



Induced genetic variability for quantitative and biochemical traits of local landraces of rajmash (*Phaseolus vulgaris*) of north-western Himalayas

SHILPA BHAGAT¹, SANJEEV KUMAR^{1*}, ARCHANA JOSHI SAHA², SUBHASH CHANDER KASHYAP¹ and RADHYASHYAM KUMAWAT¹

Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir 180 009, India

Received: 09 May 2024; Accepted: 28 August 2024

ABSTRACT

The present study was carried out during rainy (*kharif*) seasons of 2020 and 2021 at Regional Horticulture Research Station (Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, Jammu and Kashmir), Bhaderwah, Doda, Jammu, Jammu and Kashmir to evaluate genetic variability for quantitative and biochemical traits of local landraces of rajmash (*Phaseolus vulgaris* L.) of north-western Himalayas. Twenty-two induced mutant lines of rajmash generated with gamma rays and ethyl methane sulphonate were screened for quantitative, qualitative and anthracnose reactions under natural and artificial conditions in the field during *kharif* 2020 (M_3) and 2021 (M_4). The highly significant differences imply that induced mutant lines for different traits exhibited substantial amount of genetic diversity. Zinc content (55.38) and grain yield/plant (33.24) both had high phenotypic coefficients of variation, while 1000-seed weight (10.14) and protein content had low values (9.83). Zinc content had highest genetic advance, measured as a percentage of the mean, and the highest heritability, followed by grain yield/plant, iron content, number of pods/plant, number of clusters/plant, days to flowering and number of seeds/pod, whereas days to maturity and 1000-seed weight were moderate. The number of pods/plant (0.774), number of seeds/pod (0.556), number of clusters/plant (0.729), length of pod (0.648) and 1000-seed weight (0.620) all exposed a highly significant relationship with grain yield/plant but the days to flowering (-0.636) and days to maturity showed negative correlation (-0.602). It is obvious that the 9 induced mutant lines of Bhaderwah local and Poonch local i.e. R-BL- M_3 -1, R-BL- M_3 -9, R-BL- M_3 -10, R-BL- M_3 -12, R-BL- M_3 -13, R-BL- M_3 -14, R-PL- M_3 -18, R-PL- M_3 -20 and R-PL- M_3 -22 showed the minimum anthracnose disease incidence along with early emergence and superiority in grain yield/plant compared to controls. These mutant lines may be carried forward for the development of mutant genetic stocks and varietal development programmes of rajmash for north-western Himalayan region.

Keywords: Iron, Genetic advance, Heritability, Mutants, Protein, Rajmash, Seed yield, Zinc

Rajmash (*Phaseolus vulgaris* L.), is one of the generally grown pulse in the family Leguminosae having 11 pairs of chromosomes ($2n=2x=22$), a 473 Mb genome, (Schmutz *et al.* 2014). It ranks first in India among Asian nations, occupying 217.00 hectares and producing 2135.35 metric tonnes annually with an average productivity of 983 kg/ha. It is grown in Union Territory of Jammu and Kashmir across an area of over 26.75 hectares with an annual production of about 14.2 metric tonnes and a yield of about 0.8 t/ha (Jan *et al.* 2021). It is mostly grown in hilly districts with Jammu region districts such as Poonch, Bhaderwah and Kishtwar. It is a highly nutritious crop that provides a strong source of vitamins, minerals and proteins particularly

calcium, phosphorus and iron. Pulses, including rajmash have a very narrow genetic base due to small flower size, drought sensitivity, low accessibility, less diverse germplasm and very few varieties developed in India through hybridization (Kumar *et al.* 2021). Traditional plant breeding methods have limited application in the improvement of the rajmash. In more than 50 years of plant breeding studies, the ionizing radiation technique which was first utilized in the early 20th century has played a significant character in the expansion of amended plants (Kharkwal 2012). Mutation breeding is useful for producing variability in existing varieties (Khan and Goyal 2009). Although there are several mutagens for mutations breeding but one of the most popular physical mutagens is gamma ray's (Kantoglu *et al.* 2014). Structural qualities can be alienated into quantitative and qualitative into biochemical traits and the finest period for the documentation of transformed plants is the M_2 generation. However, these plants were also studied in M_4 generations. There is no alternative except

¹Sher-e-Kashmir University of Agriculture Sciences and Technology, Jammu, Jammu and Kashmir; ²Bhabha Atomic Research Centre, Trombay, Mumbai, Maharashtra. *Corresponding author email: ssalgotra@gmail.com

mutation breeding for the improvement of rajmash. Hence, the present study aims to judge the heritable variability of M_3 and M_4 mutant generations of *Phaseolus vulgaris* to screen and excellent altered plants.

