



Identification of induced mutants for morpho-physiological and biochemical traits of rajmash (*Phaseolus vulgaris*)

SHILPA BHAGAT¹, SANJEEV KUMAR^{1*} and SAPALIKA DOGRA¹

Shere Kashmir University of Agriculture Sciences and Technology, Chatha, Jammu,
Jammu and Kashmir 180 009, India

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ABSTRACT

An experiment was conducted during rainy (*kharif*) seasons of 2019 and 2020 at the Regional Horticultural Research Station, Bhaderwah of Shere Kashmir University of Agriculture Sciences and Technology, Jammu to characterize the 24 induced mutants of rajmash (*Phaseolus vulgaris* L.) to study the genetic diversity among them. Experimental material consisted of 22 induced mutants generated through gamma irradiation and 2 land races, viz. Bhaderwah Local and Poonch Local. Twenty-two induced mutants were grouped into 8 clusters, and 5 clusters on the basis of biochemical and morpho-physiological traits, respectively. Majority of which were accommodated in cluster I (13) followed by cluster II (4) and minimum in cluster V (1). Further, cluster I showed the largest intra-cluster distance ($D^2=39.10$), followed by cluster V, and the remaining clusters having the shortest intra-cluster distance ($D^2=0.00$). Cluster II and III had the least distance between them, whereas cluster III and V had the greatest inter-cluster distance ($D^2=9.36$). Among the morpho-physiological characters studied grain yield/plant (53.26%), days to 50% flowering (7.25%), days to maturity (0.72%), seeds/pod (3.62%), pods/plant (9.78%), pod length (4.35%), clusters/plant (18.84%) and 1000-seed weight (2.17%), contributed 99.99% to the total divergence. Likewise, in biochemical characters protein content (1.81%), zinc content (38.04%) and iron content (22.46%) were studied. Inter-cluster distances were larger than intra-cluster distances for biochemical traits, indicating that there was a significant degree of genetic variation among the mutant lines and these lines can be used in hybridization for rajmash improvement programme.

Keywords: Cluster distance, Genetic divergence, Inter-cluster distances, Mahalanobis D^2 technique, Tocher's method

Pulses, including rajmash (*Phaseolus vulgaris* L.), have a very narrow genetic base due to small flower size, drought sensitivity, low availability, diverse germplasm, and very few varieties developed in India through hybridization (Kumar *et al.* 2021b). In the majority of newly developed varieties, only a few parents are involved as donor parents which ultimately reduces the genetic diversity in cultivated rajmash. Hence, there is no alternative except to mutation breeding for the improvement of rajmash. Inducing mutations in pulses can be accomplished through mutagenic treatments applied to specific reproductive materials like seeds and pollen. When the natural mutation process occurs slowly, these treatments can boost the rate, variety, and range of mutations (Batan 2006). However, recent trends have seen a rise in the adoption of commercial pulse varieties while neglecting local varieties in breeding initiatives. Consequently, this has resulted in a substantial reduction in the genetic diversity

of pulse crops (Almeida *et al.* 2020).

Success through hybridization and selection is largely dependent on choosing the parents with wide genetic diversity for certain features (Kumar *et al.* 2021b). The plant breeders would be assisted in selecting suitable parents for their hybridization programmes by the knowledge of the kind and degree of divergence. The information regarding the genetic differentiation of various traits, especially those that impact yield and quality, is highly valuable when strategizing a breeding programme. A useful technique for determining the degree of divergence is the Mahalanobis D^2 statistic, which aids plant breeders in selecting appropriate parents for hybridization programmes. Several crops have had genetic divergence studied for its significance and extent, but little information has been generated on rajmash. Therefore, having knowledge about genetic diversity and comprehending the relationships among common bean varieties within and across commercial classes are crucial stages for enhancing genetics and preservation of the genetic diversity of beans (Gioia *et al.* 2019). With this understanding, the present investigation was planned to

¹Shere Kashmir University of Agriculture Sciences and Technology, Chatha, Jammu, Jammu and Kashmir. *Corresponding author email: ssalgotra@gmail.com

characterize induced mutants of rajmash in order to elucidate information on genetic divergence to formulate breeding strategies to improve the grain yield, yield attributing characters and quality features of rajmash.

