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Studies on Genetic Divergence in M_5 Generation of Chickpea Induced by Gamma Rays for Different Yield and Related Traits

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Abstract

Assessment of 30 *Kabuli* chickpea M_5 mutants was undertaken for agromorphological and quality traits following a randomised block design with three replications at the Research Sub Station, Akrot (Una), Himachal Pradesh. Genetic diversity analysis grouped 30 mutants along with four checks into ten main clusters using Mahalanobis D^2-statistics, with cluster II as the largest, comprised of fifteen mutants. Maximum inter-cluster distance was observed between cluster VII and III, indicating crosses between the parents from these clusters, viz., A8P4-1-1, C53P2-2-1, A8P4-1-1 and C48aP1-4-5, may yield superior segregants. Mutants H20P4-1-2, A8P4-1-1, C20P6-5-2, and N72P2-1-7 were found to be more diverse based on D^2 analysis. The desirable mutants viz., A5P5-5-1, C20P6-5-2, C48aP3-3-1 were observed to have rough seed surface, angular seed shape and beige seed colour and, therefore, useful as morphological markers.

Keywords: Chickpea, D^2-Statistics, Genetic diversity, Mutagen

Chickpea (*Cicer arietinum* L.) is an important grain legume and belongs to the family *Leguminosae*. Among the genus *Cicer*, it is the only cultivated species. It is the third important pulse crop in the world (Jalota *et al*. 2006). Chickpeas have hairy leaves, stems and pods that emit extremely acidic exudates and have an indeterminate and branching growth habit, with erect or spreading types of plants. Due to nitrogen fixation by rhizobium bacteria present in its root nodules, it is a significant *rabi* season pulse crop in India and helps to increase soil fertility. Albumins and globulins are the major proteins present in chickpeas, as they are in other legumes. Chickpeas possess high amounts of key vitamins such as riboflavin, niacin, thiamin, folate and beta-carotene (a precursor to vitamin A). In India, where most of the population favours a vegetarian diet, chickpeas play a critical role in overcoming protein deficiencies. It offers a significant increase in agricultural productivity even in dry terrains due to minimal water requirements and its capacity to fix atmospheric nitrogen.

Chickpeas account for over 40% of total pulse crops grown in India, but their production has not increased in recent years, resulting in decreased pulse availability per capita. One of the key reasons for the yield plateau in chickpea is its limited genetic diversity. Rapid productivity enhancement by genetic manipulation can solve the problem of falling chickpea production (Wani *et al*. 2014). To achieve genetic enhancement in chickpea, a variety of crop improvement practices such as selection, hybridisation, mutation and marker-assisted selection can be employed (Singh *et al*. 2018). The extent of genetic variability has been regarded as a crucial component, which is essential for a successful

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hybridisation intended to produce progenies with high yield (Sekhon *et al.* 2019; Thakur & Sharma 2023; Srishti *et al.* 2025). A mutation breeding programme was initiated to widen the genetic base of different crop species (Sharma *et al.* 2010a; Kishore *et al.* 2023). Among these breeding techniques, mutation breeding is a potent tool for improvements as well as broadening the genetic base (Sharma *et al.* 2024; 2025).

In chickpea, physical and chemical mutagens have been utilised to induce variability as well as release of varieties in this crop. Not only chickpea, but mutation breeding has made a substantial contribution to other legumes also (Sharma *et al.* 2010b). At the global level, 21 mutant types of chickpeas have emerged from various mutagens (http://mvgs.iaea.org). Gamma rays have been reported to be the mutagen of choice for the development of maximum variations (Kumar *et al.* 2019). Keeping this in view, the present study was undertaken to screen potential desirable mutants for agro-morphological and quality traits from *Kabuli* chickpea cultivar PG0027.

