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Analysis of Genetic Diversity in Rajmah Genotypes from the Barak Valley Zone of Assam, India

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ABSTRACT

Background: A comprehensive examination of genetic diversity is essential for informed decisions in crossing programs. This study focused on 14 diverse germplasm lines of bush/pole type Rajmah (*Phaseolus vulgaris*), conducted at the Zonal Research Station, Assam Agricultural University, during the *rabi* seasons of 2020-21 and 2021-22.

Methods: The experiment was conducted utilizing a randomized block design (RBD) and comprised three replications The aim was to assess genetic diversity using ANOVA, genetic parameters, Pearson correlation, D^2 analysis and principal component analysis (PCA).

Result: The results revealed significant differences among the genotypes for all traits measured. The phenotypic coefficient of variation (PCV) was greater than the genotypic coefficient of variation (GCV) across all traits. Key traits like plant height, seed weight per plant and the number of seeds per plant displayed high GCV and heritability, suggesting these should be prioritized in breeding programs. Correlation analysis indicated that seed yield positively correlates with the number of pods per plant, seeds per plant and seed weight, which are critical for selecting high-yield Rajmah genotypes. Cluster analysis categorized the genotypes into six clusters, with Cluster I being the largest. The largest inter-cluster distance was between Clusters IV and VI, indicating potential for heterosis. Principal component analysis highlighted important yield contributors, including days to 50% flowering and average pod weight. Overall, all genotypes exhibited sufficient genetic variability, with genotypes KMJ-R-2019-02, KMJ-R-2019-17 and KMJ-R-2017-08 performing better than the check genotypes in yield-related traits, making them valuable for breeding programs.

Key words: ANOVA, Correlation, D^2 analysis, GCV, Heritability, PCA analysis, *Phaseolus vulgaris*.

INTRODUCTION

Phaseolus vulgaris L., commonly referred to as the French bean or common bean, is a highly regarded short-duration leguminous vegetable crop cultivated globally for its dry seeds and edible pods. The varieties utilized for dry seeds, designated as dal or pulses, are known as Rajmah or Rajma. Rajmah serves as a significant source of calories and protein within human diets and is increasingly popular for its tender pods and shelled beans (Smithson *et al.,* 1993). Often referred to as the “poor man’s meat,” these beans are rich in protein, fiber and carbohydrates (Beebe *et al.,* 2000). In India, the primary cultivation regions for Rajmah encompass Himachal Pradesh, Uttarakhand, Jammu and Kashmir, Punjab, Haryana, Uttar Pradesh, Bihar, Gujarat, Madhya Pradesh, Maharashtra, Karnataka, Andhra Pradesh, Tamil Nadu and portions of the northeastern states. Within the Barak Valley Zone of Assam, which includes the districts of Cachar, Karimganj and Hailakandi, Rajmah is recognized as the second most significant crop after rice. These landraces exhibit variability in morphology, growth patterns, seed size and shape, flavor, coat color, maturity duration and yield potential. Farmers select different landraces based on personal preferences; however, this practice may result in diminished yields as many prioritize taste over productivity (Verma *et al.,* 2014). Factors such as urbanization, declining soil nutrient quality, the introduction of high-value vegetable crops and climatic constraints are rapidly eroding the biodiversity of this crop within the region. Consequently, there is an urgent need to preserve and leverage the rich genetic resources of Rajmah beans. The critical characteristics for genetic improvement include wider adaptability, shorter growth duration and enhanced resistance to pests and diseases. Seed yield, being a complex quantitative trait, necessitates selection based on component traits to develop superior varieties. Identifying the critical component traits that influence seed yield, either directly or indirectly, requires a thorough understanding of the degree and direction of association among plant characteristics. Notably, the genetic diversity of Rajmah beans from the Barak Valley region of Assam has not been documented to date. This investigation,

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therefore, aims to evaluate the genetic diversity of this crop and elucidate the associations among various traits.

