Harnessing DUS Traits for the Identification and Protection of Lentil (Lens Culinaris L.) Genotypes in Breeding Programme

Abstract

DUS (Distinctness, Uniformity, and Stability) characterization is a critical process in the identification and registration of new plant varieties, ensuring their uniqueness, consistency, and reproducibility. In lentil (*Lens culinaris*), DUS testing plays a vital role in breeding programs aimed at developing improved cultivars with enhanced agronomic traits, such as disease resistance, yield potential, and climate resilience. The present study was carried out to characterize the twenty-eight lentil genotypes on the basis of thirteen DUS characters following the guidelines from Plant Protection of Varieties and Farmer's Right Authority, GOI. The experimental material was planted in randomized block design (RBD) with three replications during *Rabi* 2022 and 2023. No variation was observed for two traits viz., flowering and leaf pubescence, whereas rest of the characters showed the variation in lentil genotypes. The cotyledon colour of four genotypes was yellow and remaining 24 genotypes showed orange cotyledon colour. Foliage intensity of green colour, leaflet size (length), Plant growth habit, flower colour, seed size, seed testa colour showed trimorphic variation while stem anthocyanin, Plant height, Pod anthocyanin colouration, Seed testa mottling and Cotyledon colour showed dimorphic variation.

Keywords: DUS, genetic variation, trimorphic variation and dimorphic variation, Testa mottling etc.

Introduction

Lentil (*Lens culinaris* L. Medik.) is a self-pollinated, annual, herbaceous diploid and cool season crop plant having seven pairs of chromosomes (2n=2x=14) (Khazaei *et al*., 2016), cultivated across the world as well as have a great source of protein for under privileged people in the developing nations (Kumar *et al*., 2012; FAOSTAT, 2019). Lentil is an autogamous crop, with lens-shaped grains. The plant grows to be 20-45 cm tall and produces many small, purse-shaped pods containing one to two seeds each. It is believed to have originated from the Near East and evolved from the wild progenitor *Lens culinaris* subsp. *orientalis* (Erskine & Sarker, 2004; Ladizinsky, 1979). The cultivated lentil has been shown to be chromosomally homogeneous, with a karyotype identical to the 'standard' chromosome arrangement of the wild *orientalis* (Ferguson & Robertson, 1996; Cubero, 1981).

Lentil (*Lens culinaris* Medik.) is one of the most important pulse crops globally, valued for its high protein content and its adaptability to diverse agro-climatic conditions. In 2021, global lentil production reached around 6.5 million tons, with major producers including

Canada, India, and Turkey (FAO, 2022). Canada is the leading global producer of lentils, contributing approximately 40% of the world's supply, with a production of 3.7 million tons. India ranks second, producing 1.2 million tons, followed by Turkey with 0.43 million tons. Other notable producers include Australia, Nepal, and the United States (FAO, 2020). In India, lentil production amounts to 1.22 million tons, cultivated over an area of 1.36 million hectares (FAOSTAT, 2019). The primary lentil-growing states in India are Uttar Pradesh, Madhya Pradesh, Chhattisgarh, Bihar, and West Bengal, which together account for 80-90% of the country's total production (Singh *et al*., 2021).

The increasing demand for improved lentil varieties has led to the development of new cultivars with higher yield, disease resistance, and climate resilience. To protect these new varieties and ensure their recognition, the Distinctness, Uniformity and Stability, DUS characterization is crucial. DUS testing is a legal requirement for plant variety protection (PVP) and follows the guidelines set by the International Union for the Protection of New Varieties of Plants (UPOV, 2019).

In recent years, advances in molecular markers such as SSR (Simple Sequence Repeats) and SNP (Single Nucleotide Polymorphisms) have been integrated into DUS testing, providing a more precise and reliable method for distinguishing varieties (Kumar *et al.*, 2022). These molecular tools enhance the efficiency of DUS testing by enabling breeders to identify genetic variations that are not always visible at the morphological level.

