DISTINCTNESS, UNIFORMITY AND STABILITY (DUS) CHARACTERIZATION USING MORPHOLOGICAL TRAITS OF LTTLE MILLET

Abstract

A research studyfor 50 accessions of little millet germplasm was carried out for DUS (Distinctness, Uniformity, and Stability) descriptors encompassing both qualitative and quantitative traits. The assessment adhered to guidelines from the International Union for the Protection of New Varieties of Plants (UPOV) and the Protection of Plant Varieties and Farmer's Rights Authority (PPV & FRA). The findings underscored substantial diversity among the germplasm accessions across all evaluated characteristics. This suggests a significant genetic diversity in the morphological profile of little millet germplasm. The outcomes of this study are valuable for breeders, researchers, and farmers in identifying and selecting germplasm accessions for crop enhancement. Additionally, they contribute to the conservation of beneficial genes and facilitate the pursuit of protection under the Protection of Plant Varieties and Farmer's Rights Act.

Keywords: Distinctness, Uniformity, Stability, Little millet, Qualitative traits, Quantitative traits

Introduction

Little millet (*Panicum sumatrense L.* Roth. Ex. Roemer and Schultes) is a staple small-seeded cereal food crop belonging to the self-pollinated crop which derives under C_4.The production of little millet dropped from the 1950s to the early 21st century. But, in recent years the crop gaining much attention because of its high climate-resilient in adapting to the diverse agro-climatic zones as well asnutrient-rich components compared to other cereal food crops [22-10]. Worldwide, little millet is cultivated in an area of 0.26 m.ha with a production of 0.12 mt [5]. In India, little millet was grown in an area of 2.34 lakh hectares with an annual production of 1.27 lakh tonnes and productivity of 544 kg ha^{-1}. In Andhra Pradesh, little millet is grown in an area of 7000 ha with a production of 3000 t and productivity of 354 kg ha^{-1}[20].

Qualitative traits play decisive factors for grouping of genotypes, as they show high heritability and stable expression[17]. If qualitative characters showed association with yield attributes, it can serve as a marker in selection process which are less influenced by environmental fluctuations.Further, characterization and evaluation are essential pre-requisites for efficient utilization of germplasm [19].To commercially introduce a new plant variety, it is essential to register it by conducting DUS tests (distinctness, uniformity, and stability) to assess its uniqueness [18]. DUS tests serve as the basis for plant variety protection and for distinguishing a new variety from existing ones in a reference collection [11]. The Indian Government enacted its own legislation, the "Protection of Plant Varieties and Farmers Act" (PPV&FRA), in 2001, as the existing UPOV models were not appropriate for Indian requirements. This act provides protection to plant varieties based on DUS tests and novelty [14].

Therefore, the concept of distinctness, uniformity, and stability is essential in characterizing a plant variety as a unique creation. In this context, a study was conducted to evaluate 50 little millet germplasm accessions based on selected DUS characters and yield attributing traits.

MATERIAL AND METHODS

The research work was comprised of 50 little millet genotypes and were investigated during *Kharif,* 2023 in Randomized Block Design with three replications at Agricultural Research Station (ARS), Perumallapalle, Andhra Pradesh, India. Details of the genotypes studied in the present investigation are presented in Table 1. Each genotype was grown in a plot of two rows of 3 m length with a spacing of 22.5 × 7.5 cm. All the recommended practices were followed to ensure a good crop. For the estimation of Distinctness, Uniformity and Stability, observations were recorded on agro morphological descriptors *viz*., growth habit, plant pigmentation at leaf sheath, leaf sheath pubescence, leaf blade pubescence, ligule pubescence, inflorescence shape, culm branching, panicle compactness, peduncle length, flag leaf width, lodging, days to 50 per cent flowering, days to maturity, basal tillers, panicle length, grain yield per plot and test weight. Observations were recorded on five randomly chosen plants of each accession for growth, leafand floral traits.Based on morphologically distinct features, a visual scoring was recorded for qualitative characters.

