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Assessment of DUS Traits in Rajmash (*Phaseolus vulgaris* L.) Genotypes: A Comprehensive Study on Genetic Diversity and Morphological Characteristics

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ABSTRACT

The study was conducted at Dry Land Agriculture Research Station (DARS), Rangreth, Srinagar, Jammu and Kashmir, India aimed to comprehensively assess 40 genotypes, alongside 5 checks, for Distinctness, Uniformity and Stability (DUS) characterization. Employing an Augmented Block Design in the field in *kharif* season of April–August, 2022 and a Completely Randomized Design (CRD) in the laboratory from September–March 2023, the genotypes underwent evaluation for 21 DUS traits and 43 morphological, maturity, yield and quality traits. The evaluation followed descriptors specific to rajmash genotypes, utilizing frequency distribution based on "state" and "code" for each trait. The results indicated that majority of genotypes exhibited early flowering (<50 days), with 91% displaying an absence of anthocyanin coloration. Erect plant types were prevalent (51.11%), with 51% and 60% being viny and determinate, respectively. Most genotypes featured medium-sized, light green, ovate leaves (46.66%, 53%, and 22%, respectively). White-colored flowers (48.88%) with striped petal surfaces (57.77%) and medium curvature (40.00%) were predominant. Seed-related traits, such as cordate pod shapes (51%) and concave pod sutures (50%), along with acute pod distal shapes (69%), were observed. Pale green pods (57.77%) and 76% of genotypes exhibiting pod stringiness were noted. Seed shapes were mainly kidney-shaped (60%), and absent pigmentation in pod shells (73%) was observed. Large-sized seeds (40%) with dark red testa color (44%) were common, and seed testa variegation was "absent" in 75.55% of genotypes.

KEYWORDS: Anthocyanin, CRD, pigmentation, quality, variegation

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1. INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) a member of the *Leguminaceae* family is the most significant legume crop for direct human consumption and has a high yielding capacity as compared to gram and pea. It is a short duration legume crop, self-pollinated and diploid in nature having chromosome no. (2n=22). This is also termed as "King of Nutrition" due to its nutritional and health benefits (Vijayarani and Sabarimathi, 2021). It is an important source of protein and provides 15% of proteins and fulfill 30% of the caloric requirements of the world's population and accounts for 50% of all the grain legumes consumed globally (McConnel, 2010). It is a staple food crop and an essential source of protein and nutrients for millions of people worldwide (Los et al., 2018). Common beans, often referred to as "Poor Man's Meat" (Jackson et al., 2020), are indeed unique from a nutraceutical perspective (Sheoran et al., 2020). They provide a cost-effective source of high-quality protein, typically containing 21–25% protein content (Singh et al., 2022).

DUS (Distinctiveness, Uniformity and Stability) characterization is a fundamental process in the evaluation and documentation of common bean varieties. Evaluating DUS (Distinctiveness, Uniformity and Stability) traits helps in identifying and protecting plant breeder's right and ensuring accurate identification of different varieties. Common bean is also called as string bean, field bean, snap bean and haricot bean. Common bean is cultivated throughout the world for its green pods as well as for dry seeds (Adikshita and Kansak, 2017). Two geographically isolated and genetically recognized wild gene pools (Andean and Mesoamerican) evolved from a common wild ancestral parent about 10,000 years ago, and from these wild gene pools, nearly 8000, years ago, common bean was independently domesticated in Mexico and South America (Bitocchi et al., 2013).