Method and Material

An experiment was conducted at Research Farm, Banda University of Agriculture and Technology, Banda during the *Rabi* season of 2022-2023. The Research is located between latitudes 24°53'–25°55' N and longitudes 80°07'–81°34' E. Its climate is semi-arid, with hot summers and cold winters. The monsoon season typically begins in mid-June and lasts until the end of September, bringing an average rainfall of 800-900 mm. About 80% of this rainfall comes from the southwest monsoon. Relative humidity peaks at around 85% in August and drops to its lowest in April. Meteorological data for the crop season is presented in Table 3.1. The soil at the experimental site is clay loam with a pH of 8.40, low organic content (0.33%), and limited nitrogen and phosphorus. The experimental material was consisting of twenty-eight lentil genotypes and these materials were grown in a Randomized Block Design with three replications for DUS (Distinctness, Uniformity, and Stability) characterization. Data were recorded at the stage of maximum trait expression, in accordance with the DUS guidelines of the PPV & FR Act, 2007.

Table 1: List of twenty-eight lentil genotypes used for DUS characterization

S.No.	Genotype	Source
1.	EC- 542186	CSA, Kanpur
2.	IPL-316 (Check)	IIPR, Kanpur
3.	L-4717	CSA, Kanpur

4.	IPL-220 (Check)	IIPR, Kanpur
5.	PDL-1	CSA, Kanpur
6.	L-4594	CSA, Kanpur
7.	95-L-34	CSA, Kanpur
8.	ILL-9913	CSA, Kanpur
9.	PDL-5	CSA, Kanpur
10.	20095-83193-16M-52	ICARDA, Hyderabad
11.	KM-1 (Check)	CSA, Kanpur
12.	KL_320	CSA, Kanpur
13.	KL-127	CSA, Kanpur
14.	KM-2 (Check)	CSA, Kanpur
15.	590/8461/2-4-S1	ICARDA, Hyderabad
16.	20095-968501-S5	ICARDA, Hyderabad
17.	LS-22	CSA, Kanpur
18.	010596134-2-S1-R1	ICARDA, Hyderabad
19.	L-112-18	CSA, Kanpur
20.	L-112-6	CSA, Kanpur
21.	LS-38	CSA, Kanpur
22.	IPL_416	IIPR, Kanpur
23.	ILL-9957	CSA, Kanpur
24.	EC_2008345	ICARDA, Hyderabad
25.	WBL_50	CSA, Kanpur
26.	PANT- 639	CSA, Kanpur
27.	L-4727	CSA, Kanpur
28.	RVL_31	CSA, Kanpur

Result and Discussion

The present investigation of all lentil genotypes were evaluated for thirteen morphological traits, with two traits found to be monomorphic. All entries exhibited leaf pubescence, a finding consistent with Choudhury *et al.* (2014). The plant height for all genotypes was recorded at less than 40 cm, classifying them as dwarf (PPV & FRA, 2007). For Distinctness, Uniformity, and Stability (DUS) characterization, the intensity of green foliage colour was identified as a distinctive, consistent, and stable trait. The genotypes were categorized into light green, medium green, and dark green. Medium green foliage was the most common, observed in 46.43% of the genotypes, while 35.71% showed light green and 17.86% had dark green foliage. According to Choudhary *et al.* (2017), dark green plants persist longer and remain green during advanced crop stages, whereas light green plants mature and turn brown, a pattern also noted in the field. Hoque *et al.* (2002) reported that one gene controls leaf colour, with dark green predominating over light green. 85.71 % genotypes exhibited orange cotyledons except for 14.28 % genotype, which had yellow cotyledons. Additionally, 32.14% of the genotypes showed stem anthocyanin, while it was absent in 67.86%. Of the genotypes, 39% genotype were erect, with the remaining being semi-erect (32%) and horizontal (29%). All the 28 genotypes displayed pubescence and medium flowering (60-80 days). Regarding leaflet size, 42.86% had large leaflets, 32.14% had medium-sized leaflets, and 25.00% had small leaflets. Violet flower colour was observed in 64.29% of genotypes, with 21.43% showing white and 14.29% having blue petals. Plant height was short in 64.29% of genotypes, with the remaining 35.71% showing medium height. For 100-seed weight, genotypes were categorized as very large-seeded (>3.0 g), large-seeded (2.6-3.0 g), and small-seeded (<2.0 g). The distribution was 42.86% medium, 32.14% small, and 25% large-seeded. Seed testa colour, mottling, and cotyledon colour were measured post-harvest. Testa colour and mottling were found to be the most stable and consistent features for genetic purity verification (Choudhary *et al.*, 2017). Grey seed testa colour was observed in 53.57% of entries, brown in 28.57%, and green in 17.85%. Twenty-four genotypes exhibited testa mottling, while four (590/8461/2-4-S1, 010596134-2-S1-R1, 20095-83193-16M-S2, and 20095-968501-S5) did not. Vandenberg and Slinkard (1990) reported that seed coat colour is controlled by two dominant genes at independent loci. A dominant gene at one locus produces gray colour, double dominant genes produce brown colour, and double recessive genes produce green colour. The trait of seed testa mottling is governed by multiple alleles at a single locus. 14.28 % genotypes showed yellow cotyledon colour, while the others had orange cotyledons.