MATERIALS AND METHODS

The mutant seeds (M_3) of 22 mutant lines (R-BL- M_3 -1, R-BL- M_3 -2, R-BL- M_3 -3, R-BL- M_3 -4, R-BL- M_3 -5, R-BL- M_3 -6, R-BL- M_3 -7, R-BL- M_3 -8, R-BL- M_3 -9, R-BL- M_3 -10, R-BL- M_3 -11, R-BL- M_3 -12, R-BL- M_3 -13, R-BL- M_3 -14, R-BL- M_3 -15, R-BL- M_3 -16, R-BL- M_3 -17, R-PL- M_3 -18, R-PL- M_3 -19, R-PL- M_3 -20, R-PL- M_3 -21, R-PL- M_3 -22) of rajmash with two controls i.e. Bhaderwah local (BL) and Poonch local (PL) were raised at Regional Horticulture Research Station, Bhaderwah (32°58'N and 75° 42'E at an altitude of 1,613 m amsl), Shere Kashmir University of Agriculture Sciences and Technology, Jammu in Randomized Block Design (RBD) with 3 replications and a row length of 3 m of each line with spacing of 30 × 40 cm² during rainy (*kharif*) seasons of 2019 and 2020. Data were recorded on 25 randomly selected plants per entry per replication for various characters namely, plant height (cm), days to 50% flowering, number of pods/plant, number of pods/cluster, number of clusters/plants, number of seeds/pod, number of pods/plant, grain yield/plant (g) and days to maturity in both the years. The analysis of genetic divergence involved the calculation of pooled mean values for each character from 25 plants in both the *kharif* seasons of 2019 and 2020. The Tocher method (Rao 1952) was employed to group the induced mutants into distinct clusters. The common dispersion matrix (Rao 1952) of a group of correlated variables (Xs) was pivotally condensed to produce a set of uncorrelated linear combinations (Ys). The error mean sum of squares and sum of products were used to form the common dispersion matrix. A connection between Ys and Xs was used to compute the mean values of many attributes for each mutant (X1 to X 24). The D^2 values for the 'X' character were calculated as follows between the i^{th} and j^{th} mutants:

$$D_{ij}^2 = \sum_{r=1}^k (Y_{ir} - Y_{jr})^2, i \neq j$$

The significance of the D^2 values was assessed by treating them as chi-squared (χ^2) values with 'k' degrees of freedom at 5% and 1% levels of significance, where 'k' represents the number of traits. In using D^2 values as a measure of generalized distance between two mutants, the approach recommended by Tocher (Rao 1952) involved initiating the formation of mutants by selecting two closely related mutants with the shortest distance. Subsequently, the third mutant was chosen based on its next D^2 value, which had the lowest average with the first two. This process continued, with the fourth mutant selected based on the smallest average D^2 value from the first three, and so forth. Mutants grouped within the same cluster were expected to display lower average D^2 values among themselves compared to other mutants in different clusters (Singh and Choudary 1985).

To calculate the intra-cluster distance for each cluster, the D^2 value of each mutant was summed with the D^2 values of all other mutants within that cluster. The resulting sum was then divided by the sum of all possible combinations within the cluster (Singh and Choudary 1985).

$$\text{Average intra cluster } D^2 = \sum_{j=1}^h D^2 / n(n-1)/2$$

where, h, Number of D^2 in a cluster; n, Number of mutants in the clusters.

The calculation of inter-cluster distance between any two clusters was achieved by adding the D^2 value of each mutant in one cluster with the D^2 value of every mutant in the other cluster. This sum was then divided by the total number of all possible combinations.