Seed storage protein, phaseolin, helps in identifying domestication patterns in common bean. Two major phaseolin types that have been largely found in common bean are "S" and "T" type, with Mesoamerican and Andean genotypes possessing "S" and "T" type phaseolin patterns, respectively (Choudhary et al., 2018). A class of antioxidants known as polyphenols is prevalent in beans (Ganesan and Xu, 2017). Consuming beans may reduce one's risk of dying from a heart attack, stroke or other cardiovascular disease (Didinger et al., 2022). In India, common bean is cultivated in the states of Himachal Pradesh, Uttar Pradesh, Jammu and Kashmir, Maharashtra, Karnataka, Kerala and Tamil Nadu (Jan et al., 2021). Western Himalayas of Jammu and Kashmir holds great diversity of common bean landraces (Choudhary et al., 2018). Use of morphological descriptors in sequential fashion is useful and convenient to discriminate the different varieties (Joshi et al., 2018) variety is said to be new if it possesses characters Evaluating DUS (Distinctiveness, Uniformity and Stability) traits helps in identifying and protecting plant breeder's right and ensuring accurate identification of different varieties (Yang et al., 2021).

Common bean is only grown as a specialty crop in Himachal Pradesh, Uttar Pradesh, Jammu & Kashmir, and the North Eastern states of India. Common beans are a significant crop in Jammu and Kashmir, especially in the rainfed highlands where they are intercropped with maize on an area of about 30 thousand hectares, yielding about 17 thousand tonnes and having a productivity of about 0.56 tons ha^{-1} (Anonymous, 2020). With an output of about 1.6 thousand tonnes and a yield of about 0.8 tons ha^{-1}, common bean is produced over an area of about 2 thousand hectares in Kashmir Valley (Saba et al., 2016). The current investigation aimed to characterize niche specific Common bean (*Phaseolus vulgaris* L.) germplasm lines of Kashmir for Distinctness, Uniformity and Stability (DUS) characterization of 40 genotypes, alongside 5 checks, 21 DUS traits and 43 morphological, maturity, yield and quality traits.

2. MATERIALS AND METHODS

The study was conducted at Dry Land Agriculture Research Station (DARS), Rangreth, Srinagar, Jammu and Kashmir, India during *kharif* season of April-August, 2022 and in the laboratory from September–March 2023. The study included a total of 45 genotypes of common beans (*Phaseolus vulgaris* L.) including Forty rajmash genotypes were evaluated along with the five checks as follows: PPR-1, PPR-2, PPR-3, PPR-4, PPR-5, PPR-6, PPR-7, PPR-8, PPR-9, PPR-10, PPR-11, PPR-12, PPR-13, PPR-14, PPR-15, ALR-3, ALR-9, ALR-12, ALR-13, ALR-20, ALR-28, ALR-55, ALR-57, ALR-62, ALR-64, ALR-67, ALR-73, ALR-74, ALR-79, ALR-89, ALR-90, ALR-103, KDR-4, KDR-40, KDR-65, KDR-77, KDR-80, KDR-96, KDR-98, KDR-105, Shalimar Rajmash-1, Shalimar Rajmash-2, Jawala, Kailash, Baspa.

According to the DUS Descriptor, (PPV and FRA, New Delhi, 2007), the material under research was characterized for DUS traits. Different traits were recorded at their recommended stages of observation. Observations were made for the 21 morphological descriptors for common beans given in Table 1 at different growth stages in accordance with the DUS test standards of the PPV & FRA Authority (PPV & FRA, 2007). Following were the four categories of evaluations:

VS: Visual assessment by observations of individual plants

Table 1: Table of characterization of Common bean

Sl. No.	Characteristics	States	Note	Stage of observations	Types of assessments
1.	Time of flowering	Early(<50 days)	3	50% plants with at least one open flower	VG
		Medium(50-75 days)	5		
		Late(76-100 days)	7		
		Very late(>100 days)	9		
2.	Stem Anthocyanin colouration	Absent	1	At the time emergence	VG
		Present	9		
3.	Leaflet : Size(at the terminal leaflet of first flowering node)	Small	3	Peak flowering	MS
		Medium	5		
		Large	7		
4.	Plant : Growth type	Erect	3	Peak flowering	VG
		Semi –erect	5		
		Spreading	7		
5.	Plant: Twinning habit	Viny	1	Peak flowering	VG
		Non-viny	9		
6.	Plant: Habitat	Determinate	1	Peak flowering	VG
		Indeterminate	3		
7.	Leaf: Intensity of green colour	Light	3	Peak flowering	VG
		Dark	7		
8.	Leaf: Shape of central leaflet	Cordate	1	Peak flowering	VG
		0val	2		
		Rhombohedric	3		
		Hastate	4		
9.	Flower: Colour of standard petal	White	1	Peak flowering	VG
		Yellow	2		
		Pink	3		
		Violet	4		
10.	Flower: Outer surface of standard petal	Striped	1	Peak flowering	VG
		Non-Striped	3		
11.	Pod: Curvature	Absent	1	Fully grown green pod	VG
		Medium	5		
		Strong	7		
12.	Pod: Shape of cross section through seed	Cordate	1	Fully grown green pod	VG
		Circular	2		
		Eight Shaped	3		
		Oval	4		
13.	Pod: Shape (in relation to suture)	Concave	1	Fully grown green pod	VG
		S-Shaped	2		
		Convex	3		

Table 1: Continue...

Sl. No.	Characteristics	States	Note	Stage of observations	Types of assessments
14.	Pod: Shape of distal part (excluding beak)	Acute	3	Fully grown green pod	VG
		Acute to truncate	5		
		Truncate	7		
15.	Pod: Colour	Pale green	1	Fully grown green pod	VG
		Green	2		
		Purple	3		
16.	Pod: Stringiness	Absent	1	Fully grown green pod	VS
		Present	9		
17.	Pod: Pigmentation of pod shell	Absent	1	Fully grown green pod	VG
		Present	9		
18.	Seed: Shape	Circular	1	Mature seed	VG
		Circular to elliptical	2		
		Elliptical	3		
		Kidney shaped	4		
	Seed: size (weight of 100 seeds)	Small (<250 g)	3	Mature seed	MG
		Medium (250-350 g)	5		
		Large (351- 450 g)	7		
		Very large (> 450 g)	9		
20.	Seed: Testa colour	White	1	Mature seed	VG
		Brown	2		
		Red	3		
		Dark Red	4		
		Black	5		
21.	Seed: Testa variegation	Absent	1	Mature seed	VG

or parts of plants. VG: Visual assessment by a single observation of a group of plants or parts of plants. MS: Measurements of a number of individual plants or parts of plants. MG: Measurements by single observations of a group of plants or parts of plants.

3. RESULTS AND DISCUSSION

Forty genotypes of rajmash (*Phaseolus vulgaris* L.) were subjected to DUS (Distinctiveness, Uniformity and Stability) characterization in accordance with the recommendations provided by the DUS descriptor of PPV & FRA Authority (2007), GOI, New Delhi. Forty-five (45) DUS traits were examined in accordance with the descriptor, "state", "code" provided for each character. For various DUS characters, the frequency distribution of these genotypes provided in Table 2, (Figure 1–21 and Plate 1–13).

The frequency of genotypes for several attributes reveals a substantial degree of variation. Analysis of data in Table 2 demonstrated that the "early" flowering period (<50 days) contributed to a frequency of (51%) for the genotypes with highest flowering time, followed by "medium "flowering period (50–75 days) contributing to frequency of 38%, followed by " late" flowering period (76–100 days) contributing to frequency of 7%, followed by "very late" flowering period (>100 days)) contributing to frequency of 4%.

For stem characters like stem anthocyanin colouration maximum genotypes showed absence of anthocyanin colouration with a frequency of (91%) followed by presence of anthocyanin colouration with a frequency of (9%). Kanwar et al. (2020) categorized 26 geographically diverse French bean genotypes for plant growth habit, stem pigmentation, hairiness on the stem, flower colour, hairiness on the flower, leaflet shape, and reported significant differences among all the genotypes. Yohannes et al. (2020) showed that the growth traits measured significantly differed due to the existence of inherent genetic variations among the French bean genotypes.