MATERIALS AND METHODS

The experiments were conducted at CSK HPKV, Research Sub Station (RSS), Akrot, Distt. Una, Himachal Pradesh. The experimental material comprised 30 desirable *Kabuli* chickpea mutants in M_5 generation derived from the variety PG0027. The mutants were generated using gamma ray treatments (150Gy, 200Gy, 300Gy), and the first selection for yield and related traits was made in M_2 generation. This was followed by the advancement of progenies and selection in each progeny. In the present study, 30 mutants were selected based on their yield performance in M_4 generation. The experiment was laid out in a Randomised Block Design (RBD) with three replications. At the time of maturity, the data were recorded on plot basis (days to 50 per cent flowering, days to maturity) and on plant basis [plant height (cm), number of primary branches, biological yield (g), number of pods per plant, number of seeds per pod, 100-seed weight (g), harvest index (%) and seed yield per plant (g)] from five competitive plants. The genetic divergence between populations was estimated using the Mahalanobis D^2-Statistic (1936), and all the mutants used were clustered into different groups following Tocher's method (Rao 1952). For analysis of qualitative traits *viz*., seed surface, seed shape and seed colour, data were recorded as per the guidelines for the conduct of test for Distinctiveness, Uniformity and Stability (DUS) on chickpea by Protection of Plant Varieties and Farmers' Rights Authority, Government of India (PPV&FRA). The classification of seed surface and seed shape was recorded as depicted in Plates 1 and 2, while seed colour was determined as Beige and Creamy beige.

RESULTS AND DISCUSSION

D^2-Statistics is a powerful tool for estimating genetic diversity among germplasm lines and to identify the parents for hybridisation to obtain desirable

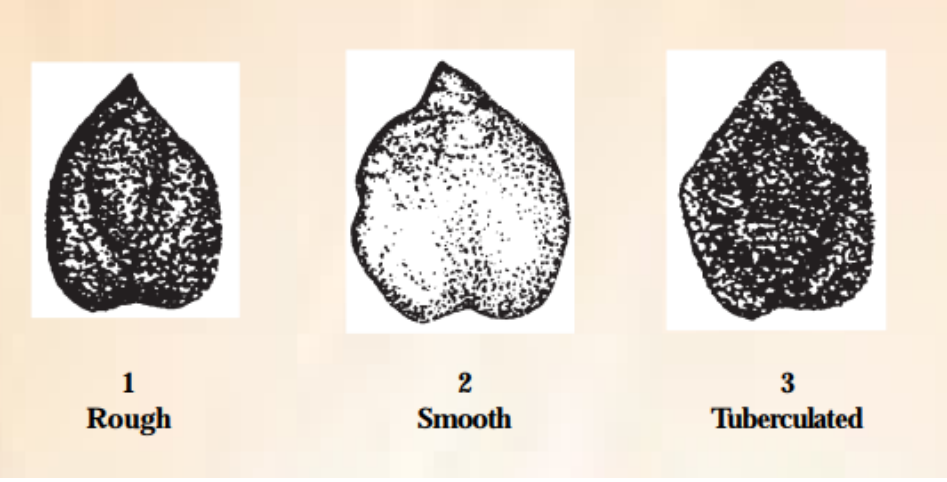


Plate 1. Seed surface.

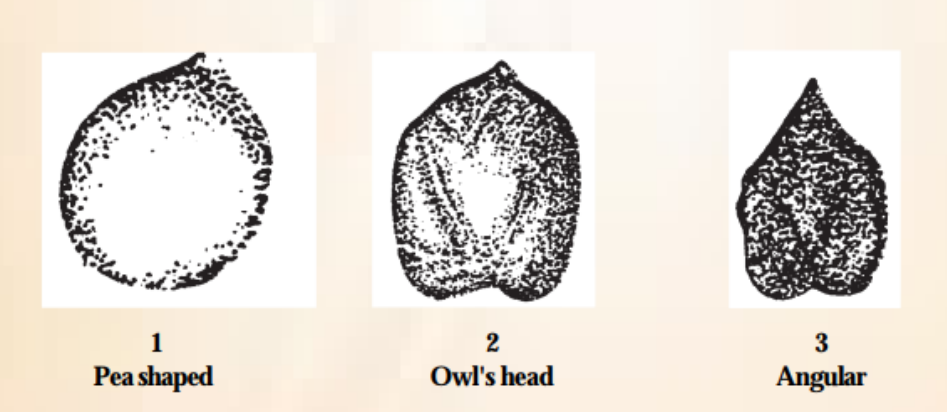


Plate 2. Seed shape.