Results and Discussions

The morphological traits were evaluated according to the DUS (Distinctiveness, Uniformity, and Stability) guidelines revealed significant variability across the studied germplasm. Based on the distinctiveness of these traits, the germplasm lines were classified into various groups. Detailed results for each trait can be found in Table 2, with graphical representation provided in Figure 1. The characterization was conducted at different growth stages of the crop, as discussed below.

Qualitative Characters

Growth habit

In 50 little millet germplasm accessions, three growth habits were identified: erect, decumbent, and prostrate. Among these, 25 accessions (50%) exhibited an erect growth habit, 20 accessions (40%) were decumbent, and only 5 accessions (10%) showed a prostrate growth habit. Similar findings regarding the predominance of the erect growth habit have been previously documented by Reddy et al. [16] and Harshiya Banu et al.[8] in foxtail millet.

Leaf traits

Among 50 little millet genotypes studied, only two entries, namely DhLtMV-36-3 and WV-126, exhibited plant pigmentation at the leaf sheath. In contrast, the remaining 48 genotypes (96%) showed no plant pigmentation at the leaf sheath.Regarding leaf sheath pubescence, the majority of accessions (49 out of 50, 98%) did not exhibit pubescence, indicating a lack of hairiness. One genotype, DhLtMV-36-3, showed pubescence, which is known to confer resistance to insect pests (Jagadeesh Babuet al. [9]. These findings are in consistent with those reported by Harshiya Banu et al. [8] in foxtail millet germplasm.

In terms of leaf blade and ligule pubescence, all 50 genotypes (100%) showed absence of pubescence. Similar results were previously documented in foxtail millet by Reddy et al. [15] and in finger millet by Chandrasekhar Hardari[6].

Floral traits

Regarding inflorescence shape, 31 accessions (62%) of little millet exhibited a diffused type, while 19 accessions (38%) showed an arched type, consistent with findings in foxtail millet as reported by Vetriventhan[21].Inflorescence compactness, an important trait influencing grain yield by Reddy et al.[16], was categorized into three types: open (25 accessions, 50%), compact (20 accessions, 40%), and intermediate (5 accessions, 10%) at the dough stage. This distribution aligns with earlier findings by Amarnath et al. [2].

Genotypes were assessed for degree of lodging, resulting in three categories: absent in 39 genotypes (78%), semi lodging in nine genotypes, and lodging observed in four genotypes (DhLtMV-28-4, RLM-238, TNAU-152, and DLM-14) at flowering. These observations corroborate with those reported by Reddy et al.[16].

Quantitative Characters

50 little millet germplasm accessions evaluated in the study, all genotypes (100%) exhibited short peduncle length (<20 cm), aligning with findings from Reddy *et al*. (2006), Amgaiet al. [3], and Amarnath et al. [2]. Culm branching was observed in 38 genotypes (76%), consistent with Ashok et al.[4]. All genotypes (100%) displayed narrow flag leaf width (cm), was similar to results reported in pearl millet by Ahmed et al. [1] and Nehra et al. [12].

Days to 50% flowering ranged from 46 days (OLM-233, OLM-203, TNAU-159, TNAU-152, and TNAU-160) to 83 days (GPUL-4), with a mean of 53 days. Plant height varied, with 26 accessions tall (>120 cm) and 24 medium (80-120 cm), similar to findings by Nehra *et al.*[12]. Basal tiller number ranged from 5 (IIMRLM-7012) to 9 (RLM-238 and TNpsu-170), with an average of 7.00. Panicle length spanned from 21.5 cm (IIMRLM-7162) to 32.8 cm (DhLtMV-14-1), with a mean of 26.9 cm. Test weight (g) ranged between 1.50 (DhLtMV-36-3) and 2.91 (Nallasama), with a mean of 2.24. Grain yield per plot (g) ranged from 70 (GPUL-2) to 280 (BL-6), averaging 0.18, consistent with findings in foxtail millet by Nirmala Kumari et al. [13] and in pearl millet by Nehra *et al.*[12].

