two types of cumin GC 3 and GC 4 made up the essential components of the experiment. The seeds for GC 3 and GC 4 were acquired from the Seed Spices Research Station, Jagudan. The first M_1 generation of the mutagen-treated seeds was grown in *rabi* 2017-18. During the *rabi* 2018-19, the seeds of a few chosen M_1 generation progeny were sowed for the M_2 generation. During the 2019-20 *rabi*, the selected M_2 generation progeny's seeds were sown for the next M_3 generation. The chosen offspring of the M_3 generation were developed for the M_4 generation, which was assessed for its yield and characteristics during the rabbis of 2020-21. Both physical and chemical mutagens were applied to the fresh seeds of the two kinds. The Department of Genetics and Plant Breeding, CPCA, SDAU, Sardarkrushinagar, created 0.2%, 0.4%, and 0.6% concentrations of EMS formulated in phosphate buffer for treatment of the seeds of both types. At the Bhabha Atomic Research Centre in Trombay, seeds from both kinds were exposed to doses of gamma rays of 130, 160, and 190 Gy. The EMS was freshly made in 0.1 M phosphate buffer that had its pH set to 7.0. The presoaked seeds of both kinds were soaked in distilled water for six hours prior to being placed in a mutagen solution and incubated for six hours with periodic shaking. After six hours, the treatment was stopped and the seeds were thoroughly washed under running water. The surface-dried seed samples were immediately sown in the field after being treated. The two varieties' control seeds, which had just been presoaked in distilled water, had been treated similarly to the treated seeds but without the use of mutagens. These control seeds were sown alongside the treated seeds. In the field, gamma-treated dried seeds of both kinds were planted.

Together beds measuring 4.0 m x 2.5 m, the treated seeds were mixed in with the untreated controls in a Randomized Block Design with Factorial Concept and three replications. In each bed, 300 seeds were put in rows that were 45 cm apart, with a 10 cm space between plants. The LD_{50} result for germination among the three EMS dosages was 0.6%. For EMS (0.6%), this conclusion was also supported by Krishna and Yadav (2013) [13]. The LD_{50} value for germination was 190 Gy for each of the three gamma treatments. (Verma, 2017[a]) [30] discovered a similar outcome for gamma rays (190 Gy). Single plants from the M_1 generation were harvested. For the purpose of growing the M_2 generation, the M_1 plants that had a normal appearance were randomly chosen and selfed. Each individual M_1 plant produced offspring for M_2 offspring. `In order to prevent out crossing, 30 healthy-appearing plants from the M_1 generation were chosen and bagged before to flowering. Only the bagged M_1 plants were used to harvest the M_2 seeds. The M_2 offspring and control were planted on a 0.9 square metre plot with a single row (each of 2.0 m long). Every 30 rows of M_2 progenies in the experiment, the control was performed once.

The M_3 generation was developed using an Augmented Randomized Complete Block Design (Federer, 1956), whereas the M_4 generation was developed using a Randomized Block Design with three replications (Panse and Sukhatme, 1978) [21]. The plant distance was kept at 10 cm within each row and each row was 2.0 m long with a 45 cm spacing between rows. To determine the extent of genetic diversity for seed yield, the progeny were assessed for nine features, including days to blooming, days to maturity, plant height (cm), branches per plant, umbels per plant, umbellets per umbel, seeds per umbel, seed output per plant (g), and test weight (g). Five randomly chosen plants from each progeny (family) were observed in the M_3 and M_4 generations. The means for all the characters aside from days to flowering, days to maturity, and test weight were determined.

Results and Discussions

In order to address the issue of crop improvement, the many forms of plant breeding techniques are typically alternatives and should be utilised in conjunction with one another (Micke, 1995) [19]. However, mutation breeding has proven to be more effective than an alternative since the results of mutagenesis have more frequently been issued immediately as a variety (Ahloowalia *et al.*, 2004) [1]. Mutagenesis offers the breeder two significant opportunities: first, it provides an unselected genetic diversity; and second, it can be used to increase genetic variability for selection and cross-breeding. Cumin is a good option for addressing mutant breeding because of its small flower and lack of useable variation, even if later opportunity has typically been a reason for mutagenesis strategy for quantitative trait improvement.