recombinants (Sharma & Devi 2013). The assessment of genetic diversity helps in reducing the number of breeding lines and the progenies derived from diverse parents, and provides a better scope to isolate superior recombinants (Sharma *et al.* 2013; Sharma *et al.* 2025a; Sharma *et al.* 2025b). The non-hierarchical Euclidean cluster analysis, Tocher's method, was followed to group the entries into different clusters considering the estimated D^2 values (Singh & Chaudhary 1977). The 30 mutants, along with 4 checks, were grouped into ten clusters (Figure 1). Of the ten clusters, Cluster II comprising of fifteen mutants (A5P5-5-1, B32P6-1-2, M50P3-1-3, N20P3-1-3, H20P4-1-2, H20P4-1-1, B25P1-3-2, C48aP1-4-3, D15P2-3-1, C20P6-5-2, N72P2-1-7, C20P6-2-1, A5P5-1-2, C53P2-2-1, C48aP3-3-1) was the largest followed by cluster V [(G13P1-1-1, L555(Check 4), L552(Check 3), B47bP1-1-2, C26P1-1-2, N55P6-2-3)], cluster I [(G13P1-1-3, PG0027 (Check1), L556 (Check 2), H19P4-22-1)], cluster III (A8P4-1-1, N22P1-1-5), cluster X (B53P7-6-1, C48aP1-4-6), cluster IV (C57P4-1-2), cluster VI (H20P4-22-2), cluster VII (F14P1-1-2), cluster VIII (M50P3-15-2) and cluster IX (C48aP1-4-5) (Figure 1, Table 1).

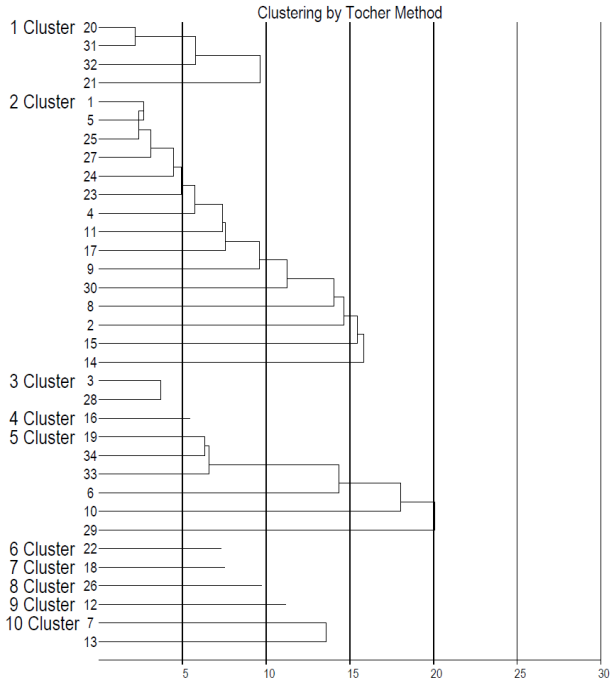


Figure 1. Phenogram showing diversity of chickpea mutants generated using Mahalanobis D2-cluster analysis.

The cluster means of chickpea mutants falling under different clusters are presented in Table 2. The average cluster mean values for different traits showed that the mutants in cluster III had maximum value for plant height and minimum for number of seeds per pod, cluster IV showed maximum value for 100-seed weight and minimum for number of seeds per pod, cluster VI showed maximum value for biological yield and minimum for seed yield, cluster VII showed maximum value for number of pods per plant and minimum for plant height while cluster IX and X showed maximum values for days to 75 per cent maturity and minimum value in cluster IX was observed for number of pods per plant and in cluster X for number of primary branches. Among the ten clusters, cluster X showed maximum cluster mean values for the maximum number of traits, suggesting that the cluster X individuals were superior