Table 2: Frequency distribution of DUS traits in rajmash (*Phaseolus vulgaris* L.) genotypes based on DUS desrcriptor of PPV&FRA, GOI, New Delhi (2007)

Sl. No.	Characteristics	States	Code	Absolute number	Frequency
1.	Time of flowering	Early(<50 days)	3	23	51%
		Medium(50-75 days)	5	17	38%
		Late(76-100 days)	7	3	7%
		Very late(>100 days)	9	2	4%
2.	Stem Anthocyanin colouration	Absent	1	41	91%
		Present	9	4	9%
3.	Leaflet : Size(at the terminal leaflet of first flowering node)	Small	3	19	42.22%
		Medium	5	21	46.66%
		Large	7	5	11%
4.	Plant : Growth type	Erect	3	23	51.11%
		Semi-erect	5	12	27%
		Spreading	7	10	22.22%
5.	Plant: Twinning habit	Viny	1	27	60%
		Non-viny	9	18	40%
6.	Plant: Habitat	Determinate	1	23	51%
		Indeterminate	3	22	49%
7.	Leaf: Intensity of green colour	Light	3	24	53%
		Dark	7	21	47%
8.	Leaf: Shape of central leaflet	Cordate	1	18	40%
		0val	2	22	48.88%
		Rhombohedric	3	3	7%
		Hastate	4	2	4%
9.	Flower: Colour of standard petal	White	1	26	57.77%
		Yellow	2	0	0%
		Pink	3	5	11%
		Violet	4	14	31.11%
10.	Flower: Outer surface of standard petal	Striped	1	26	57.77%
		Non-Striped	3	19	42.22%
11.	Pod: Curvature	Absent	1	17	38%
		Medium	5	18	40.00%
		Strong	7	8	17.77%
12.	Pod: Shape of cross section through seed	Cordate	1	23	51%
		Circular	2	14	31.11%
		Eight Shaped	3	0	0%
		Oval	4	8	17.77%
13.	Pod: Shape (in relation to suture)	Concave	1	26	58%
		S-Shaped	2	0	0%
		Convex	3	19	42%

Table 2: Continue...

Sl. No.	Characteristics	States	Code	Absolute number	Frequency
14.	Pod: Shape of distal part (excluding beak)	Acute	3	31	69%
		Acute to truncate	5	12	27%
		Truncate	7	2	4%
15.	Pod: Colour	Pale green	1	26	57.77%
		Green	2	17	37.77%
		Purple	3	2	4.44%
16.	Pod: Stringiness	Absent	1	11	24.44%
		Present	9	34	76%
17.	Pod: Pigmentation of pod shell	Absent	1	33	73%
		Present	9	12	27%
18.	Seed: Shape	Circular	1	2	4.44%
		Circular to elliptical	2	8	18%
		Elliptical	3	15	33%
		Kidney shaped	4	20	44.44%
	Seed: size (weight of 100 seeds)	Small (<250 g)	3	14	31%
		Medium (250–350 g)	5	7	15.55%
		Large (351–450 g)	7	18	40%
		Very large (> 450 g)	9	6	13.33%
20.	Seed: Testa colour	White	1	8	42%
		Brown	2	2	4%
		Red	3	15	33%
		Dark red	4	20	44%
		Black	5	0	0%
21.	Seed: Testa variegation	Absent	1	34	75.55%
		Present	9	11	24%

For plant characters like growth habit, maximum genotypes were of “erect” and “semi-erect” type with a frequency of (51.11%), (27%) and (22.22%) were “spreading” type respectively. In the character plant twining habit, maximum genotypes showed “viny” type of twining habit with a frequency of (60%) and the rest genotypes showed “non-viny” type of twining habit with a frequency of (40%), in the character plant habitat, maximum genotypes showed “determinate” plant habitat with a frequency of (51%) followed by “ indeterminate” plant habitat with a frequency of (49%). Duran et al. (2005) utilized morphological descriptors, including leaf width, leaf length, leaf shape (cordate, ovate, rhombohedric, or hastate), growth habit, and length of the fifth internode, to characterize French bean landraces and cultivars from the Caribbean.