Average inter cluster D^2 between k^{th} and i^{th} cluster

$$\text{Cluster} = \sum_{i=1}^{n_1 n_2} D^2 / n_1 n_2$$

where n_1 , Number of mutants in K^{th} cluster; n_2 , Number of mutants in i^{th} cluster.

To express the total in terms of D, the square root of D^2 values representing intra and inter cluster distance were computed as follows:

$$D = \sqrt{D^2}$$

Determination of cluster mean: The cluster mean for a certain trait is the sum of the average values of mutants present in a cluster divided by the number of mutants in the cluster. These values were computed independently for each cluster and characteristic.

Grouping of mutants: Tocher's method was used to group 22 mutants of rajmash and two controls into separate clusters. The relative distance between the two rajmash mutants was used to compute the two closest mutants of rajmash one by one until they were all sorted in the same order using this approach. D^2 values were utilized to create clusters following this sorting. The closest two rajmash mutants were chosen initially, succeeded by a 3rd with the slightest average D^2 value from the 1st (two) rajmash mutants, and so on. When it was shown that introducing a certain rajmash mutants resulted in a sudden rise in the average D^2 , those rajmash mutants were removed from the cluster. A second cluster was established in the same way, and the procedure was repeated until all 24 rajmash mutants.

Average intra and inter-cluster distances: The square root of intra and inter-cluster distance (D^2) values was used to calculate the average intra and inter-cluster distance (D^2). The whole analysis was programmed and carried out on computer in INDOSTAT software.

Average intra-cluster distances: For the measurement of intra-cluster distance, the following formula was used:

$$D^2 = \sum D^2 / \sqrt{nD^2}$$

where $\sum D^2$, Sum of distance between all possible combination; (n) Population included in a cluster.

Average inter-cluster distances:

$$D^2 = \frac{\sum \text{distance between the population of cluster } i \text{ and } j}{n_i n_j}$$

where n_i , No. of populations in cluster i ; n_j , No. of populations in cluster j

Protein content (%): Estimation of Protein content was done by Micro-Kjeldhal's method (Johann Kjeldahl 1883).

Iron content and zinc content (mg 100/g): Iron content and Zinc content were estimated as the method suggested by Jackson 1973.

RESULTS AND DISCUSSIONS

Genetic diversity studies (D² Analysis): The achievement of a breeding programme via hybridization is incredibly dependent on the genetic divergence of the parents involved. The more diverse the parents, the greater the possibility of pronounced heterotic effects and an accelerated spectrum of variability in the segregating generations. The present study also aimed to investigate the genetic divergence among the 24 rajmash lines which includes 22 induced mutant lines and 2 local controls (BL and PL) during *kharif* 2019 and 2020 to identify the superior and divergent lines for the hybridization programme.

Contribution of each character towards total divergence: Maximum contribution of each character towards genetic divergence (Table 1) was showed by grain yield/plant (53.26%), followed by clusters/plant (18.84%), pods/plant (9.78%), days to 50% flowering (7.25%), pod length (4.35%), seeds/pod (3.62%), 1000-seed weight (2.17%) and maturity days (0.72%) contributing 99.99% to the total divergence. Likewise, in biochemical characters studied zinc content (38.04%) followed by iron content (22.46%) and minimum in protein content (1.81%) was observed. Similar results of highest contribution towards the divergence by seed yield/plant, pods/plant, clusters/plant, maturity days and hundred seed weight, while grain yield/plant, days to maturity and hundred seed weight were primarily responsible for contributing to genetic divergence as reported by Henry *et al.* (2005) in guar. Kumar *et al.*

Table 1 Contribution of individual characters towards genetic divergence

Source	Times ranked 1 st	Contribution (%)
<i>Morpho-physiological parameter</i>		
Days to flowering	20	7.25%
Days to maturity	2	0.72%
Seeds/pod	10	3.62%
Pods/plant	27	9.78%
Pod length (cm)	12	4.35%
Clusters/plant	52	18.84%
1000-seed weight (g)	6	2.17%
Grain yield/plant (g)	147	53.26%
<i>Biochemical Parameters</i>		
Protein content (%)	5	1.81%
Iron content (mg 100/g)	62	22.46%
Zinc content (mg 100/g)	105	38.04%
Grain yield/plant (g)	104	37.68%