MATERIALS AND METHODS

The present study was carried out during rainy (*kharif*) seasons of 2020 and 2021 at Regional Horticulture Research Station (Sher-e-Kashmir University of Agricultural Sciences and Technology, Jammu, Jammu and Kashmir), Bhaderwah, Doda, Jammu, Jammu and Kashmir. Twenty-two induced mutant lines of rajmash generated with gamma rays and ethyl methane sulphonate were screened for quantitative, qualitative and anthracnose reactions under natural and artificial conditions in the field during *kharif* 2020 (M_3) and 2021 (M_4). The mutant breeding materials were generated from the two widely cultivated landraces of rajmash namely Bhaderwah Local (BL) and Poonch Local (PL) under the Ad-hoc Research Project funded by Bhaba Atomic Research Centre, Mumbai, Maharashtra. Both the local landraces (controls) and 22 mutant lines constituted the breeding material for the present study. Ten randomly selected plants per mutant lines were chosen to record the following data in each year i.e. *kharif* 2020 (M_3 generation) and 2021 (M_4 generation) and the pooled mean value of each mutant line in both the generations was computed for statistical analysis.

Morphological characters: Days to 50% flowering were recorded for 10 randomly selected plants in every plot in each year and generation and an average count of days to 50% flowering was computed. Number of days from days to 50% flowering to biological maturity of the crop was used to compute the number of maturity days. Number of pods/plant enumerate after quantifying the total number of pods detected on individual plant at maturity. Number of seeds/pod was estimated by counting the number of seeds/pod of selected pods of observational plants. The length of the pod taken at each designated plant was measured in centimeters and the mean of length of the pod was computed. On the basis of designated experimental plants, number of clusters/plant was counted and an average was taken. 1000-seeds were counted and weight was recorded in grams. The total grain weight/plant after drying was used to calculate grain yield/plant which was recorded phenotypically after harvesting of the crop.

Protein content (%): It was estimated by Micro-Kjeldhal's method (Kjeldahl 1883).

Iron content and zinc content (mg 100/g): As per the method given by (Davies and Reid 1979).

Anthracnose resistance scoring: Disease rating is given to randomly selected observational plants according to 0–9 scale projected by (Mayee and Dattar 1986).

Components of variance: Johnson *et al.* (1955) provided a method for calculating the genotypic and phenotypic coefficient of variation.

Estimation of coefficient of variation: Burton (1952) proposed a method for calculating phenotypic and genotypic variations.

Estimation of heritability and genetic advance: The formula accessible by Allard (1960) was used to compute heritability (h^2) in the broad wisdom.

Correlation coefficient: Genotypic correlation was planned bestowing to Johnson *et al.* (1955).

Statistical analysis: Pooled values of designated experimental plants for various characteristics in both the years were utilized in the statistical analysis. To represent data on various quantitative attributes, the following statistical measures have been computed by Panse and Sukhatme (1985).

RESULTS AND DISCUSSION

Analysis of variance: For each of the eleven (11) characters under the present investigation, the study of variance indicated highly significant differences 0.01 (1%) and 0.05 (5%) in all 22 induced mutant lines (M_3) of rajmash and the two control namely BL and PL (Table 1). The pooled mean sum of squares due to treatments was extremely significant for all the traits. As a result, there was a clearly significant difference between the rajmash induced mutant lines. Ketema and Geleta (2022) showed that the varieties were significantly different for all traits except for days to 50% flowering and number of seed per pod.

Anthracnose resistance: These lines were appraised for anthracnose resistance under normal and artificial environment in the field and glass house conditions respectively, out of which six mutant lines of Bhaderwah Local and Poonch Local namely R-BL- M_3 -1, R-BL- M_3 -9, R-BL- M_3 -10, R-BL- M_3 -12, R-BL- M_3 -13, R-BL- M_3 -14 showed resistance reaction against anthracnose and rest of the mutants are moderately resistant except two controls which are susceptible to anthracnose (Table 2). The anthracnose resistance studied with 0–9 scale and mutant lines showed resistance with the scale bearing from 1 (anthracnose resistance)–9 (anthracnose susceptible). Mutant lines of rajmash in the existing study were primarily categorized into three modules i.e. resistant (disease score 0 and 1), susceptible (disease score 2 and 3) and highly susceptible lines (disease score 7 and 9). Kumar *et al.* (2021) also conveyed comparable results that mutants in M_2 generation of Bhaderwah Local screened under 200Gy and 250Gy showed high resistance against anthracnose and moderate resistance by the mutants induced by EMS. Maibam *et al.* (2015) also reported three genotypes, viz. Rajma Gold, ML-D and ML-F found moderately resistant, whereas 10 and 7 genotypes categorized as moderately susceptible and susceptible, respectively.