MATERIALS AND METHODS

The genetic materials examined consist of 13 Rajmah genotypes sourced from the Zonal Research Station, Assam Agricultural University in Karimganj, Assam, along with a check variety named Arun (Table 1). The evaluation of these Rajmah genotypes was conducted at the main research farm of the Zonal Research Station during the rabi seasons of 2020-21 and 2021-22. The seeds were sown on raised beds with a row spacing of 30 cm and a plant spacing of 10 cm. The experiment was conducted utilizing a randomized block design (RBD) and comprised three replications. All recommended agronomic practices were followed to ensure optimal crop development (PoP of rabi crops of Assam, 2015). Data were collected from five randomly selected plants per replication, focusing on sixteen morphological characters, which include: - Days to fifty per cent flowering (D50F), leaf length (LL), leaf breadth (LB), plant height (cm) (PH), number of branches per plant (NBP), number of pods per plant (PPP), pod length (cm) (PL), pod breadth (cm) (PB), number of seeds per pod (NSP), seed weight per pod (gm) (SWP), average pod weight (gm) (APW), number of seeds per plant (NSPP), seed weight per plant (gm) (SWPP), hundred seed weight (g) (HSW), seed yield (kg per hectare) (SYH).

Statistical analysis

The average values of the quantitative traits were analyzed using ANOVA with the 'metan' package in R software. Genetic parameters were estimated using the formulas proposed by Burton (1952) for genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), by Hanson *et al.* (1956) for heritability and by Allard (1960) for the expected genetic advance as a percentage of the mean, calculated in MS Excel 2007. Additionally, Pearson correlation coefficients, Mahalanobis D^2 and principal component analysis (PCA) were conducted using R software.

RESULTS AND DISCUSSION

The pooled ANOVA analysis for the two years (Table 2a and 2b) showed that the mean squares due to years were significant for seven characteristics: plant height, number of branches per plant, number of pods per plant, pod length, pod breadth, number of seeds per pod and number of seeds per plant. This suggests that the years had a significant influence on the phenotypic expression of these traits. Additionally, there were noticeable genotypic differences for all the traits, as indicated by highly significant mean squares. Furthermore, the interaction between years and genotype was substantial for the number of branches per plant, pod length and hundred seed weight, indicating a significant influence of the years on the phenotypic expression of these traits.

The variation observed among various traits under study demonstrated the levels of free variability in populations of different genotypes, highlighting the potential impacts of this variability on yield. The performance of the genotypes was assessed based on the pool mean values of the observed traits. The mean performances of the cultivars, averaged across different environments, are presented in Table 3. The highest mean values for various traits were recorded in specific genotypes: KMJ-R-2019-01 exhibited the earliest days to 50% flowering; KMJ-R-2019-19 had the longest leaf length; KMJ-R-2017-08 showed the widest leaf breadth, along with the longest pod length, the greatest number of seeds per pod, the heaviest seed weight per pod, the average pod weight, the total seed weight per plant and the hundred seed weight. KMJ-R-2019-06 was noted for having the shortest plant height, while KMJ-R-2019-17 recorded the highest number of branches per plant. KMJ-R-2019-20 attained the greatest pod breadth; Arun had the highest number of seeds per plant and KMJ-R-2019-02 achieved the highest seed yield

Table 1: List of Rajmah genotypes used for evaluation during *Rabi* 2020-21.

Accession no./Name	Growth habit	Seed colour	Shape	Hilum colour
KMJ-R-2016-04	Bush	Purple black	Kidney shaped, medium sized seeds	White
KMJ-R-2017-08	Bush	Gulf red	Rectangular, big sized seeds	White
KMJ-R-2019-01	Bush	Brown speckled	Kidney shaped, medium sized seeds	White
KMJ-R-2019-02	Bush	White with brown streak	Kidney shaped, big sized seed	White
KMJ-R-2019-05	Bush	Black mottled	Kidney shaped, medium sized seeds	White
KMJ-R-2019-06	Bush	Purple mottled	Kidney shaped, medium sized seeds	White
KMJ-R-2019-07	Bush	Reddish mottled	Kidney shaped, medium sized seeds	White
KMJ-R-2019-16	Bush	Blackish	Rectangular, big sized seeds	White
KMJ-R-2019-17	Bush	Gulf red	Rectangular, medium sized seeds	White
KMJ-R-2019-18	Bush	Blackish	Rectangular, big sized seeds	White
KMJ-R-2019-19	Bush	Brown speckled	Kidney shaped, medium sized seeds	White
KMJ-R-2019-20	Bush	Blackish	Oval shaped, small sizeed seeds	White
KMJ-R-2019-22	Bush	Blackish	Rectangular, medium sized seeds	White
Arun (Check)	Semi pole	Gulf red	Kidney shaped, medium sized seeds	White

in kilograms per hectare, followed closely by KMJ-R-2019-17 and KMJ-R-2017-08.