In leaf characters, like size of terminal leaflet of first flowering node, intensity of green colour in leaf and the leaf shape. In case of leaflet size, maximum frequency of (46.66%) was observed for “medium” state of expression (42.22%) was observed for “small” State of expression and (11%) was observed for “large” state of expression, whereas intensity of “light green” colour of leaf was shown by maximum genotypes with a frequency of (53%) and intensity of “dark green colour” of leaf was shown by rest of the genotypes with a frequency of (47%) and shape of central leaflet was cordate, ovate, rhombohedric and hastate. Maximum frequency was observed for “ovate” (22%) followed by “cordate” (40%), “rhombohedric” (7%) and “hastate” (2%). Jan et al. (2021) identified significant variations in the twinning habit among French bean genotypes. Furthermore, Jan et al. (2021) and Sofi et al. (2014) both noted the prevalence of indeterminate vinyl/climbing types in French bean. Prakash and Singal (1997) classified seven grain and vegetable pea cultivars based on leaf colours, including yellow-green, blue-green, and green. In the screening of 109 French bean genotypes, Jan et al. (2021) revealed a frequent occurrence of dark green leaf colour.

Figure 1: Continue...

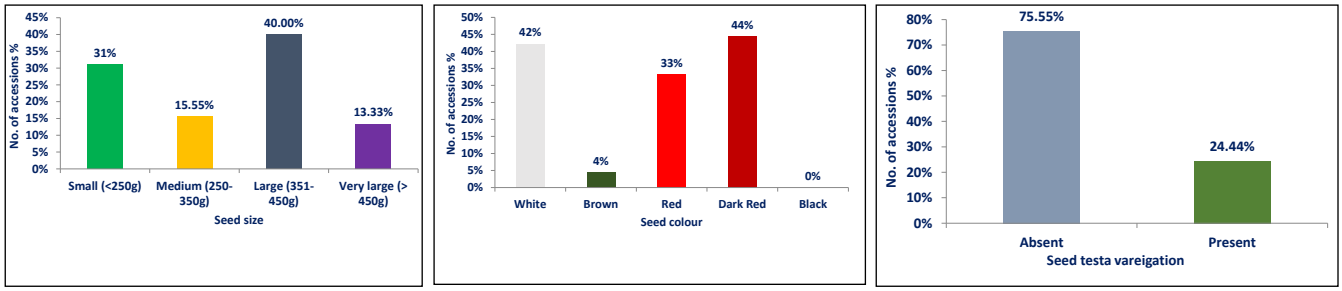


Figure 1: Frequency distribution of DUS traits in rajmash (*Phaseolus vulgaris* L.) genotypes

The frequency for "striped" outer surface of standard petal was recorded in (57.77%) genotypes followed by "non-striped" (42.22%). In pod characters like, curvature of pod, shape of pod *viz.*, cross-section of pod through seed, in relation to suture and shape of distal part of pod excluding beak, pod colour, pod stringiness and pigmentation of pod shell. "Medium" curvature was recorded in maximum genotypes with the frequency of (40.00%), curvature of pod was "absent" in (38%) of genotypes and "strong" curvature was recorded in (17.77%) of genotypes. Shape of cross section of pod through seed was cordate, circular, eight shaped and oval. Maximum genotypes showed "cordate" shape with the frequency of (51%), followed by "circular" (31.11%), "oval" (17.77%) with no any eight shaped structure. (58%) of genotypes showed "concave" shape in relation to its suture followed by "convex" with a frequency of (42%) and "S-shaped" suture was absent.

Vinyl Non-vinyl

Plate 1: Recording the twining habit of rajmash (*Phaseolus vulgaris* L.)

Large Medium Small

Plate 2: Recording the leaf size in rajmash (*Phaseolus vulgaris* L.)