Genetic variability: Assessment of heritable variability is essential for any crop breeding programme. Significant variations between rajmash mutants were found in the current study. The outcomes showed that all the variables had a high level of genetic variability (Table 2). It is vitally essential to start a breeding programme with genetic diversity such as genetic coefficient of variation, heritability estimates and genetic gain. For all the parameters examined in the current study, it was found that the PCV surpassed the GCV.

Table 1 Study of variance (ANOVA) for quantitative and biochemical traits of rajmash

Source of variation	Df	Mean sum of squares (MSS)										
		Days to flowering	Days to maturity	No. of seeds/ pod	No. of pods/ plant	Pod length (cm)	No. of clusters/plant	1000-seed weight (g)	Grain yield/ plant (g)	Protein content (%)	Fe content (mg/100 g)	Zn content (mg/100 g)
Replication	2	35.959	57.473	0.074	5.788	0.181	1.190	185.916	0.027	1.540	0.122	0.001
Treatment	23	314.830**	319.198**	1.072**	101.901**	1.745**	24.574**	1235.563**	19.159**	18.496**	5.554**	0.864**
Error	46	16.944	32.820	0.077	2.268	0.121	0.467	119.917	0.224	1.181	0.079	0.006

*and**, indicates significance at 5% and 1% (0.01) highly significant levels, respectively.

The maximum genotypic coefficient of variation was found in the zinc content (54.73) which was trailed by the grain yield/plant (32.66), iron content (29.69), number of pods/plant (25.02) and number of clusters/plant (23.23). The characters like days to maturity (8.98), protein content (8.95) and 1000-seed weight (8.82) showed low values of GCV whereas the number of seeds/pod (12.74), days to flowering (12.70) and pod length (10.55) had a moderate GCV value. This showed the existence of environmental influences in the expression of these traits but these differences were small in most of the traits studied. The improvement of rajmash mutants would be rewarded by selection for phenotype-based traits.

However, highest phenotypic coefficient of variation was for zinc content (55.38) which was trailed by grain yield/plant (33.24), iron content (30.33), pods/plant (25.86) and clusters/plant (23.89). Protein content (9.83) displayed a small value of PCV in contrast to traits such as seeds/pod (14.15) trailed by flowering days (13.74), length of pod (11.68), maturity days (10.41) and 1000-seed weight (10.14). Iron content, grain yield/plant, number of clusters/plant, number of pods/plant, days to flowering, number of seeds/pod, and pod length exposed high to reasonable PCV and GCV values, yet zinc content had the highest phenotypic coefficient of variance. The conclusions of Singh *et al.* (2017), who described high estimates of the genotypic coefficient of variance and phenotypic coefficient of variance for zinc and iron content are reliable with these outcomes. The traits such as number of pods/plant and grain yield/plant were also the theme of maximal GCV and PCV reports by Gangadhara *et al.* (2018). While high GCV and PCV were conveyed by Ramya *et al.* (2014), for the number of clusters/plant. Similar results were found by Gangadhara *et al.* (2018) who exposed that the GCV and PCV for pod length were moderate. Likewise, Ghimire *et al.* (2019) discovered a reasonable genotypic and phenotypic coefficient of variance for days to flowering. Correspondingly, Mammo *et al.* (2019) observed low GCV and PCV for days to maturity. Days to maturity, 1000-seed weight and protein content all have low phenotypic and genotypic coefficients of variation inferring that these appearances have a lower probability of phenotypic selection owed to the greater effect of the environment. Comparable results were also reported by Bhagat *et al.* (2023) in rajmash.