The general mean, genotypic and phenotypic coefficients of variation, heritability and genetic advance as a percentage of the mean are summarized in (Table 4). The highest genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) were found in plant height (27.29 for GCV and 28.03 for PCV), seed weight per plant (20.74 for GCV and 23.50 for PCV), hundred seed weight (13.18 for GCV and 13.46 for PCV) and average pod weight (11.54 for GCV and 13.20 for PCV), indicating these traits' strong contribution to Rajmah bean improvement. A high level of heritability (over 60%) was observed in eight of the fifteen traits, ranging from 73.00% to 95.89%. The highest heritability was found in hundred seed weight (95.89%) and plant height (94.76%). High heritability suggests these traits are primarily controlled by genetics rather than environmental factors. Significantly, six traits exhibited high genetic advance as a percentage of the mean, from 20.76% to 54.72%, with the greatest advance in plant height (54.72%) and seed weight per plant (37.69%). These findings indicate that selecting for high heritability and genetic advance will be beneficial for breeding efforts. The traits to prioritize for Rajmah breeding include plant height, seed weight per plant, number of seeds per plant, hundred seed weight, number of pods per plant and average pod weight. This study's findings are consistent with previous research by Sofi *et al.* (2011); Verma *et al.* (2014) and Devi *et al.* (2015) that reported high heritability and genetic advance in common beans.

Understanding the relationship between yield and its components is crucial for developing effective plant selection strategies. The current study revealed both positive and negative correlations between yield and its components (Fig 1). The results indicated that traits such as the number of pods per plant (0.69), number of seeds per pod (0.68), pod length (0.66), number of seeds per plant (0.65), seed weight per plant (0.61) and average pod weight (0.54) were significantly and positively correlated with grain yield per hectare at the 0.01 probability level. Conversely, traits like days to 50% flowering (0.44), plant height (0.26) and number of seeds per plant (0.20) showed no significant but negative correlations with hundred seed weight at the 0.05 probability level. Specifically, the negative correlation observed for plant height suggests that shorter parental lines tend to produce greater seed weight and vice versa. Overall, the seed yield per hectare is greatly influenced by the number of pods per plant, number of seeds per pod, pod length, number of seeds per plant, seed weight per plant and average pod weight. Similar findings have been documented by Kamaludin (2011) and Singh *et al.* (2011). These studies indicate that seed yield exhibits a significant positive correlation with the number of pods per plant, the number of seeds per plant and seed weight. Therefore, the associations among these yield and yield components are crucial for selecting desirable Rajmah genotypes with high yield potential.

Based on the divergence analysis, 14 genotypes were categorized into 6 clusters using estimated D^2 values

Table 2a: Pooled analysis of variance for fifteen quantitative traits in Rajmah genotypes.

Source	df	Mean square						
		D50F	LL	LB	PH	NBP	PPP	PL
Year	1	74.30**	0.05	0.20	61.40**	0.49**	1.55**	13.90**
Rep within the year	4	5.45	0.06	0.12	23.48	0.10	1.10	0.90
Genotype	13	62.05**	2.14**	1.32**	1153.98**	0.60**	13.45**	3.40**
Gen × Year	13	4.19	0.05	0.04	14.29	0.25*	0.87	2.61**
Pooled error	52	1.62	0.71	0.24	10.54	0.09	0.45	0.46
CV (%)		1.70	6.88	5.81	6.42	6.52	5.63	5.08

* and ** indicates significant at P<0.05 and 0.01 respectively.

Table 2b: Pooled analysis of variance for fifteen quantitative traits in Rajmah genotypes.