Table 2: Frequency distribution for different DUS characters of lentil genotypes

S.No.	**Characters**		**No of genotypes**	**Frequency %**	**Name of genotypes**
1.	Foliage; Intensity of Green Colour	Light	5	17.86	20095-83193-16M-52, 590/8461/2-4-S1, 20095-968501-S5, 010596134-2-S1-R1, PANT- 639
		Medium	13	46.43	IPL-316, PDL-1, L-4594, PDL-5, KM-1, KL-320, KM-2, L-112-18, LS-38, IPL-416, ILL-9957, EC-2008345, RVL-31
		Dark	10	35.71	EC- 542186, L-4717, IPL-220, 95-L-34, ILL-9913, KL-127, LS-22, L-112-6,

					WBL-50, L-4727
2.	Stem: Anthocyanin colouration	Present	9	32.14	L-4717, IPL-220, 95-L-34, PDL-5, L-4594, LS-22, L-112-18, WBL-50, 20095-83193-16M-5
		Absent	19	67.86	EC-542186, IPL-316, L-4594, ILL-9913, KM-1, KL-320, KL-127, KM-2, 590/8461/2-4-S1, 20095-968501-S5, 010596134-2-S1-R1, L-112-6, LS-38, IPL-416, ILL-9957, EC-2008345, PANT- 639, L-4727, RVL-31
3.	Flowering	Early (<60 days)	-	-	-
		Medium (60-80 days)	28	100.00	EC-542186, L-4717, IPL-220, 95-L-34, ILL-9913, KL-127, LS-22, L-112-6, WBL_50, L-4727, IPL-316, PDL-1, L-4594, PDL-5, KM-1, KL_320, KM-2, L-112-18, LS-38, IPL_416, ILL-9957, EC-2008345, RVL-31, 20095-83193-16M-52, 590/8461/2-4-S1, 20095-968501-S5, 010596134-2-S1-R1, PANT- 639
		Late (>80 days)	-	-	-
4.	Leaf pubescence	Present	28	100.00	EC- 542186, L-4717, IPL-220, 95-L-34, ILL-9913, KL-127, LS-22, L-112-6, WBL-50, L-4727, IPL-316, PDL-1, L-4594, PDL-5, KM-1, KL-320, KM-2, L-112-18, LS-38, IPL-416, ILL-9957, EC-2008345, RVL-31, 20095-83193-16M-S2, 590/8461/2-4-S1, 20095-968501-S5, 010596134-2-S1-R1, PANT- 639
		Absent	-		-
5.	Leaflet size	Small	7	25.00	DL-1, ILL-9913, 590/8461/2-4-S1, 20095-968501-S5, LS-38, IPL-416, WBL-50
		medium	9	32.14	EC- 542186, IPL-316, PDL-5, 20095-83193-16M-S2, KM-1, 010596134-2-S1-R1, L-112-18, L-112-6, ILL-9957
		Large	12	42.86	L-4717, IPL-220, L-4594, 95-L-34, KL-320, KL-127, KM-2, LS-22, EC-2008345, PANT- 639, L-4727, RVL-31
6.	Plant growth habit	Erect (<30%)	11	39.28	PDL-5¸ EC- 542186, IPL-316, KM-1, KL-320, LS-22, ILL-9957, EC-2008345, WBL-50, PANT- 639, RVL-31
		Semi erect	9	32.14	KM-2, L-4717, PDL-1, IPL-220, 590/8461/2-4-S1, 20095-968501-S5,