Conclusions

Characterizing qualitative and quantitative traits in 50 little millet germplasm accessions demonstrated notable polymorphism. These findings indicate that morphological DUS descriptors are valuable for identifying, documenting, and categorizing varieties. Plant breeders can effectively use these descriptors to visually assess and select desirable genotypes. Accessions showing significant differences in specific traits can serve as valuable resources for developing mapping populations to map QTLs. Morphological descriptors thus hold great potential to support efforts in crop improvement.

References

1. Ahmed I, Molla R,Rohman Md,Motiar Md, Hossain A. Morphological characterization of foxtail millet germplasm. International Journal of Applied Research. 2017; 3(2):1-9.
2. Amarnath K, Durga Prasad AVS, Chandra Mohan Reddy CV. DUS characterization of germplasm accessions in foxtail millet (*Setariaitalica* (L.) Beauv.). International Journal of Current Microbiology and Applied Science. 2019; 8(02): 2410-2422.
3. Amgai RB, Pantha S, Chhetri TB, Budhathoki SK, Khatiwada SP,Mudwari. Variation on agromorphological traits in Nepalese foxtail millet (*Setariaitalica* (L) P Beauv). Agronomy Journal of Nepal. 2011; 2 (1):133-138.
4. Ashok S, Patro TSSK, Divya M, Sandhya Rani, Y, Triveni U,Subba Rao M. Distinctness, uniformity and stability (DUS) characterization using morphological traits of fox tail millet (*Setariaitalica* (L.) Beauv). Progressive Research – An International Journal Society for Scientific Development. 2016; 11 (4): 306-313.
5. Bhat BV, Dayakar Rao B, Tonapai VA. The Story of Millets, published by Karnataka State Department of Agriculture, Bangalore, India with ICAR-Indian Institute of Millets Research, Hyderabad, India.2018; 1-124.
6. Chandrashekhar Hardari, B. Formation of core set in African and Indian *finger millet* (Eleusinecoracana (L.) Gaertn) accessions. M.Sc. (Agri) Thesis, University of Agricultural Science. Bangalore.2009.
7. Dalsaniya D, Das A, Parmar DJ. Variability Study and DUS Characterization in Kodo Millet (*Paspalumscrobiculatum* L.). International Journal of Plant and Soil Science. 2024;36(4):408-17.
8. Harshiya Banu, Jayarame Gowda,Channabyre Gowda MV. Characterization and identification of DUS traits in reference set of foxtail millet germplasm (*Setariaitalica* (L.) Beauv.). International Journal of Current Microbiology and Applied Science.2018; 7(12): 175-183.
9. Jagadeesh Babu CS, Byre Gowda M, Girish G, Gowda TKS. Screening of Dolichos germplasm for pod borers and bruchids. Environment and Ecology. 2008; 26 (4): 2288-2290.
10. Joshi DC, Meena RP,Chandora R. Genetic resources: collection, characterization, conservation, and documentation. in millets and pseudo cereals, genetic resources and breeding advancements. Woodhead Publishing.2021; Pp. 19–31.
11. KwonYS, Lee JM, Yi GB. Use of SSR markers to complement tests of distinctiveness, uniformity, and stability (DUS) of pepper (*Capsicum annuum* L.) varieties. Molecules and Cells. 2005; 19(3): 428–435.
12. Nehra M, Kumar M, Vart D, Sharma RK, Choudhary M. DUS characterization and diversity assessment in pearl millet inbreds. Electronic Journal of Plant Breeding. 2016; 7(4): 925-933.
13. Nirmala kumari A, Vetriventhan M. Characterization of foxtail millet germplasm collections for yield contributing triats. Electronic Journal of Plant Breeding. 2010; 1(2): 140-147.
14. Patra BC.Collection and characterization of rice genetic resources from Keonjhar district of Orissa. Oryza. 2000; 34:324-326.