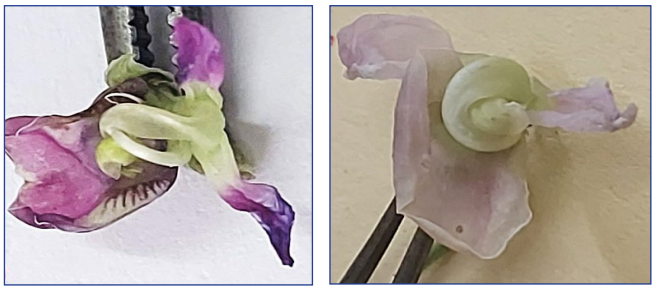
The shape of distal part of pod excluding beak was a cute, acute to truncate and truncate. Maximum genotypes had "acute" shape with a frequency of (69%) followed by "acute to truncate" (27%) and "truncate" (4%). The frequency of "Pale green" colour was recorded in (57.77%) followed by "green" (37.77%) and purple colour (4.44%), the pod

Light green Dark green

Plate 3: Recording the intensity of green colour in rajmash (*Phaseolus vulgaris* L.)

Cordate Rhombohedric Ovate Hastate

Plate 4: Recording the shape of leaves in rajmash (*Phaseolus vulgaris* L.)

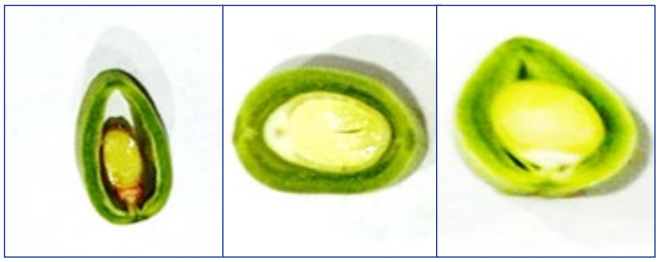


Striped Non-striped

Plate 5: Recording the surface of standard petal in rajmash (*Phaseolus vulgaris* L.)

Absent Medium Strong

Plate 6: Shape of curvature of pod in rajmash (*Phaseolus vulgaris* L.)



Oval Circular Chordate

Plate 7: Shape of pod cross-section through seed in rajmash (*Phaseolus vulgaris* L.)

Concave Convex

Plate 8: Shape of pod in relation to its suture in rajmash (*Phaseolus vulgaris* L.)

Acute Truncate Acute to truncate

Plate 9: Shape of distal part of pod excluding beak in rajmash (*Phaseolus vulgaris* L.)

stringiness was "present" in (76%) of genotypes and was "absent" in (24.44%) and pigmentation of pod shell was "absent" in (73%) of genotypes and was "present" in (27%).

Pale green Green Purple

Plate 10: Colour of pod in rajmash (*Phaseolus vulgaris* L.)

Present Absent

Plate 11: Shape of pod stringiness in rajmash (*Phaseolus vulgaris* L.)

Circular Circular to elliptical Elliptical Kidney shaped

Plate 12: Shape of seed in rajmash (*Phaseolus vulgaris* L.)

White Brown Red Dark red

Plate 13: Colour of seed in rajmash (*Phaseolus vulgaris* L.)

Islam et al. (2006) assessed 1105 French bean accessions, considering the position of pod tip, pod form, and various pod attributes traits. Zaccardelli et al. (2013) identified statistically significant differences in pod morphological traits, with a prevalence of slightly curved pods. Bode et al. (2013) noted considerable variability in French bean pods concerning pod colour, pod cross-section, pod curvature, pod beak position, and pod beak orientation.

The seed shape showed variation for all states however the state "Kidney" shaped was having highest frequency

of (44.44%) followed by "elliptical" (33%), "circular to elliptical" (18%) and circular (4.44%). Seed size 100-seed weight also revealed variability for all the state, "large" state was having highest frequency of (40%) followed by "small"(31%), "medium" (15.55%) and "very large" (13.33%) . The frequency of "dark red" seed was having maximum frequency of (44%) followed by "red" and "brown" with the frequency of (33%) and (4%) respectively and "black" Coloured seed was absent. Seed testa vareigation was "absent" in (75.55%) of genotypes and "present" in (24%) of genotypes. The results obtained depict high variability in the studied common bean (*Phaseolus vulgaris* L.) germplasm, hence, can be used for further breeding programs to develop new and improved varieties for the benefit of farmers.