Table 1. Cluster distribution of 30 chickpea mutants along with four checks based on Mahalanobis D^2 analysis

Clusters	No. of mutants	Mutants
I	4	G13P1-1-3, PG0027 (Check1), L556 (Check 2), H19P4-22-1
II	15	A5P5-5-1, B32P6-1-2, M50P3-1-3, N20P3-1-3, H20P4-1-2, H20P4-1-1, B25P1-3-2, C48aP1-4-3, D15P2-3-1, C20P6-5-2, N72P2-1-7, C20P6-2-1, A5P5-1-2, C53P2-2-1, C48aP3-3-1
III	2	A8P4-1-1, N22P1-1-5
IV	1	C57P4-1-2
V	6	G13P1-1-1, L555 (Check 4), L552 (Check 3), B47bP1-1-2, C26P1-1-2, N55P6-2-3
VI	1	H20P4-22-2
VII	1	F14P1-1-2
VIII	1	M50P3-15-2
IX	1	C48aP1-4-5
X	2	B53P7-6-1, C48aP1-4-6

to other clusters and could be selected directly and used in a hybridisation programme.

The inter- and intra-cluster distances (Table 3) revealed that the maximum inter-cluster distance was between cluster VI and III (12.24), followed by cluster X and III (10.87), cluster III and I (10.25). Minimum cluster distance was observed between cluster IV and II (4.10) and cluster IX and VI (4.15). Intra-cluster distance ranged from 0 to 4.43. Maximum intra-cluster distance was observed for cluster V (4.43), followed by cluster X (3.68), cluster II (3.48), cluster I (3.11) and cluster III (1.92).

The selection of suitable diverse parents for hybridisation is an important feature of any crop improvement programme for getting desired recombinants. The present D^2-Statistical study had grouped the thirty chickpea mutants along with four checks into ten clusters. The cluster pattern observed in the present study was also reported by Syed *et al.* (2012), Singh *et al.* (2012) and Boparai and Katna (2021). These studies suggested that the germplasm lines belonging to the clusters separated by high statistical distance could be used in a hybridisation programme for obtaining a wide spectrum of variation among the segregants (Lata & Sharma 2022).

The classification of quality traits was recorded as per the guidelines for the conduct of tests for (DUS) on chickpea by (PPV&FRA). Variability for these traits existed among the mutants (Table 4). The desirable mutants, *viz.*, A5P5-5-1, C20P6-5-2 and C48aP3-3-1, were observed to have rough seed surface, angular seed shape and beige seed colour, while mutant A8P4-1-1 had tuberculated seed surface, angular seed shape and creamy beige colour. The mutant M50P3-1-3 was observed with a rough seed surface, seed shape being that of an owl's head and seed colour was creamy beige, and the mutant N20P3-1-3 had a smooth seed surface, pea-shaped seed and beige-coloured seeds.

Table 2. Cluster means of ten clusters for different traits in chickpea mutants

Clusters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII	Cluster IX	Cluster X
Days to 50% flowering	125.75	122.2	113.5	122.67	123.94	108	113	116.67	127.67**	106.83*
Days to 75% maturity	172.92	171.38	164	173	170.22	164.33	164.67	164.33	174.67**	158.83*
Plant height(cm)	80.26	72.08	54.7*	74.13	72.96	75.33	83.77**	65.03	78	77.07
Primary branches	3.1	2.89	2.63	4.47	3.24	2.8	4.87	5.2**	3.77	2.6*
Biological yield(g)	91.17	38.6	32.5	30	71.39	27.67*	95.33	91.67	32	97.17**
Pods per plant	83.55	30.9	25.6	24.87	63.56	20.47	89.67**	86.33	20*	88.75
Seeds per pod	0.99	1.04	1.06**	0.94*	1.01	1.04	0.97	0.98	0.97	1.02
100-seed weight(g)	39.73	40.92	40.45	35.83*	38.64	35.94	37.53	37.96	39.92	48.58**
Harvest index (%)	35.45	33.56	33.47	33.75	33.91	27.62	33.74	35.08	24.36*	42.77**
Seed yield(g)	32.38	12.98	10.94	10.03	24.16	7.69*	32.12	32.17	7.87	41.67**