15. Reddy GV, Upadhyaya HD, Gowda, CLL.Characterization of world's foxtail millet germplasm collections for morphological traits. International Sorghum and Millets Newsletter. 2006; 47: 107- 109.
16. Reddy GV, Upadhyaya HD, Gowda CLL, Singh S. Characterization of eastern African finger millet germplasm for qualitative and quantitative characters at ICRISAT. SAT ejournal/ ejournal.icrisat.org. 2009; 3(1):1-4.
17. Smith JSC, Smith OS. Finger printing crop varieties. Advanced Agronomy. 1992; 47: 85-140.
18. Tommasini L, Batley J, Arnold GM. The development of multiplex simple sequence repeat (SSR) markers to complement distinctness, uniformity and stability testing of rape (Brassica napus L.) varieties. Theoretical and Applied Genetics. 2003; 106(6): 1091–1101.
19. Upadhyaya HD, Yadav D, Dronavalli N, Gowda CLL, Singh S. Mini core germplasm collections for infusing genetic diversity in plant breeding programs. Electronic Journal of Plant Breeding. 2010; 1 (4): 1294-1309.
20. Venkata Ratnam T, Latha LM, Sekhar MR, Nirmal ARK. Genetic divergence studies in Little Millet (*Panicum sumatrense*). Agricultural Science Digest. 2019; 39(3): 210-214.
21. Vetriventhan M. Phenotypic and genotypic diversity in the foxtail millet (*Setariaitalica*(L.) Beauv.) core collection. Ph.D. Thesis, Tamil Nadu. Agricultural University. Coimbatore. 2011.
22. Vetriventhan M, Azevedo VCD, Upadhyaya HD, Nirmalakumari A, Potaka JK, Anitha S,CeasarAS, Muthamilarasan M, Venkatesh VB, Hariprasanna BK, Bellundagi A, Cheruku D, Backiyalakshmi C, Santra D, Vanniarajan C and Tonapi VA. Genetic and genomic resources, and breeding for accelerating improvement of small millets: current status and future interventions. Nucleus.2020;63 (1):217–239.
23.

Table 1 Pedigree details of little millet genotypes used in the study

S. No.	Genotypes	Pedigree	Centre
1	BL-2	CO-2 × OLM-56	Jagdalpur
2	BL-4	CO-2 × TNAU-97	Jagdalpur
3	BL-8	CO-2 × OLM-56	Jagdalpur
4	BL-41-3	Paiyur-2 × TNAU-97	Jagdalpur
5	BL-150	Paiyur-2 × DLM-369	Jagdalpur
6	CO-2	Pure line selection	Coimbatore
7	DhLtMV-10-2	-	Hanumanamatti
8	DhLtMV-14-1	CO-2 × TNAU-110	Hanumanamatti
9	DhLtMV-21-1	CO-2 × TNAU-26	Hanumanamatti
10	DhLtMV-28-4	-	Hanumanamatti
11	DhLtMV-36-3	CO-4 × Paiyur-2	Hanumanamatti
12	DhLtMV-39-1	CO-4 × Paiyur-2	Hanumanamatti
13	DLM-8	-	Dindori
14	DLM-14	Pure line selection from Local Germplasm	Dindori
15	DLM-89	Pure line selection from Local Germplasm	Dindori
16	DLM-95	Selection from Local Germplasm	Rewa
17	DLM-103	Pure line selection from Local Germplasm	Dindori
18	DLM-186	-	Dindori
19	DhLt-28-4	CO-2 × TNAU-26	Hanumanamatti
20	GPUL-1	-	Bangalore
21	GPUL-2	Pure line selection from Peddasame	Bangalore
22	GPUL-3	-	Bangalore
23	GPUL-4	JK-8 × Peddasame	Bangalore
24	GPUL-5	JK-8 × Peddasame	Bangalore
25	GV-2-1	Mutant of Gujarat Vari-1	Waghai
26	IIMRLM-7012	Selection from IPMR-699	IIMR, Hyderabad
27	IIMRLM-7162	Selection from GPMR-1153	IIMR, Hyderabad
28	KADIRI-1	Selection from Kadiri local	Bangalore
29	KOPLM-53	IPS from local germplasm	Kolhapur

Table 1 (Cont.).