The parent material selected for mutagenesis is very important. A common rule is to choose the best genotype that is available because it would require fewer steps or grades to enhance (Ahloowalia *et al.*, 2004) [1]. This norm would persist for polygenic traits because there is little information on how genotypes affect the range of induced mutations (Micke, 1995) [19]. However, the Brock's theory (Brock, 1965), which contends that the outcome of the mutation would be contrary

to the prior history of selection for quantitative features, may provide some insight. Furthermore, an open pollinated population makes sense from the perspective of inducing polygenic variation. Sharma (1995) [19] successfully showed in pea that hybrids exposed more micromutational diversity than their pure line parents when both groups were subjected to mutagenic treatments. This was attributable to the hybridization-induced reorganisation of minor genes. Individuals from open pollinated populations offer a comparable reorganisation of minor genes, and as a result, are likely to exhibit greater variety than inbreds. However, when precise genetic analysis is done through forced mutagenesis, inbreds are unavoidable.

Knowing which mutagens to utilise is crucial for a systematic mutation breeding effort. Due to the existence of mutagen specificity, the use of many mutagens is plausible (Micke, 1995) [19]. Alkylating agents (EMS) and gamma rays were included in the study because they were thought to be justified due to three factors: (1) the potential existence of mutagen specificity, if any; (2) the distinct modes of action of two types of mutagens; and (3) the potential impact of the test material's genotypic background.

The goal of the current research was to characterise the segregating generations, or M_4 generation, of two kinds of cumin, GC 3 and GC 4, in terms of yield and yield qualities. This involved performing extensive physical and chemical mutagenesis.

High producing M_3 progenies were used to select specific M_4 plants (those showing significantly higher mean than the control). So, 65 M_4 descendants in total were raised using a randomised block design with checks. Five competing plants randomly chosen in a progeny were given data on a variety of quantitative traits, and the results were then submitted to a variance analysis.

Table 1: Analysis of variance (mean square) for different characters in M_4 generation of cumin

Sr. No.	Character	Mean sum of square			C.D. at 5%	C.V. %
		Replications	Treatments	Error		
	Degree of freedom	2	66	132		
1	Days to flowering	1.80	30.67**	8.51	4.67	4.39
2	Days to maturity	7.18	52.11**	2.56	2.56	1.43
3	Plant height	8.93	69.66**	8.83	4.76	8.67
4	Branches per plant	0.02	2.07**	0.07	0.41	4.99
5	Umbels per plant	22.57	116.98**	9.07	4.82	12.26
6	Umbellets per umbel	0.03	0.61**	0.03	0.29	3.74
7	Seeds per umbel	5.94	30.53**	8.51	4.67	10.95
8	Seed yield per plant	0.14	6.31**	0.39	1.01	16.06
9	Test weight	0.20	3.52**	0.20	0.71	7.50

*, ** Significant at 0.05 and 0.01 level of significance, respectively.

Table 2: Genetic variability analysis for different characters in M_4 mutant lines of cumin

Sr. no.	Characters	Range	Mean	σ^2_g	σ^2_p	σ^2_e	GCV (%)	PCV (%)	ECV (%)	h^2_b (%)	GA	GAM (%)
1	Days to flowering	61.00-73.67	66.54	7.39	15.91	8.52	4.09	5.99	4.39	46.45	3.82	5.74
2	Days to maturity	101.00-120.00	112.04	16.51	19.07	2.56	3.63	3.90	1.43	86.57	7.79	6.95
3	Plant height (cm)	22.38-41.69	34.27	20.28	29.11	8.83	13.14	15.75	8.67	69.66	7.74	22.60
4	Branches per plant	3.26-7.43	5.11	0.67	0.74	0.07	16.00	16.77	4.99	91.13	1.61	31.47
5	Umbels per plant	15.23-45.68	24.56	35.97	45.04	9.07	24.42	27.32	12.26	79.87	11.04	44.95
6	Umbellets per umbel	4.04-5.77	4.84	0.19	0.22	0.03	9.05	9.79	3.74	85.40	0.83	17.23
7	Seeds per umbel	19.53-33.40	26.63	7.34	15.85	8.51	10.18	14.95	10.95	46.33	3.80	14.27
8	Seed yield per plant (g)	1.86-8.62	3.91	1.97	2.36	0.39	35.91	39.34	16.06	83.33	2.64	67.53
9	Test weight (g)	4.21-7.81	5.90	1.11	1.31	0.20	17.83	19.34	7.50	84.98	2.00	33.86

Where,

σ^2_g, σ^2_p and σ^2_e are the genotypic, phenotypic and environmental variance, respectively.

GCV (%), PCV (%) and ECV (%) are genotypic, phenotypic and environmental coefficient of variation, respectively.

h^2_b (%), GA and GAM are broad sense heritability, genetic advance and genetic advance expressed as per cent of mean, respectively.