*Minimum value** Maximum value

Table 3. Inter and intra-cluster values of D^2 among clusters

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	3.11									
II	7.57	3.48								
III	10.25	5.08	1.92							
IV	8.33	4.15	6.04	0						
V	4.8	5.55	7.5	6.25	4.43					
VI	8.57	4.26	6.07	5.01	6.97	0				
VII	4.56	9.47	12.24	9.33	6.69	9.75	0			
VIII	5.71	8.35	9.55	7.95	5.65	9.59	5.09	0		
IX	8.15	4.53	7.09	3.9	6.52	4.1	9.13	8.6	0	
X	5.18	8.87	10.87	9.92	6.81	9.01	6.21	7.01	9.57	3.68

Table 4. Analysis of qualitative traits in chickpea mutants

Mutants	Seed Surface	Seed Shape	Seed Colour	Mutants	Seed Surface	Seed Shape	Seed Colour
A5P5-5-1	Rough	Angular	Beige	C57P4-1-2	Tuberculated	Owl's head	Beige
A5P5-1-2	Rough	Angular	Beige	D15P2-3-1	Rough	Owl's head	Beige
A8P4-1-1	Tuberculated	Angular	Creamy Beige	F14P1-1-2	Smooth	Pea shaped	Creamy Beige
B25P1-3-2	Rough	Owl's head	Beige	G13P1-1-1	Rough	Owl's head	Beige
B32P6-1-2	Rough	Owl's head	Beige	G13P1-1-3	Rough	Angular	Creamy Beige
B47bP1-1-2	Rough	Owl's head	Beige	H19P4-22-1	Tuberculated	Angular	Cream Beige
B53P7-6-1	Rough	Angular	Creamy Beige	H20P4-22-2	Tuberculated	Owl's head	Beige
C20P6-2-1	Smooth	Pea shaped	Beige	H20P4-1-1	Rough	Pea shaped	Beige
C20P6-5-2	Rough	Angular	Beige	H20P4-1-2	Rough	Angular	Creamy Beige
C26P1-1-2	Tuberculated	Angular	Beige	M50P3-1-3	Rough	Owl's head	Creamy Beige
C48aP1-4-3	Rough	Owl's head	Beige	M50P3-15-2	Tuberculated	Angular	Beige
C48aP1-4-5	Rough	Owl's head	Beige	N20P3-1-3	Smooth	Pea shaped	Beige
C20P6-5-2	Rough	Angular	Beige	N22P1-1-5	Rough	Angular	Beige
C26P1-1-2	Tuberculated	Angular	Beige	N55P6-2-3	Rough	Owl's head	Beige
C48aP1-4-3	Rough	Owl's head	Beige	N72P2-1-7	Tuberculated	Angular	Creamy Beige
C48aP1-4-5	Rough	Owl's head	Beige	PG0027	Rough	Angular	Creamy Beige
C48aP1-4-6	Smooth	Pea shaped	Beige	L556	Rough	Owl's head	Beige
C48aP3-3-1	Rough	Angular	Beige	L552	Smooth	Pea shaped	Beige
C53P2-2-1	Rough	Angular	Beige	L555	Rough	Angular	Beige

CONCLUSION

Genetic diversity analysis based on Mahalanobis D^2-Statistics helped in grouping 30 mutants along with four checks into ten main clusters, among which Cluster II was the largest, comprising fifteen mutants. Maximum inter-cluster distance was observed between cluster VII and cluster III, which suggests progenies A8P4-1-1, C53P2-2-1, A8P4-1-1, and C48aP1-4-5 will be involved in hybridisation to yield superior segregants. The desirable mutants, viz., A5P5-5-1, C20P6-5-2, C48aP3-3-1, were observed to have rough seed surface, angular seed shape and beige seed colour according to DUS classification, which can be used as a morphological marker.

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