(2020) reported the mutants of cluster III & IV shall be utilized for the hybridization programme for the development of high yielding varieties (HYVs) of mungbean. However, Kumar *et al.* (2021a) reported that maximum contribution towards genetic divergence is exhibited by plant height (40.33), followed by grain yield/plant (15.33g) and days to 50% flowering (15%). These three characters together recorded for more than 70% of the total divergence in the 22 mutants. Kumar *et al.* (2017) reported that maximum contribution towards genetic diversity was exhibited by pod length (48.53%) followed by grain yield/plant (30.15%).

Cluster formation: The multivariate analysis grouped 24 lines of rajmash into 5 clusters based on morpho-physiological traits and 8 clusters based on biochemical traits (Table 2). Among these 5 clusters, cluster I was the largest containing 13 mutant's lines followed by cluster II with 4 mutant lines, cluster III and IV with 3 mutant lines each and cluster V had only single mutant line whereas, among 8 cluster, cluster I was the largest containing 16 mutant lines whereas all other clusters having only single mutant except cluster V having two mutants.

Average intra and inter-cluster distance: The average D² value intra and inter clusters among 24 lines calculated by Tocher's method (Table 2 and Fig 1 and 2) showed a dendrogram based on genetic distance between mutant lines of rajmash based on morpho-physiological and biochemical parameters, respectively. The inter-cluster distances were larger than the intra-cluster for morphological characteristics, indicating that there was a significant degree of genetic variation among the mutant lines of rajmash examined. Grouping of mutants into cluster I (13), cluster II (4), cluster III (3), and cluster IV (3), cluster V (1) indicated that these mutants within the cluster are more closely related to each other.

In morphological character cluster III (15.63) showed the highest intra-cluster distance, while the lowest intra-cluster distance was exhibited by cluster V (0.00) likewise, the highest inter-cluster distance was observed between cluster IV and V (342.66), followed by cluster II and IV (204.33), cluster III and V (137.12), cluster I and IV (106.91) and cluster I and V (103.65) and least inter-cluster distance was recorded between cluster I and III (25.67). The most genetically varied mutants came from clusters with the largest inter-cluster distance and hybridization between the mutants from divergent clusters is likely to result in a broad range of variability.

However, for biochemical characteristics inter-cluster distances were larger than intra-cluster, indicating that there was a significant degree of genetic variation among the induced mutant lines of rajmash. As per biochemical characters, cluster I had the greatest intra-cluster distance (39.10), succeeded by cluster V, and all other clusters had the smallest intra-cluster distance (0.00). The highest inter-cluster distance was observed between cluster III and V (342.93), succeeded by cluster II and V (274.51), cluster III and IV (255.20), cluster II and IV (205.42) and cluster III and VIII (201.99). Cluster II and III had the shortest distance

Table 2 Average intra and inter-cluster distance (D^2 - values) on the basis of two years pooled data

	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	-	-	-
<i>Morpho-physiological traits</i>								
Cluster I	<u>13.41</u>	32.99	25.67	106.91	103.65	-	-	-
Cluster II		<u>5.30</u>	58.61	204.33	29.91	-	-	-
Cluster III			<u>15.63</u>	59.92	137.12	-	-	-
Cluster IV				<u>7.08</u>	342.66	-	-	-
Cluster V					<u>0.00</u>	-	-	-
<i>Biochemical traits</i>								
	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Cluster VII	Cluster VIII
Cluster I	<u>39.10</u>	76.43	107.85	70.01	118.52	70.16	84.14	128.58
Cluster II		<u>0.00</u>	9.36	205.42	274.51	77.62	113.01	164.91
Cluster III			<u>0.00</u>	255.20	342.93	98.55	158.36	201.99
Cluster IV				<u>0.00</u>	34.79	82.24	78.56	113.67
Cluster V					<u>19.02</u>	149.72	148.56	95.69
Cluster VI						<u>0.00</u>	25.12	67.34
Cluster VII							<u>0.00</u>	118.23

