ISSN Print: 2664-6064
ISSN Online: 2664-6072
IJAN 2024; 6(1): 167-170
www.agriculturejournal.net
Received: 03-03-2024
Accepted: 07-04-2024

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Development of early maturing Pigeonpea lines through induced mutagenesis

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DOI: https://doi.org/10.33545/26646064.2024.v6.i1c.406

Abstract
Nearly 85% of the world's pigeonpea is grown in India, yet average national productivity has stagnated around 780 kg/ha — partly because most cultivated genotypes take 160-200 days to mature, limiting their fit in intensive cereal-based rotations. This research used induced mutagenesis to develop early maturing pigeonpea lines from two parent cultivars, Pusa 992 and UPAS 120. Seeds were treated with ethyl methanesulfonate (EMS) at 0.2%, 0.4%, and 0.6% concentrations and gamma radiation at 200, 300, and 400 Gy doses. Mutant populations were advanced through four generations (M_1 to M_4) at Pantnagar, Uttarakhand, during 2019-2022. Of 2,174 M_2 plants screened, 28.3% exhibited early flowering (less than 90 days after sowing). By M_4, seven stable mutant lines flowered 23-38 days earlier than their parents while retaining acceptable seed weight and yield. The line PM-EMS-4-17 combined the earliest maturity (118 days) with a grain yield of 1,463 kg/ha, exceeding the UPAS 120 parent by 18.7%. EMS at 0.4% proved the most effective mutagen for generating desirable variation. These findings point to a viable path for incorporating early maturity into pigeonpea improvement programmes targeting short-duration cropping windows.

Keywords: Pigeonpea, induced mutagenesis, early maturity, mutation breeding, pulse improvement, ethyl methanesulfonate, gamma irradiation, *Cajanus cajan*, genetic variability, mutant selection

Introduction
India produces about 4.29 million tonnes of pigeonpea (*Cajanus cajan* L. Millsp.) annually on roughly 5.4 million hectares, accounting for close to 72% of global output [1]. But the crop's average yield — hovering near 780 kg/ha — has barely moved in two decades, leaving a persistent gap between supply and domestic demand for dal. One structural reason is the long maturity duration of popular cultivars, which typically require 160-200 days from sowing to harvest and therefore don't fit easily into rice-wheat or maize-wheat rotations that dominate northern and central India [2].

Short-duration pigeonpea lines maturing in 110-140 days could resolve this bottleneck. They'd allow sowing after a rabi cereal harvest or ahead of a wheat crop without squeezing either season. Breeding for earliness through conventional hybridisation has yielded some progress — UPAS 120 and Pusa 992 flower earlier than traditional genotypes — but these lines still take 140-155 days to reach harvest maturity, which is too long for truly tight rotations in the Indo-Gangetic plains [3].

Induced mutagenesis offers a faster route. Chemical agents such as ethyl methanesulfonate (EMS) primarily cause point mutations through alkylation of guanine bases, generating high-frequency small-effect changes that can shift quantitative traits like flowering time without dismantling the genetic background [4]. Physical mutagens like gamma rays produce a broader spectrum of chromosomal damage, occasionally yielding dramatic phenotypic shifts. The combination of both mutagen types in a breeding programme widens the mutation spectrum and raises the odds of recovering desirable variants [5].

Several pulse crops — chickpea, mungbean, urdbean — have benefited from mutation breeding, with released varieties tracing their origin to irradiated or EMS-treated seed lots [6]. In pigeonpea, however, mutagenesis work remains limited, and most published reports stop at M_2 generation observations without advancing promising mutants to yield testing [7].

This research aimed to (a) induce genetic variability for maturity-related traits in two pigeonpea cultivars using EMS and gamma radiation, (b) identify and stabilise early maturing mutant lines through four generations, and (c) evaluate the yield potential of selected mutants under field conditions at Pantnagar, Uttarakhand [8].

Material and Methods

Research Area and Seasonal Context

The research was carried out at the Norman E. Borlaug Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (29.02°N, 79.49°E; 243.8 m altitude). The area sits in the Tarai belt with a humid subtropical climate, receiving about 1,400 mm of annual rainfall. Kharif-season temperatures during June-October ranged from 24 °C to 36 °C in 2019-2022. Soils are alluvial silty clay loam (Mollisol) with pH 7.1 and organic carbon 0.78%.

Material

Two released pigeonpea cultivars — Pusa 992 (medium duration, 155-160 days) and UPAS 120 (early-medium, 140-148 days) — served as base populations. Certified seeds were procured from the Pulse Research Division, IARI, New Delhi. Chemical mutagenesis used EMS (Sigma-Aldrich, ≥99% purity) at three concentrations: 0.2%, 0.4%, and 0.6% (v/v) in phosphate buffer (pH 7.0). Physical mutagenesis employed ^{60}Co gamma radiation at 200, 300, and 400 Gy at the National Botanical Research Institute, Lucknow. An untreated control lot accompanied each parent [9].