Source	df	Mean square							
		PB	NSP	SWP	APW	NSPP	SWPP	HSW	SYH
Year	1	0.21**	0.88**	0.02	0.00	104.00**	4.57	0.76	236237.00
Rep within the year	4	0.01	0.02	0.09	0.23	9.75	1.41	3.56	112844.25
Genotype	13	0.01**	0.28**	0.38**	0.81**	381.92**	98.27**	254.09**	723866.31**
Gen × Year	13	0.00	0.05	0.01	0.03	24.08	3.54	6.79*	35106.69
Pooled error	52	0.00	0.05	0.02	0.04	9.33	4.45	1.80	191766.81
CV (%)		3.67	5.02	6.17	6.42	6.85	11.06	2.73	18.60

* and ** indicates Significant at P< 0.05 and 0.01 respectively.

D50F- Days to 50% flowering, LL- Leaf length, LB- Leaf breadth, PH- Plant height, NBP- No. of branches per plant, PPP-No of pods per plant, PL- Pod length, PB- Pod breadth, NSP- No. of seeds per pod, SWP- Seed weight per pod, APW- Average pod weight, NSPP- No. of seeds per plant, SWPP- Seed weight per plant, HSW- Hundred seed weight, SYH- Seed yield kg per hectare.

Table 3: Pooled mean performance of fourteen Rajmah genotypes evaluated during *rabi* season 2020-21.

Genotype	D50F	LL	LB	PH	NBP	PPP	PL	PB	NSP	SWP	APW	NSPP	SWPP	HSW	SYH
KMJ-R-2016-04	72.67	11.55	8.08	45.65	4.60	11.09	13.98	1.12	4.77	2.50	3.27	40.82	19.07	53.00	2465.11
KMJ-R-2017-08	73.67	12.60	9.10	48.90	4.17	14.20	14.26	1.14	5.12	2.68	4.08	45.58	25.43	60.67	2841.56
KMJ-R-2019-01	70.00	13.02	8.73	46.67	4.30	11.03	13.94	1.11	4.57	2.37	3.33	46.93	14.58	48.67	2454.44
KMJ-R-2019-02	74.17	12.15	8.20	48.73	4.83	14.20	13.69	1.21	4.53	2.70	3.23	54.42	24.78	55.33	2883.56
KMJ-R-2019-05	77.00	13.23	8.75	47.18	4.98	14.24	13.84	1.20	4.73	2.53	2.87	53.98	23.20	46.83	2651.11
KMJ-R-2019-06	71.67	11.60	7.82	43.27	4.40	12.12	12.78	1.13	4.50	1.88	2.62	52.75	16.07	43.50	1919.56
KMJ-R-2019-07	73.67	11.98	8.83	45.93	4.03	9.99	13.88	1.18	4.53	2.45	3.02	34.92	15.30	45.83	2174.89
KMJ-R-2019-16	72.83	11.72	7.70	48.15	4.40	11.32	12.84	1.20	4.27	2.55	2.95	41.88	19.55	59.00	2168.89
KMJ-R-2019-17	73.17	12.60	8.55	49.82	5.00	13.22	13.68	1.15	4.88	2.65	3.17	45.20	22.27	49.33	2872.22
KMJ-R-2019-18	75.17	12.28	8.82	49.55	4.32	11.86	12.96	1.19	4.48	2.53	3.17	34.57	23.55	53.50	1979.11
KMJ-R-2019-19	75.00	12.87	8.73	46.22	4.73	10.87	13.23	1.16	4.53	2.47	3.33	33.90	17.03	49.33	2075.78
KMJ-R-2019-20	76.67	11.17	7.78	42.45	4.83	11.88	11.30	1.29	4.40	2.18	2.65	34.30	15.75	44.17	1987.06
KMJ-R-2019-22	76.67	12.35	8.48	47.57	4.22	9.90	13.35	1.15	4.62	2.27	3.03	49.80	13.85	38.33	2103.78
Arun (Check)	83.50	12.32	7.87	98.18	4.32	10.97	13.67	1.17	4.43	1.97	2.77	54.90	16.53	41.50	2380.00

Table 4: Estimates of genetic parameters in Rajmah genotypes.