between them (9.36). Grouping of mutants into cluster I (16), cluster II (1), cluster III (1) and cluster IV (1), cluster V (2), cluster VI (1), cluster VII (1), cluster VIII (1), indicated that these mutants within the cluster are closely related to each other. The mutants from lonely clusters showed high divergence and can be used in breeding. The formation of solitary clusters may be due to total isolation preventing the gene flow or intensive natural selection for diverse adaptive

complexes. These mutants may be unique and useful from breeding point of view. Such kind of lonely clusters were observed by Singh *et al.* (2003) in cluster bean.

As a result, it is predicted that if the various mutant lines of rajmash from different groups are utilized in a breeding programme, a wide range of segregants will be produced. The shortest inter-cluster distance between clusters implies that the lines in these clusters are genetically less vigorous

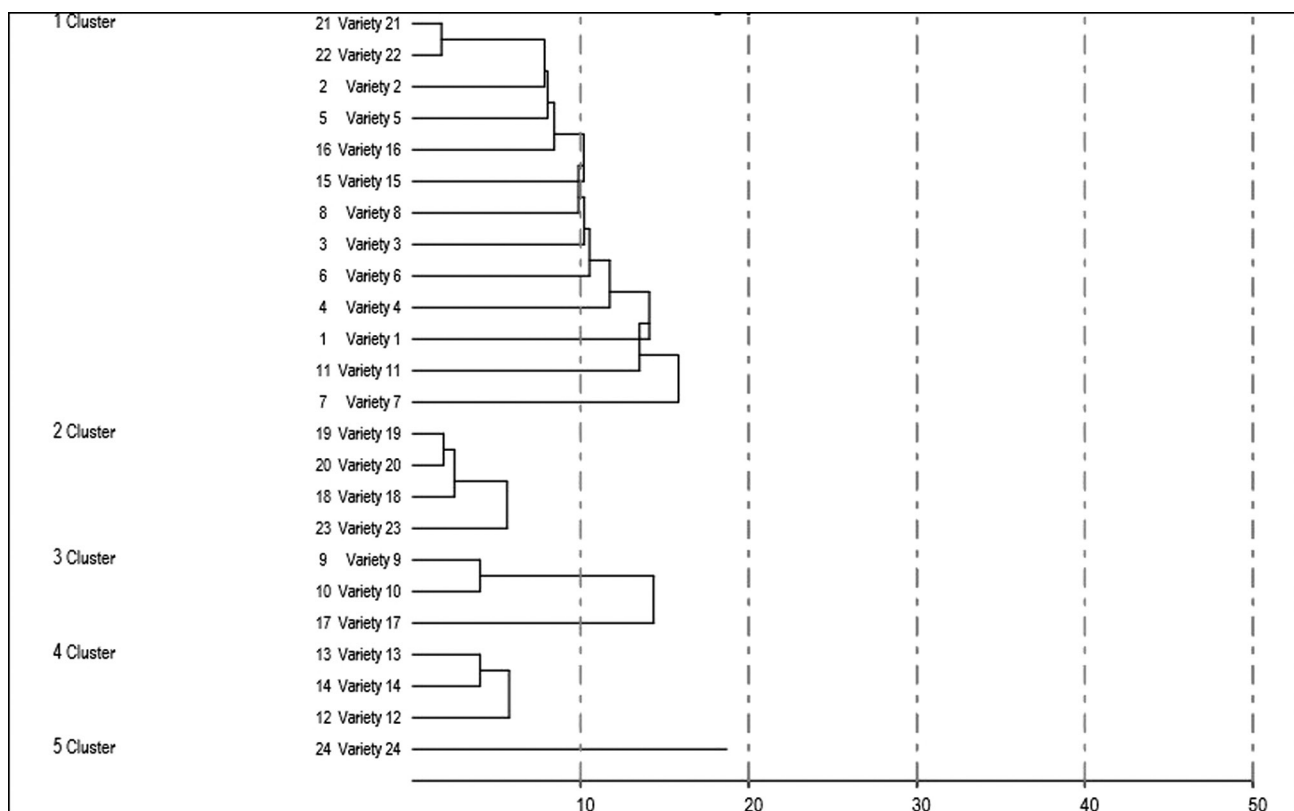


Fig 1 Dendrogram exhibited distribution of mutants in various clusters for morpho-physiological traits.