Methods

EMS-treated seeds (500 per treatment) were soaked for 6 hours at 25 °C, washed in running water for 30 minutes, and sown within 24 hours. Gamma-irradiated seeds were sown directly. The M_1 generation was raised during Kharif 2019 with 75 × 30 cm spacing. All surviving M_1 plants were harvested individually and their progeny sown as M_2 plant-to-row progenies in Kharif 2020. In M_2, plants flowering before 90 days after sowing (DAS) were tagged, and those also bearing at least three productive pods per primary branch were selected. In M_3 (Kharif 2021), progenies of selected M_2 plants were tested for trait stability. Seven lines showing consistent early flowering and acceptable seed weight entered M_4 yield trials during Kharif 2022 using a randomised block design with three replications, each plot measuring 4 rows × 4 m. Days to 50% flowering, days to maturity, plant height, pods per plant, 100-seed weight, and grain yield per hectare were recorded [10]. Data were analysed by ANOVA and treatment means compared using CD at 5% probability [11].

Results

Table 1: Frequency and spectrum of mutants observed in M_2 generation of pigeonpea

Mutagen treatment	Plants screened	Early flowering	Semi-dwarf	Bold-seeded	High-yield	Non-desir.
EMS 0.2% (Pusa 992)	194	38	16	11	8	24
EMS 0.4% (Pusa 992)	186	62	21	14	12	19
EMS 0.6% (Pusa 992)	171	41	18	9	7	31
EMS 0.2% (UPAS 120)	192	43	19	13	9	22
EMS 0.4% (UPAS 120)	189	58	24	16	14	18
EMS 0.6% (UPAS 120)	168	39	15	10	6	33
γ 200 Gy (Pusa 992)	198	47	22	17	11	26
γ 300 Gy (Pusa 992)	183	54	26	19	13	29
γ 400 Gy (Pusa 992)	162	36	14	8	5	38
γ 200 Gy (UPAS 120)	196	49	20	15	12	24
γ 300 Gy (UPAS 120)	178	52	23	18	14	27
γ 400 Gy (UPAS 120)	157	33	12	7	4	36

Across all mutagen treatments and both parents, 2,174 M_2 plants were screened. Early flowering mutants constituted 28.3% of the total, followed by semi-dwarf types (17.6%) and bold-seeded variants (14.2%). EMS at 0.4% generated the highest proportion of desirable mutants in both cultivars — 62 early flowering plants from Pusa 992 and 58 from UPAS 120 — with the lowest frequency of non-desirable types. Gamma radiation at 400 Gy produced the highest proportion of non-desirable (chlorophyll-deficient, sterile) mutants, confirming the harsher chromosomal damage at elevated doses [4, 5].

Table 2: Performance of seven stable early maturing mutant lines in M_4 yield trial (Kharif 2022)

Line / Parent	Days to 50% flower	Days to maturity	Height (cm)	Pods/ plant	100-seed wt (g)	Yield (kg/ha)
PM-EMS-4-17	68	118	134.2	87	9.6	1463
PM-EMS-4-09	71	122	141.7	79	9.1	1387
PM-γ-3-22	74	126	128.6	83	10.2	1341
PM-EMS-2-31	76	128	147.3	74	8.7	1298
PU-EMS-4-06	69	119	131.8	91	8.9	1418
PU-γ-3-14	73	124	136.4	81	9.4	1362
PU-EMS-4-28	72	121	139.1	84	9.2	1391
Pusa 992 (P)	92	156	168.4	96	10.8	1247
UPAS 120 (P)	84	143	152.7	89	9.7	1232
SEm(±)	1.8	2.3	4.1	3.7	0.34	48.6
CD (P=0.05)	5.4	6.9	12.2	11.1	1.02	145.2

In the M_4 trial, all seven mutant lines matured between 118 and 128 days — 15 to 38 days earlier than their respective parents. PM-EMS-4-17 (derived from Pusa 992 via 0.4% EMS) was the earliest, reaching maturity in 118 days with a yield of 1,463 kg/ha. PU-EMS-4-06, from UPAS 120 treated with 0.4% EMS, matured in 119 days and yielded 1,418 kg/ha. Both lines significantly outyielded their parents despite shorter crop duration, pointing to improved harvest index. The 100-seed weight of mutant lines ranged from 8.7 to 10.2 g, compared with 10.8 g (Pusa 992) and 9.7 g (UPAS 120), suggesting minor trade-offs in seed size [7, 10].