Parameters	D50F	LL	LB	PH	NBP	PPP	PL	PB	NSP	SWP	APW	NSPP	SWPP	HSW	SYH
GM	74.70	12.25	8.39	50.59	4.51	11.92	13.39	1.17	4.60	2.41	3.11	44.57	19.07	49.21	2354.08
SeM	0.52	0.34	0.20	1.33	0.12	0.27	0.28	0.02	0.09	0.06	0.08	1.25	0.86	0.55	178.78
GCV	4.25	3.99	5.06	27.29	6.49	12.35	5.23	3.15	4.23	10.14	11.54	17.68	20.74	13.18	12.65
PCV	4.58	7.95	7.70	28.03	9.20	13.57	7.29	4.84	6.56	11.86	13.20	18.96	23.50	13.46	22.50
H2bs	86.15	25.15	43.20	94.76	49.77	82.79	51.48	42.34	41.52	73.00	76.35	86.94	77.86	95.89	31.62
GA % of mean	8.12	4.12	6.86	54.72	9.43	23.15	7.73	4.22	5.61	17.84	20.76	33.96	37.69	26.58	14.65

(Table 5). Cluster I included six genotypes: KMJ-R-2019-16, KMJ-R-2019-19, KMJ-R-2019-18, KMJ-R-2019-17, KMJ-R-2019-20 and KMJ-R-2016-04. Clusters II, III and IV each contained two genotypes, while Clusters V and VI had one each. The grouping appeared random, indicating no direct link between genetic distance and clustering. Intra-cluster distances showed that Cluster IV had the highest distance at 135.79, followed by Cluster III at 126.48, Cluster II at 125.58 and Cluster I at 95.12 (Table 6). Inter-cluster analysis revealed that the largest distance was between Clusters IV and VI at 902.72, followed by Clusters III and IV at 685.67, indicating potential for effective hybridization among these clusters. Among the 15 traits studied, hundred seed weight contributed the most to total diversity at 41.9%, followed by days to 50% flowering (9.2%) and pods per plant (8.8%) (Table 7). The clustering of genotypes suggests that parents from Clusters III, V and VI can be utilized to breed high-yielding, early-flowering types with better pod quality. These findings are consistent with earlier research by Gangadhara *et al.* (2014), Kumar *et al.* (2014) and Gelaw (2017).

Principal component analysis (PCA), a technique utilized for dimensionality reduction, was implemented using the dataset encompassing the horticultural characteristics under investigation. The analysis yielded five principal components, with their corresponding eigen values, percentage variance and cumulative percentage variance detailed in Table 8. The first principal component (PC1) exhibited the highest eigenvalue of 5.74, accounting for 38.30% of the total variation. The second and third principal components explained 17.51% and 16.67% of the total variation, respectively. Furthermore, the fourth and fifth components contributed 8.23% and 7.41% to the total variation, respectively. Collectively, these five principal components accounted for 88.12% of the cumulative total variation, thereby indicating that they adequately represent the variance within the reduced dimensions. The PCA further facilitated the interpretation of the relative weight of variables within each component. Significant variables are characterized by pronounced positive or negative weights. According to Girgel (2021), eigenvalues exceeding 1 suggest that the weights associated with the evaluated principal components are robust (Fig 2). The contributions of various traits to the principal components relevant to Rajmah are presented in (Table 7 and Fig 3). In the first component (PC1), positive weights were assigned to average pod weight (0.348), seed yield per hectare (0.339), seed weight per pod (0.335), number of seeds per pod (0.334), seed weight per plant (0.313) and pod length (0.302). For the second component (PC2), high positive weights were recorded for plant height (0.378), pod length (0.363) and number of seeds per plant (0.306). The third component (PC3) displayed significant positive weights for the number of seeds per plant (0.394), days to 50% flowering (0.392) and number of branches per plant (0.371). In summary, the principal component analysis identified key variables pertaining to yield-

Table 5: Cluster composition and classification of Rajmah genotypes.

Cluster identity	No. of genotypes	Name of genotypes
I	6	KMJ-R-2019-16, KMJ-R-2019-19, KMJ-R-2019-18, KMJ-R-2019-17, KMJ-R-2019-20, KMJ-R-2016-04
II	2	KMJ-R-2019-05 and KMJ-R-2019-06
III	2	KMJ-R-2017-08 and KMJ-R-2019-02
IV	2	KMJ-R-2019-22 and Arun
V	1	KMJ-R-2019-01
VI	1	KMJ-R-2019-16