Heritability and genetic advance: In this study, high heritability estimates and high genetic advance percentage of mean for the succeeding traits were found, viz. zinc content (97.7, 111.42), iron content (95.8, 59.87) grain yield/plant (96.6, 66.12), 1000-seed weight (75.6, 15.79), number of clusters/plant (94.5, 46.52), days to flowering (85.4, 24.18), number of pods/plant (93.6, 49.87) and number of seeds/pod (81.6, 23.63), respectively (Table 2). This recommends that additive gene effects are more noteworthy in influential these traits and that enhancement can be attained through phenotypic selection based on these traits. Conferring to Singh *et al.* (2017), zinc content has high heritability as well as a robust genetic advance as a percentage of the mean. Kumar and Singh (2017) described similar results for all the traits under studied except for days to flowering and grain yield/plant. Kumar *et al.* (2020) also exhibited high heritability with high genetic advance percent of mean for 1000-seed weight, number of clusters/plant and grain yield/plant. Kumar *et al.* (2016) showed high heritability with high genetic advance as % mean for pod/length, number of seeds/pod and grain yield/plant. Days to maturity, pod length, and protein content, on the other hand, showed high heritability composed with low genetic progress, demonstrating that phenotypic selection for such features was ineffective in earlier generations. High heritability and high genetic progress as a percentage of mean for days to maturity and pod length, conferring to Mammo *et al.* (2019), recommend that these features are powerfully influenced by the environment and that phenotypic selection is unproductive.

Correlation coefficient analysis: Grain yield is a polygenic trait regulated by several genes that have cumulative gene effects. Correlation studies revealed higher estimates of genotypic correlation coefficients equated to corresponding

Tables 2 Genetic parameters for morpho-physiological, biochemical traits and disease resistance reaction of mutants of rajmash

Treatment	Doses	ADR	Character	Range		Mean (SEm ±)	GCV (%)	PCV (%)	Hbs (%)	GA	GA as percentage of mean
				Min.	Max.						
R-BL-M ₃ -1	100 GyBL	R (1)	Days to flowering	62.57	103.89	78.44 ± 2.37	12.70	13.74	85.4	18.97	24.18
R-BL-M ₃ -2	100 GyBL	MR (3)	Days to maturity	92.36	133.79	108.73 ± 3.30	8.98	10.41	74.4	17.36	15.96
R-BL-M ₃ -3	100 GyBL	MR (3)	No. of seeds/pod	2.77	5.5	4.51 ± 0.16	12.74	14.15	81.1	1.06	23.63
R-BL-M ₃ -4	100 GyBL	MR (3)	No. of pods/plant	12.36	37.64	23.02 ± 0.86	25.02	25.86	93.6	11.48	49.87
R-BL-M ₃ -5	100 GyBL	MR (3)	Pod length (cm)	4.65	8.32	6.96 ± 0.20	10.55	11.68	81.7	1.36	19.65
R-BL-M ₃ -6	100 GyBL	MR (3)	No. of clusters/plant	5.40	17.86	12.20 ± 0.39	23.23	23.89	94.5	5.67	46.52
R-BL-M ₃ -7	100 GyBL	MR (3)	1000-seed weight (g)	170.25	242.58	218.64 ± 6.32	8.82	10.14	75.6	34.54	15.79
R-BL-M ₃ -8	100 GyBL	MR (3)	Grain yield/plant (g)	4.59	13.5	7.69 ± 0.27	32.66	33.24	96.6	5.08	66.12
R-BL-M ₃ -9	100 GyBL	R (1)	Protein content (%)	20.55	30.13	26.81 ± 0.62	8.95	9.83	83.0	4.5	16.81
R-BL-M ₃ -10	100 GyBL	R (1)	Iron content (mg/100g)	2.41	7.50	4.54 ± 0.16	29.69	30.33	95.8	2.72	59.87
R-BL-M ₃ -11	100 GyBL	MR (3)	Zinc content (mg/100g)	0.23	1.80	0.97 ± 0.04	54.73	55.38	97.7	1.08	111.42
R-BL-M ₃ -12	150 GyBL	R (1)	-	-	-	-	-	-	-	-	-
R-BL-M ₃ -13	150 GyBL	R(1)	-	-	-	-	-	-	-	-	-
R-BL-M ₃ -14	150 GyBL	R (1)	-	-	-	-	-	-	-	-	-
R-BL-M ₃ -15	250 GyBL	MR (3)	-	-	-	-	-	-	-	-	-
R-BL-M ₃ -16	250 GyBL	MR (3)	-	-	-	-	-	-	-	-	-
R-BL-M ₃ -17	250 GyBL	MR (3)	-	-	-	-	-	-	-	-	-
R-PL-M ₃ -18	150 GyPL	MR (3)	-	-	-	-	-	-	-	-	-
R-PL-M ₃ -19	250 GyPL	MR (3)	-	-	-	-	-	-	-	-	-
R-PL-M ₃ -20	250 GyPL	MR (3)	-	-	-	-	-	-	-	-	-
R-PL-M ₃ -21	0.5% PL	MR (3)	-	-	-	-	-	-	-	-	-
R-PL-M ₃ -22	0.5% PL	MR (3)	-	-	-	-	-	-	-	-	-
BL Control	BL Control	S (7)	-	-	-	-	-	-	-	-	-
PL Control	PL Control	S (7)	-	-	-	-	-	-	-	-	-