S. No.	Genotypes	Pedigree	Centre
30	Nallasama	Selection from Local	ARS, Perumallapalle
31	OLM-217	Selection from Udayagiri Local	Berhampur
32	OLM-233	Selection from L55	Berhampur
33	RLM-37	Selection from Local germplasm No. 37	Rewa
34	RLM-238	-	Rewa
35	RLM-367	Selection from Local germplasm No. 367	Rewa
36	TNAU-152	Paiyur-1 × PM-29	Coimbatore
37	TNAU-159	TNAU-81 × TNAU-25	Coimbatore
38	TNAU -160	TNAU-91 × MS-4729	Coimbatore
39	TNPsu-167	CO-2 × TNAU-26	Coimbatore
40	TNPsu-170	CO-4 × IPM-113	Coimbatore
41	TNPsu-171	CO-2 × TNAU-28	Coimbatore
42	TNPsu-174	CO-2 × IPM-113	Coimbatore
43	TNPsu-183	CO-2 × MS-4729	Athiyandal
44	TNPsu-186	MS-507 × MS-1211	Athiyandal`
45	WV-125	Local collection from Waghai	Waghai
46	WV-126	Local collection from Dangas	Waghai
47	WV-167	Local selection from Subir	Waghai
48	BL-6	Paiyur-1 × OLM-29	Jagdalpur
49	JK-8	Selection from Local germplasm	Rewa
50	OLM-203	Pure line selection from Lakshmipur local	Berhampur

Table 2. Genotyping of 50 little millet genotypes based on DUS characters

S. No.	Name of the descriptor	Stage of	Descriptor state	No. of	Frequency

		observation		accessions	(%)
1	Growth habit	Two to Four leaf stage	Erect	25	50
			Decumbent	20	40
			Prostrate	5	10
2	Plant pigmentation at leaf sheath	Flowering	Present	2	4
			Absent	48	96
3	Leaf sheath pubescence	Flowering	Present	1	2
			Absent	49	98
4	Leaf blade pubescence	Flowering	Present	0	0
			Absent	50	100
5	Ligule pubescence	Flowering	Present	0	0
			Absent	50	100
6	Inflorescence shape	Flowering	Diffused	31	62
			Arched	19	38
7	Lodging	Maturity	Absent	39	78
			Semi lodge	9	18
			Lodging	4	8
8	Culm branching	Dough	Present	38	76
			Absent	12	24
9	Panicle compactness	Dough	Open	25	50
			Compact	20	40
			Intermediate	5	10
10	Peduncle length (cm)	Flowering	Short (<20)	50	100
			Medium(20.0-30.0)	0	0
			Long (30.0-40)	0	0
			Very long (>40)	0	0
11	Flag leaf width (cm)	Flowering	Narrow (<1.5)	50	100
			Medium (1.5-3.0)	0	0
			Long (>3.0)	0	0
12	Panicle length (cm)	Maturity	Short (<10)	0	0
			Medium(10.0-15.0)	0	0
			Long (>15)	50	100
13	Plant height (cm)	Maturity	Short (<80)	0	0
			Medium (80-120)	25	50
			Compact (> 120)	25	50