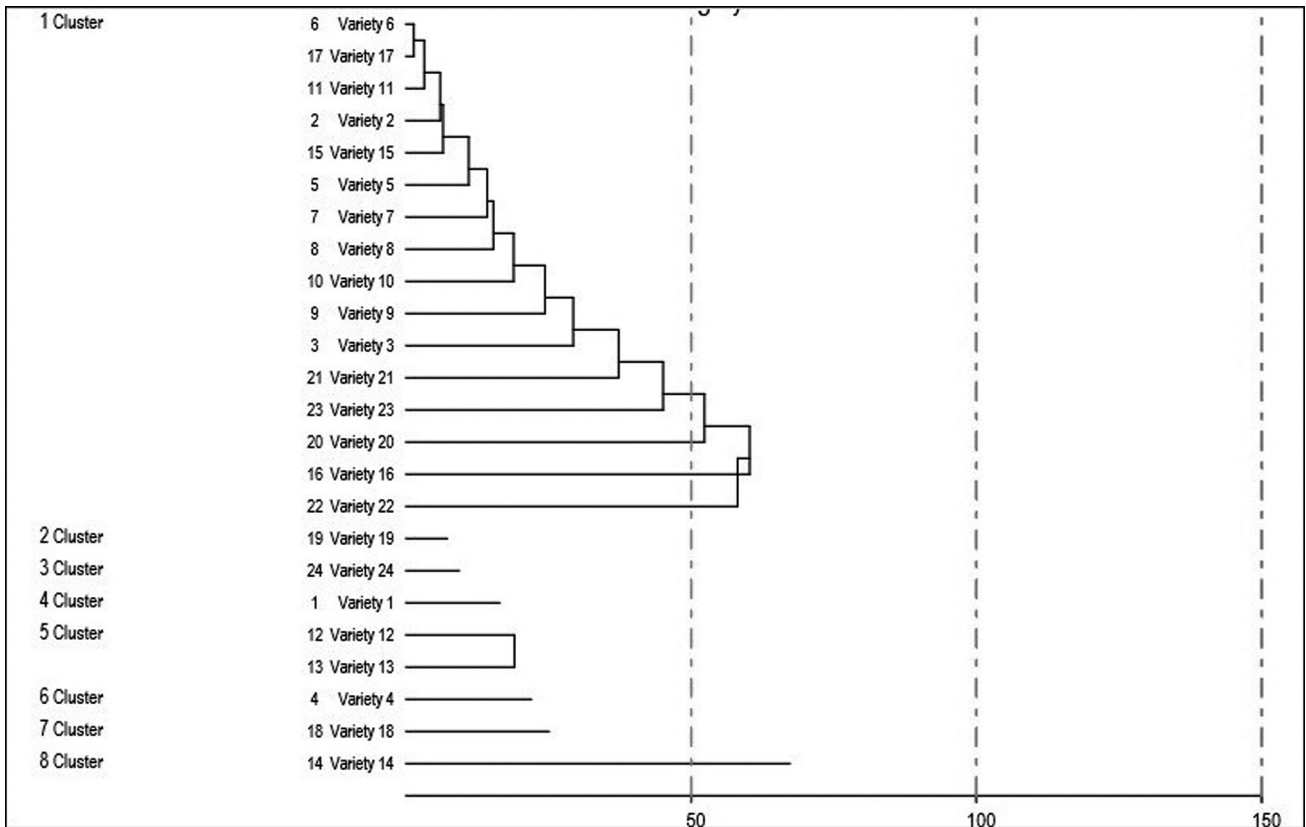


Fig 2 Dendrogram exhibited distribution of mutants in various clusters for biochemical traits.

and almost identical and hybridization in such lines are not rewarding for creating genetic variability. As suggested by Rai and Dharmatti (2014) the maximum distances between clusters are the right choice of diverse parents for hybridization programme.