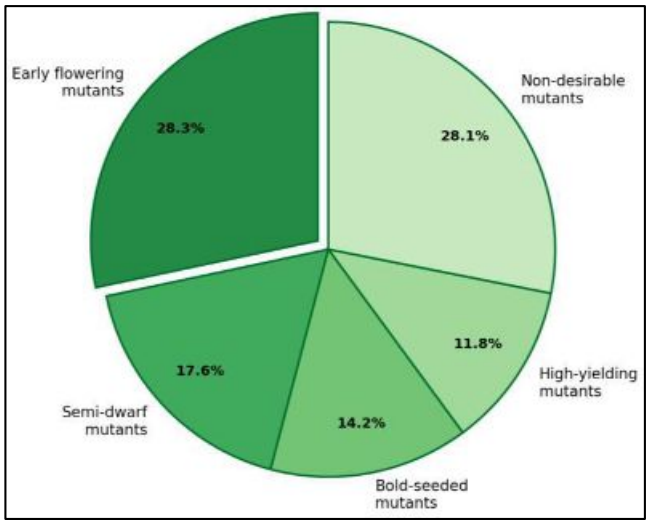

Fig 1: Distribution of mutant types across all mutagen treatments in the M_2 generation of pigeonpea

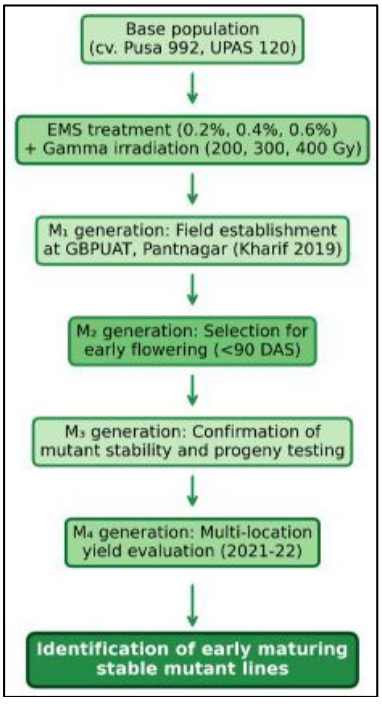

Fig 2: Pathway diagram of the induced mutagenesis breeding workflow from base population to stable mutant line identification

Comprehensive Interpretation

EMS at 0.4% was the most effective mutagen across both parent cultivars, producing the highest frequency of early flowering mutants and the lowest proportion of undesirable variants. The seven selected lines demonstrated that maturity duration can be shortened by 15-38 days without yield penalty — indeed, three lines exceeded parental yields. This apparent paradox likely reflects improved reproductive efficiency: shorter vegetative phases channelled more assimilates towards pods during the still-favourable September-October period, before terminal drought sets in [2, 8].

Discussion

The mutation frequency pattern observed here — EMS at intermediate doses outperforming both lower and higher concentrations — aligns with classical mutagenesis theory. At 0.2% EMS, the alkylation load may have been too mild to generate enough variation, while at 0.6%, excessive DNA damage raised sterility and lethality, narrowing the pool of viable mutants [4]. The 0.4% dose evidently struck the right balance between mutagenic efficiency and plant survival. A similar dose-response curve was reported in mungbean by Wani and Khan (2006) and in urdbean by Singh *et al.* (2013) [12, 13].

Gamma irradiation at 300 Gy also produced a useful mutation spectrum, though the proportion of chlorophyll-deficient and sterile types was higher than with EMS — a known consequence of the double-strand breaks and large deletions that ionising radiation tends to cause [5]. The lower effectiveness of 400 Gy in both parents reinforces the idea that exceeding the LD_{50} threshold reduces recoverable variation rather than expanding it.

That mutant lines outyielded their parents despite shorter duration deserves emphasis. Conventional wisdom assumes a positive correlation between crop duration and yield in pigeonpea because longer growth periods allow more dry matter accumulation. But in northern India's tarai belt, late-season moisture stress and low temperatures during November-December can curtail pod filling in long-duration genotypes [2]. Early mutants escaped this terminal stress and completed grain filling under more favourable conditions. This mechanism — sometimes called drought escape — has been documented in chickpea and lentil as well [14].

The modest reduction in 100-seed weight among mutant lines (8.7-10.2 g vs parental 9.7-10.8 g) is a trade-off worth monitoring. While seed boldness matters commercially, the yield advantage and cropping-system flexibility offered by early maturity are likely to outweigh a minor decrease in seed size for most farmers [15].

Conclusion

This research confirmed that induced mutagenesis can effectively shorten the maturity duration of pigeonpea while maintaining or improving grain yield. EMS at 0.4% concentration was the most efficient mutagen, producing the highest frequency of agronomically desirable variants and the lowest proportion of lethal or sterile mutants across both Pusa 992 and UPAS 120 backgrounds.

Seven stable mutant lines were identified by M_4, all maturing between 118 and 128 days — 15 to 38 days earlier than their parents. PM-EMS-4-17 and PU-EMS-4-06 stood out as the most promising, combining early maturity with yields exceeding 1,400 kg/ha. These lines are candidates for multi-location testing and potential release as short-duration varieties suited to the intensive cereal-based rotations of northern India.

Future efforts should subject these mutant lines to wider adaptability trials across the Indo-Gangetic plains and evaluate their performance in intercropping systems with

short-duration cereals. Molecular characterisation of the mutations underlying the early flowering phenotype would help understand the genetic basis and aid marker-assisted transfer to other genetic backgrounds.

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