Table 3: Genotypic and phenotypic correlation coefficient for different characters in M_4 mutant lines of cumin

Sr. no.	Character		Days to flowering	Days to maturity	Plant height (cm)	Branches per plant	Umbels per plant	Umbellets per umbel	Seeds per umbel	Test weight (g)	Seed yield per plant (g)
1	Days to flowering	r_g	1.0000	0.9998 **	-0.7400 **	-0.4639 **	-0.4657 **	-0.3850 **	-0.5458 **	-0.5955 **	-0.5649 **
		r_p	1.0000	0.6884 **	-0.5641 **	-0.3363 **	-0.2971 **	-0.2505 **	-0.2775 **	-0.3473 **	-0.3365 **
2	Days to maturity	r_g		1.0000	-0.6957 **	-0.5229 **	-0.5026 **	-0.3862 **	-0.5217 **	-0.5241 **	-0.5606 **
		r_p		1.0000	-0.5521 **	-0.4659 **	-0.4247 **	-0.3328 **	-0.3635 **	-0.4586 **	-0.4925 **
3	Plant height (cm)	r_g			1.0000	0.6481 **	0.5885 **	0.4558 **	0.5468 **	0.7158 **	0.6826 **
		r_p			1.0000	0.5473 **	0.4411 **	0.3523 **	0.4059 **	0.4977 **	0.4943 **
4	Branches per plant	r_g				1.0000	0.8436 **	0.6330 **	0.7370 **	0.6057 **	0.7384 **
		r_p				1.0000	0.7316 **	0.5669 **	0.5238 **	0.5227 **	0.6476 **
5	Umbels per plant	r_g					1.0000	0.8337 **	0.9995 **	0.7051 **	0.9164 **
		r_p					1.0000	0.6638 **	0.5990 **	0.5834 **	0.7593 **
6	Umbellets per umbel	r_g						1.0000	0.9508 **	0.4817 **	0.7247 **
		r_p						1.0000	0.5966 **	0.4226 **	0.6163 **
7	Seeds per umbel	r_g							1.0000	0.7208 **	0.8454 **
		r_p							1.0000	0.4053 **	0.5445 **

8	Test weight (g)	r_g								1.0000	0.8990 **
		r_p								1.0000	0.7781 **
9	Seed yield per plant (g)	r_g									1.0000
		r_p									1.0000

Table 4: Direct and indirect effects of yield component on seed yield in M_4 mutant lines of cumin

Sr. no.	Character	Days to flowering	Days to maturity	Plant height (cm)	Branches per plant	Umbels per plant	Umbellets per umbel	Seeds per umbel	Test weight (g)	Seed yield per plant (g)
1	Days to flowering	-0.2589	0.3345	-0.0304	-0.1105	0.1217	0.0514	-0.4101	-0.2626	-0.5649 **
2	Days to maturity	-0.2783	0.3112	-0.0285	-0.1246	0.1311	0.0516	-0.3920	-0.2311	-0.5606 **
3	Plant height (cm)	0.1916	-0.2165	0.0410	0.1544	-0.1537	-0.0609	0.4109	0.3158	0.6826 **
4	Branches per plant	0.1201	-0.1628	0.0266	0.2383	-0.2203	-0.0845	0.5538	0.2672	0.7384 **
5	Umbels per plant	0.1206	-0.1564	0.0240	0.2010	-0.2611	-0.1113	0.7886	0.3110	0.9164 **
6	Umbellets per umbel	0.0997	-0.1202	0.0187	0.1507	-0.2177	-0.1335	0.7145	0.2125	0.7247 **
7	Seeds per umbel	0.1413	-0.1623	0.0224	0.1755	-0.2740	-0.1269	0.7515	0.3179	0.8454 **
8	Test weight (g)	0.1542	-0.1631	0.0294	0.1442	-0.1841	-0.0643	0.5417	0.4410	0.8990 **

*,** significant at 0.05 and 0.01 level of significance, respectively
Residual effect = 0.3586

In Table 1, the analysis of variance showing the mean sum of squares for nine quantitative features is shown. Days to flowering, days to maturity, plant height (cm), branches per plant, umbels per plant, umbellets per umbel, seeds per umbel, seed yield per plant (g), and test weight (g) were the nine characters for which the analysis of variance revealed significant differences among the genotypes, indicating the presence of variability in the experimental material. Each character's genotypic and phenotypic variations (σ^2_g and σ^2_p) were calculated. Analysis of variance was used to determine the genotypic (σ^2_g) and phenotypic (σ^2_p) variance estimates for each character. The additional genetic parameters, including genotypic coefficient of variation (GCV%), phenotypic coefficient of variation (PCV%), broad sense heritability (h^2_b), and genetic advance as a percentage of mean (GA% mean), were calculated from variance components and mean values. Table 2 provides a summary of all these estimations.