Cluster mean: The cluster means for morphological and biochemical traits are given in Table 3. The mutant lines of cluster IV had the lowest mean values for days to 50% flowering (64.30) and days to maturity (93.39) and the highest mean values was exhibited for number of seeds/plant (5.23), number of pods/plant (34.33), pod length (7.99), number of clusters/plant (16.82), 1000 seed weight

(239.70) and grain yield/plant (13.04). Similarly, cluster V had lowest mean value for trait, viz. number of seeds/pod (2.77), number of pods/plant (12.36), pod length (4.66), number of clusters/plant (5.40), 1000-seed weight (170.25) and grain yield/plant (4.59). Our results were reliable with Kumar *et al.* (2017) in which cluster-V showed highest mean performance for days to maturity followed by days to 50% flowering in chickpea mutants.

The cluster means for biochemical traits was studied and results showed that Cluster III had high protein content (30.13) followed by cluster IV (28.43), cluster I (27.54). The mean of cluster VII (20.55) and cluster VIII (23.72)

Table 3 Mean performances of pooled data of different clusters for morpho-physiological and biochemical traits

Clusters	Morpho-physiological traits						Biochemical traits					
	Days to flowering	Days to maturity	Seeds/ pod	Pods/ plant	Pod Length (cm)	Clusters/ plant	1000-seed weight (g)	Grain yield/ plant (g)	Protein content (%)	Iron content (mg 100/g)	Zinc content (mg 100/g)	Grain yield/ plant (g)
I	77.48	108.18	4.64	22.83	7.10	12.78	222.33	6.89	27.54	4.22	1.09	7.17
II	88.12	118.01	3.98	17.29	6.41	8.58	195.03	5.66	25.52	6.49	1.67	5.59
III	75.40	105.73	4.61	23.80	6.92	12.16	229.29	9.55	30.13	7.50	1.75	4.59
IV	64.30	93.39	5.23	34.33	7.99	16.82	239.70	13.04	28.43	3.10	0.23	8.56
V	103.89	133.79	2.77	12.36	4.66	5.40	170.25	4.59	23.74	3.10	0.44	12.94
VI	-	-	-	-	-	-	-	-	27.11	6.44	0.48	6.52
VII	-	-	-	-	-	-	-	-	20.55	4.50	0.27	5.56
VIII	-	-	-	-	-	-	-	-	23.72	7.40	0.70	13.23

indicated low protein content. Although cluster mean for the high iron content mutants were found in cluster III (7.50) followed by cluster VIII (7.40), cluster II (6.49) whereas low iron content mean values of mutants were found in cluster IV and cluster V (3.10). Whereas, cluster III (1.75) showed high zinc content followed by cluster II (1.67) whereas cluster IV (0.23) revealed low zinc content. The grain yield/plant was grouped into cluster VIII (13.23) and cluster V (12.94) likewise, minimum cluster mean value was observed in cluster III (4.59).

Therefore, in order to enhance a specific trait, one can select a donor from a suitable cluster. Mutants from different clusters, characterized by high mean values for desired traits and maximum divergence, can be effectively employed in hybridization programmes. For morphological traits, the maximum inter-cluster distance was observed between cluster IV and V followed by cluster II and IV. However, for biochemical traits, the highest inter-cluster distance was observed between cluster III and V followed by cluster II and V, cluster III and IV and mutants from these can be used in hybridization for rajmash improvement programme. Mutant lines (M_3) selected from various clusters for a targeted attribute, such as grain yield/ plant, protein content and other traits should be utilized as donor parents in breeding programmes with the objective of enhancing the specified trait.

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