Table 3 Quantitative variations for seven descriptors of little millet genotypes

S. No.	Genotypes	Days to 50 per cent flowering	Days to maturity	Plant height (cm)	Panicle length (cm)	Basal tillers	Test weight (g)	Grain yield plot^{-1} (g)
1	BL-2	58	88	123.7	32.5	6	2.37	140
2	BL-4	58	88	104.0	23.3	6	2.09	130
3	BL-8	52	83	114.0	29.8	7	2.84	220
4	BL-41-3	49	79	124.0	32.2	7	2.58	220
5	BL-150	59	90	120.7	25.4	7	2.09	200
6	CO-2	55	85	134.7	26.9	7	2.46	230
7	DhLtMV-10-2	61	91	141.0	25.0	8	2.52	170
8	DhLtMV-14-1	54	82	135.0	32.8	7	2.39	210
9	DhLtMV-21-1	60	93	122.7	29.4	8	2.21	230
10	DhLtMV-28-4	65	95	128.0	24.7	6	1.82	90
11	DhLtMV-36-3	54	86	117.3	26.0	8	1.50	180
12	DhLtMV-39-1	59	89	114.3	27.5	8	2.53	180
13	DLM-8	49	79	108.0	27.3	6	2.34	180
14	DLM-14	50	80	116.7	27.9	8	2.27	220
15	DLM-89	55	85	111.3	23.9	7	2.15	200
16	DLM-95	54	84	117.3	25.4	7	1.88	180
17	DLM-103	66	96	114.3	23.0	7	1.71	150
18	DLM-186	50	80	126.3	30.5	7	2.50	200
19	DhLt-28-4	52	82	111.7	25.7	7	2.81	170
20	GPUL-1	54	87	124.0	25.0	6	2.41	210
21	GPUL-2	77	107	99.0	23.3	7	2.27	70
22	GPUL-3	74	104	90.0	24.2	6	1.73	160
23	GPUL-4	84	110	115.7	27.9	6	2.42	120
24	GPUL-5	49	79	125.0	26.0	6	2.26	190
25	GV-2-1	48	78	122.3	24.2	6	1.91	160
26	IIMRLM-7012	78	108	111.7	21.7	5	1.81	70
27	IIMRLM-7162	61	95	114.3	21.5	7	1.94	130
28	KADIRI-1	60	90	122.3	26.7	7	2.47	230
29	KOPLM-53	47	77	125.7	26.8	7	2.37	140
30	Nallasama	64	93	127.3	29.9	6	2.91	170
31	OLM-217	72	102	116.0	27.0	6	2.28	120
32	OLM-233	46	77	110.0	28.0	8	2.18	160
33	RLM-37	75	105	98.3	22.2	7	1.88	80
34	RLM-238	79	109	111.0	24.9	9	1.78	150
35	RLM-367	48	77	115.7	24.8	7	2.31	170
36	TNAU-152	46	76	112.7	23.7	7	2.17	150
37	TNAU-159	46	76	112.7	28.0	7	2.26	160
38	TNAU -160	46	76	106.3	25.2	7	2.09	110
39	TNPsu-167	54	84	128.7	28.8	7	2.34	160
40	TNPsu-170	54	84	138.3	32.2	9	2.14	180
41	TNPsu-171	51	81	121.0	26.3	8	2.59	210
42	TNPsu-174	69	99	125.7	23.9	7	2.15	220
43	TNPsu-183	52	82	127.3	32.0	7	2.35	190
44	TNPsu-186	51	81	127.0	31.8	6	2.42	200
45	WV-125	52	81	129.0	30.8	8	2.70	240
46	WV-126	63	93	123.3	32.5	7	2.56	270
47	WV-167	52	82	127.0	25.5	6	2.50	190
48	BL-6	80	110	125.7	27.2	7	1.71	280
49	JK-8	72	102	121.3	27.2	7	1.83	120
50	OLM-203	46	76	115.0	24.5	7	2.08	250
	Mean	58	88	119.1	26.9	7	2.24	175
	Minimum	46	76	90.0	21.5	5	1.50	70
Range	Maximum	84	110	141.0	32.8	9	2.91	280
	CV%	5.92	4.04	4.77	6.49	9.32	6.46	12.73