A new genotype must meet three basic criteria before it can be licensed as commercial variety: it must be distinct (D), Uniform (U) and stable (S) Tommassini et al. (2003). DUS testing serves as the foundation for both plant variety protection and the discovery of new varieties from the reference set (Yang et al., 2021). Based on character classification, the *Phaseolus vulgaris* L. genotypes used for DUS assessment were divided into the number of classes. For days to flowering, maximum genotypes (23) took <50 days to flowering followed by medium *i.e,* 50 to 75 days (17), late 76–100 days (3) and very late >100 days (2).

Maximum genotypes were of erect type (23) and semi-erect (12) followed by spreading type (10), viny (27) and determinate (23) type of plants were predominant over non-viny (18) and indeterminate type (22). Maximum genotypes had medium sized leaves (21) followed by small (19) and large (5). Maximum foliage colour was light green (24) followed by dark green (21) and ovate leaves (22) were maximum than other shapes of leaf. Flower colour was mostly white (22) followed by violet (14) and there were more striped (26) flowers as compare to non-striped (19).

Maximum genotypes were having medium pod curvature (18) and absent in (17) genotypes followed by strong pod curvature (8). Pod shape through seed was cordate (23) followed by circular (14) and oval (8). For pod shape in relation to suture maximum number of genotypes was concave (26) followed by convex (19). Leaflet shape varies among the cultivars, but in general, leaflets exhibit broad bases and pointed tips Duran et al. (2005) utilized the morphological descriptor "leaf shape" (cordate, ovate, rhombohedric, or hastate) to characterize French bean landraces and cultivars from the Caribbean. Similarly, Islam et al. (2006) assessed 1105 French bean accessions for leaf shape.

Pod distil shape was acute (31) followed by acute to truncate (12) and truncate (2). Pod stringiness was present in most of the genotypes (34) and absent in (11) genotypes. Maximum genotypes showed absence (33) of pigmentation of pod shell followed by presence (12) of pigmentation of pod shell. The pod colour was pale green (26) in maximum genotypes followed by green (17) and purple (2) with a frequency of (44. 44%) for kidney shaped followed by elliptical (33%), circular to elliptical (18%) and circular (4.44%). 100 seed weight was large for maximum number of genotypes (18) having of total frequency (40%). Maximum genotypes showed dark red seeds (20) followed by red coloured seeds (15). Seed testa variegation was absent in maximum genotypes (34) and present in (11) genotypes. Zaccardelli et al. (2013) identified statistically significant differences in pod morphological traits, highlighting a prevalence of slightly curved pods. Bode et al. (2013) observed substantial variability in French bean pods, encompassing traits such as pod colour, pod cross-section, pod curvature, pod beak position, and pod beak orientation. Singh et al. (2022) characterized 18 French bean genotypes and concluded that pod curvature, pod shape of cross-section (through seed), pod shape (in relation to suture), pod shape of the distal part (excluding beak), pod colour, pod stringiness, and pod pigmentation on the pod shell serve as important morphological markers.

Similarly, Singh et al. (2014) and Saba et al. (2016) observed huge variability in accessions. For every trait, variations resulting from different genotypes were highly significant. To enhance the performance of genotypes, variation resulting from genetic and non-genetic sources must be taken into account throughout the selection process (Comstock and Moll, 1963). Sofi et al. (2014) reported findings that were similar for yield and yield attributing traits among 75 germplasm lines (Long et al., 2020) likewise found significant heterogeneity and also similar findings was recorded by Sajad et al. (2014).

4. CONCLUSION

The findings revealed prevalent early flowering, erect plant types and predominance of medium-sized, light green, ovate leaves. Seed-related traits, including cordate pod shapes and kidney-shaped seeds, highlighted notable patterns, underscoring the diversity within the evaluated genotypes highlighted their potential uses in breeding programme.

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