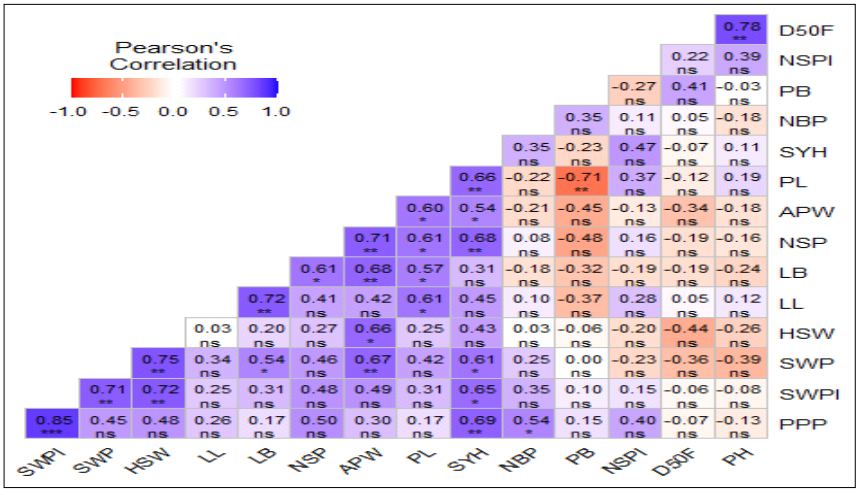


Fig 1: Graphical representation of pearson correlation of fifteen quantitative traits of Rajmah.

Table 6: Intra and inter cluster distance of fourteen Rajmah genotypes.

Cluster identity	I	II	III	IV	V	VI
I	95.12	249.41	233.38	357.39	140.10	247.76
II	249.41	125.58	383.77	205.52	196.82	610.14
III	233.38	383.77	126.48	685.67	272.72	160.97
IV	357.39	205.52	685.67	135.79	374.02	902.72
V	140.10	196.82	272.72	374.02	0.00	333.64
VI	247.76	610.14	160.97	902.72	333.64	0.00

Table 7: Cluster mean performance and contribution of the characters toward divergence of fourteen Rajmah genotypes.

Characters	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI	Contribution of characters towards divergence (%)
D50F	74.39	74.33	73.92	80.08	70.00	72.83	9.2
LL	12.08	12.42	12.38	12.33	13.02	11.72	3.3
LB	8.47	8.28	8.65	8.18	8.73	7.70	2.7
PH	46.60	45.23	48.82	72.87	46.67	48.15	4.6
NBP	4.59	4.69	4.50	4.27	4.30	4.40	4.9
PPP	11.48	13.18	14.20	10.43	11.03	11.32	8.8
PL	13.17	13.31	13.98	13.51	13.94	12.84	2.5
PB	1.18	1.17	1.17	1.16	1.11	1.20	3.3
NSP	4.60	4.62	4.83	4.53	4.57	4.27	1.2
SWP	2.46	2.21	2.69	2.12	2.37	2.55	5.4
APW	3.10	2.74	3.66	2.90	3.33	2.95	3.3
NSPP	37.28	53.37	50.00	52.35	46.93	41.88	8.7
SWPP	18.83	19.63	25.11	15.19	14.58	19.55	0.2
HSW	49.19	45.17	58.00	39.92	48.67	59.00	41.9
SYH	2259.03	2285.34	2862.56	2241.89	2454.44	2168.89	0

Table 8: Eigen value, contribution of variability and factor loadings for the principal components of fourteen Rajmah genotypes.

	PC1	PC2	PC3	PC4	PC5
Eigen value	5.74	2.63	2.50	1.23	1.11
Proportion of variance	0.38	0.18	0.17	0.08	0.07
Cumulative proportion	0.38	0.56	0.72	0.81	0.88
	PC1	PC2	PC3	PC4	PC5
D50F	-0.143	0.211	0.392	-0.512	-0.042
LL	0.246	0.278	0.053	-0.209	0.443
LB	0.285	0.129	-0.230	-0.356	0.363
PH	-0.087	0.378	0.325	-0.296	-0.363
NBP	0.059	-0.291	0.371	0.148	0.437
PPP	0.268	-0.206	0.367	0.141	0.037
PL	0.302	0.363	-0.045	0.049	-0.104
PB	-0.166	-0.387	0.258	-0.387	0.074
NSP	0.334	0.114	-0.011	0.086	0.130
SWP	0.335	-0.248	-0.060	-0.197	-0.013
APW	0.348	0.037	-0.215	-0.159	-0.188
NSPP	0.063	0.306	0.394	0.429	-0.051
SWPP	0.313	-0.225	0.245	-0.116	-0.190
HSW	0.273	-0.293	-0.078	-0.041	-0.485
SYH	0.339	0.058	0.269	0.121	-0.060