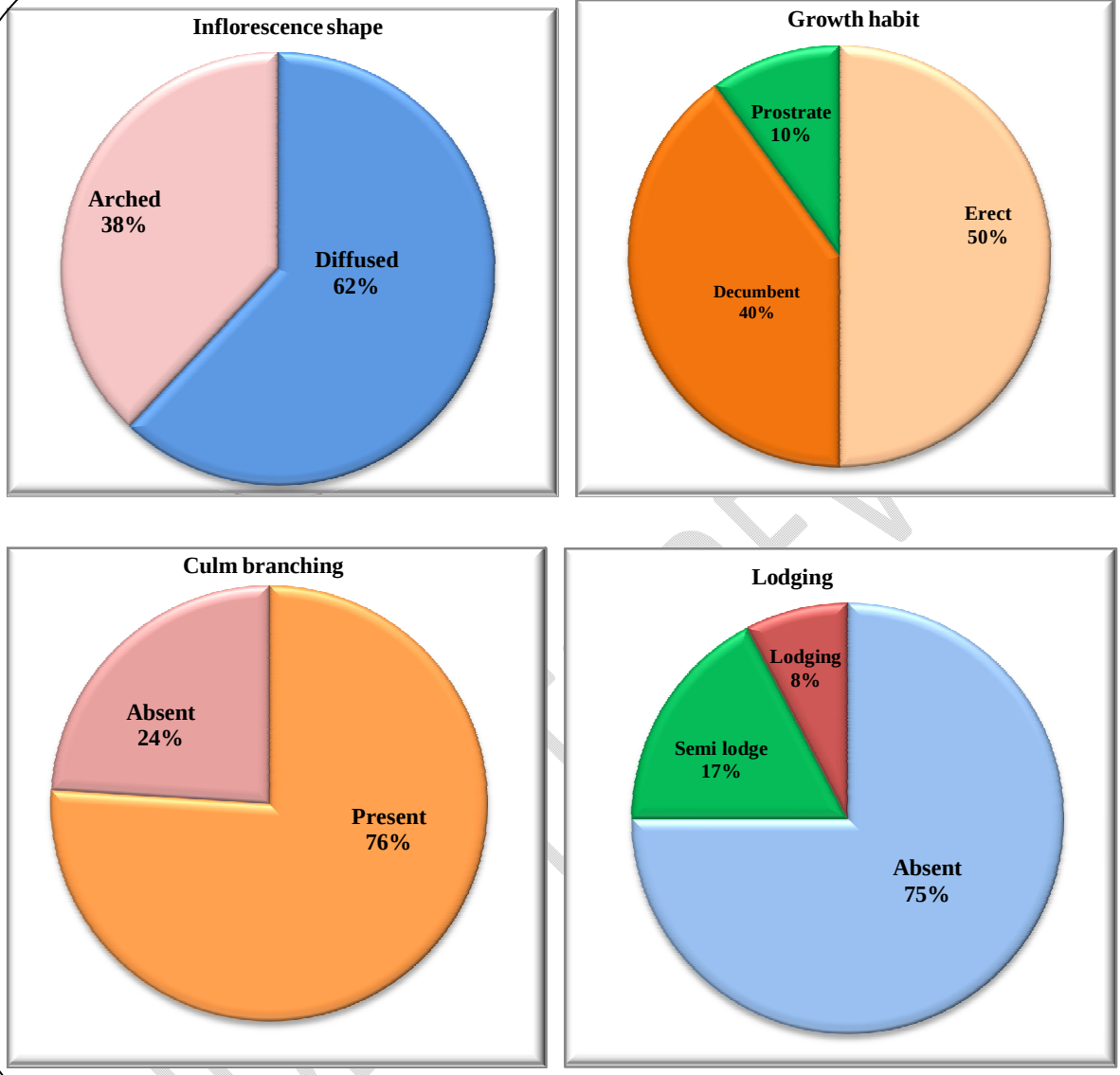

Fig.1. Pie diagram depicting variability for qualitative traits

Plate 1. Phenotypic variation observed in inflorescence shape and colour in different Little millet genotypes