R, Resistant; MR, Moderately resistant; ADR, Anthracnose resistant rating; GyBL, Gray Bhaderwah Local; GyPL, Gray Poonch Local; PCV, Phenotypic coefficient of variation; GCV, Genotypic coefficient of variation; GA, Genetic advance; Hbs, Heritability in broad sense; Fe, Iron; Zn, Zinc.

phenotypic correlation coefficients signifying a strong inherent relationship between the different traits and that environmental factors did not play a major role in the heredity of the considered traits. In the present study grain yield/plant (Table 3) was positively and significantly correlated with the number of seeds/pod, number of pods/plant, pod length, number of clusters/plant, and 1000-seed weight, nonetheless destructively correlated with days to flowering and days to maturity signifying that these characteristics are important in determining grain yield/

plant. Our outcomes were in consistent with the decisions of Lekshmanan and Vahab (2018), that grain yield/plant was positively and significantly associated with the number of seeds/pod, number of pods/plant, pod length, number of clusters/plant and 1000-seed weight and also reported that number of clusters/plant had positive and significant correlation with number of pods/plant. Likewise, our result also presented that grain yield/plant exhibited an adverse, non-significant correlation with protein content, iron content and zinc content. Our conclusions were in consistent

Table 3 Correlation coefficients at phenotypic and genotypic levels among morpho-physiological and biochemical traits of mutants of rajmash

Character	Days to maturity	No. of seeds/pod	No. of pods/plant	Pod length	No. of clusters/plant	1000-seed weight	Grain yield/plant	Characters	Fe content (mg/100 g)	Zn content (mg/100 g)	Grain yield/plant (g)
Days to flowering	r_g 0.999** r_p 0.957**	-0.860** -0.728**	-0.746** -0.684**	-0.787** -0.664**	-0.779** -0.702**	-0.905** -0.764**	-0.699** -0.636**	Protein content	-0.016 0.009	0.235 0.207	-0.286 -0.278
Days to maturity	r_g - r_p -	-0.891** -0.689**	-0.778** -0.680**	-0.792** -0.622**	-0.795** -0.675**	-0.891** -0.683**	-0.714** -0.602**	Iron content	- 0.391**	0.382**	-0.169 -0.156
No. of seeds/pod	r_g - r_p -	- -	0.752** 0.666**	0.888** 0.706**	0.882** 0.784**	0.759** 0.626**	0.655** 0.556**	Zinc content	- -	- -	-0.336 -0.321
No. of pods/plant	r_g - r_p -	- -	- -	0.755** 0.657**	0.894** 0.850**	0.665** 0.576**	0.815** 0.774**	-	- -	- -	- -
Pod length(cm)	r_g - r_p -	- -	- -	- -	0.899** 0.796**	0.777** 0.643**	0.705** 0.648**	-	- -	- -	- -
No. of clusters/plant	r_g - r_p -	- -	- -	- -	- -	0.772** 0.627**	0.765** 0.729**	-	- -	- -	- -

*and**, indicates significance at 5% and 1% levels, respectively.

with Singh *et al.* (2017) testified iron content exhibited a positive and noteworthy correlation with zinc content. Also, Asaduzzaman *et al.* (2014) reported that protein content exhibited a negative correlation through grain yield/plant. Laskar *et al.* (2024) reported the M₂ generation showcased substantial improvements in both yield and associated traits within the french bean cultivar, registering the highest pod yield per plant under the 0.2% EMS treatment. The character association study uncovered robust correlations between pod yield and specific traits, providing a strategic approach to selecting mutants within treated populations influenced by EMS, thus significantly enhancing crop yield.

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