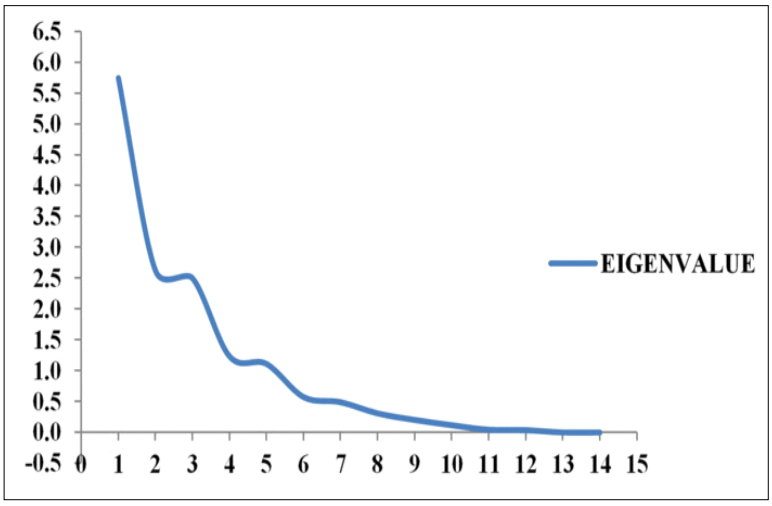


Fig 2: Graphical representation of eigen value of fourteen genotypes of Rajmah.

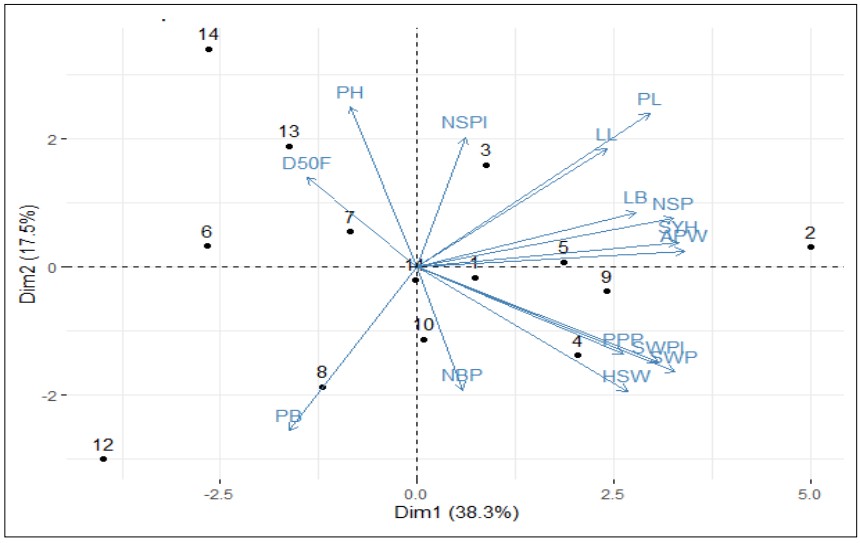


Fig 3: Biplot of fourteen genotypes and fifteen quantitative traits of Rajmah.

contributing traits within Rajmah genotypes, notably days to 50% flowering, plant height, pod length, pod breadth, seed weight per pod, average pod weight and number of seeds per plant. These variables warrant consideration for effective selection of parental lines during hybridization programs. Previous studies, including those by Sharma *et al.* (2019); Reddy *et al.* (2021) and Girgel (2021), have reported analogous selection indices for the enhancement of French bean varieties.

CONCLUSION

From the present investigation, it is concluded that all genotypes exhibited sufficient genetic variability for the traits studied. Genotypes KMJ-R-2019-02, KMJ-R-2019-17 and KMJ-R-2017-08 outperformed the check genotypes in terms of yield and yield-attributing traits. These genotypes could be valuable for breeding programs aimed at improving specific traits.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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