The absolute variability may not be shown by analysis of variance (ANOVA), but it can be found by normalising the phenotypic and genotypic variances and calculating the coefficient of variability. Separating the impact of the environment from overall variability is also crucial. This shows how precisely a genotype may be determined based on its phenotypic behaviour. Heritability estimations by themselves are unable to capture the effect of selection. The heritability estimates therefore seemed to have more significance when they were combined with estimates of genetic advancement.

All nine of the characters' estimates of phenotypic variance were higher than those for genotypic variance, indicating a greater environmental influence on this character's expression and pointing to the possibility that character selection may be based on phenotypic expression. There was less environmental influence, therefore the estimations of GCV and PCV were high in umbels per plant and seed output per plant, providing superior selection potential. Meena *et al.* (2013) [16] and Subramaniyan *et al.* (2019) [29] both came to similar conclusions.

Seed yield per plant, followed by umbels per plant, test weight, branches per plant, and plant height, showed strong heritability and high genetic progress. High genetic progress in conjunction with high heritability suggests that selection may be effective and that the heritability is most likely caused by additive gene effects. Yogi *et al.* (2014) [33], Patahk *et al.* (2014) [22], Akoijam *et al.* (2019), Subramaniyan *et al.* (2019) [29], Kumhar *et al.* (2020) [14], and Shiwangi *et al.* (2020) [28] all came to similar conclusions.

According to Falconer (1989) [8], heritability aids in determining the similarity between parents and their offspring, whereas genetic progress informs us of the projected gain for a given feature after selection. With the exception of days to flowering and seeds per umbel, which showed the least environmental influence, all characteristics in the current study had substantial heritabilities. The GCV and heritability estimations would be an efficient selection approach (Burton, 1952) [5].

Phenotypic correlation is the link between traits that may be seen directly. The selection programme for improving agricultural production benefits from knowledge of the phenotypic association between yield-contributing traits. The genotypic correlation is useful in the building of selection indices as it enables the prediction of correlated responses and evaluates the relative influence of one character on another. 67 cumin genotypes were used to evaluate the phenotypic and genotypic correlation coefficients (Table 3) for 9 variables to determine the relationship between yield and other yield-contributing characters.

For every character, the genotypic correlation coefficients were greater than their phenotypic counterparts (Table 3). Additionally, Kassahun *et al.* (2011) [12] and Mengesha *et al.* (2013) [18] have found that genotypic correlation values were higher than their phenotypic equivalents. This showed a strong genotypic link between the two factors; however, the influence of the environment may have an impact on how the two variables manifest phenotypically.

At both the genotypic and phenotypic levels, characteristics including umbels per plant, test weight, seeds per umbel, branches per plant, umbellets per umbel, and plant height revealed a strong positive correlation with seed yield per plant. Numerous studies, including Gurjar *et al.* (2016) [10], Wojo *et al.* (2016) [31], Lemma *et al.* (2020) [15], Narendra *et al.* (2020) [20] and Zigyalew *et al.* (2020) [34] have reported on the beneficial correlation between seed yield and its characteristics in cumin. Therefore, while making choices to increase seed yield per plant, more attention should be placed on these factors. Days to flowering and days to maturity had a significantly significant negative association with seed yield per plant.

Direct and indirect effects of various features on seed yield were assessed using path co-efficient analysis in order to get a clear picture of how they interact with seed yield at the genotypic level (Table 4). The remaining eight characters served as the causal variables in the current study, with seed yield per plant serving as the resulting variable. It was found that the factors seeds per umbel, test weight, days to maturity, branches per plant, and plant height all had significant positive direct influence on seed yield. Thus, these five characteristics were crucial in producing features that contributed to the current population. The path co-efficient analyses of cumin conducted by Dyulgerov and Dyulgerova (2013[a]), Yadav *et al.* (2013) [13], Meena *et al.* (2014) [33], and Narendra *et al.* (2020) [20] similarly revealed significance for these five features. Seeds per umbel, test weight, days to maturity, branches per plant, and plant height are good indicators of seed yield per plant in cumin and can be used for indirect selection for seed yield, according to path analysis and correlation studies.

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