		(30-60%)			010596134-2-S1-R1, 20095-83193-16M-S2, KL-127
		Horizontal (>60%)	8	50.00	L-4594, 95-L-34, L-112-6, L-112-18, LS-38, IPL-416, L-4727, ILL-9913
7.	Flower colour	Violet	18	64.29	EC-542186, IPL-316, L-4717, IPL-220, PDL-1, L-4594, 95-L-34, ILL-9913, PDL-5, 20095-83193-16M-S2, KL-320, KL-127, KM-2, 590/8461/2-4-S1, L-112-18, IPL-416, EC-2008345, PANT-639
		White	5	17.85	KM-1, 20095-968501-S5, LS-22, 010596134-2-S1-R1, ILL-9957
		Blue	5	17.85	L-112-6, LS-38, WBL-50, L-4727, RVL-31
8.	Plant height	Short (<40 cm)	18	64.29	EC-542186, IPL-316, L-4717, L-4594, PDL-5, 20095-83193-16M-52, KL-320, 590/8461/2-4-S1, 20095-968501-S5, LS-22, 010596134-2-S1-R1, L-112-18, IPL-416, ILL-9957, EC-2008345, PANT- 639, L-4727, RVL-31
		Medium (40-60 cm)	10	35.71	IPL-220, PDL-1, 95-L-34, ILL-9913, KM-1, KL-127, KM-2, L-112-6, LS-38, WBL-50,
9.	Pod: anthocyanin colouration	Present	7	25.00	IPL-220, 95-L-34, KM-2, L-112-18, LS-38, IPL-416, EC-2008345
		Absent	21	75.00	EC- 542186, IPL-316, L-4717, PDL-1, L- PDL-5, 20095-83193-16M-52, KM-1, KL KL-127, 590/8461/2-4-S1, 20095-968501 LS-22, 010596134-2-S1-R1, L-112-6, ILL-9957, WBL-50, PANT- 639, L-4727, RVL-31
10.	Seed size	Small (<2 g)	9	32.14	PDL-1, L-4594, 95-L-34, 20095-83193-16 KM-1, KM-2, LS-22, L-112-6, LS-38
		Medium (2-2.5 g)	12	42.86	EC- 542186, ILL-9913, KL-127, 20095-968501-S5, PDL-5, 010596134-2-S1-R1, L-112-18, IPL-416, ILL-9957, EC-2008345, WBL-50, PANT- 639,
		Large (2.6-3 g)	7	25	IPL-316, L-4717, IPL-220, KL-320, 590/8461/2-4-S1, L-4727, RVL-31

11.	Seed: Testa colour	Green	5	17.85	0105961342-S1-R1, 20095-83193-16M-S2, 590/8461/2-4-S1, EC- 542186, 20095-96501-S5
		Gray	15	53.57	IPL-220, KL-127, IPL-316, PDL-1, PDL-5, KL-320, LS-22, L-112-6, IPL-416, ILL-9957, EC-2008345, WBL-50, PANT- 639, L-4727, RVL-31
		Brown	8	28.57	PANT- 639, L-112-18, KM-1, KM-2, L-4594, 95-L-34, ILL-9913, LS-38,
12.	Seed: Testa mottling	Present	24	85.71	EC- 542186, L-4717, IPL-220, 95-L-34, ILL-9913, LS-22, L-112-6, WBL-50, L-4727, IPL-316, KL-127, PDL-1, L-4594, PDL-5, KM-1, KL-320, KM-2, L-112-18, LS-38, IPL-416, ILL-9957, EC-2008345, RVL-31, PANT- 639
		Absent	4	14.28	590/8461/2-4-S1, 010596134-2-S1-R1, 20095-968501-S5, 20095-83193-16M-S2
13.	Cotyledon: colour	Yellow	4	14.28	20095-83193-16M-52, 010596134-2-S1-R1, 590/8461/2-4-S1, 20095-968501-S5
		Orange	24	85.71	EC- 542186, L-4717, IPL-220, 95-L-34, ILL-9913, LS-22, L-112-6, WBL-50, L-4727, IPL-316, PDL-1, L-4594, PDL-5, KM-1, KL-320, KM-2, L-112-18, LS-38, IPL-416, ILL-9957, EC-2008345, RVL-31, , PANT- 639, KL-127

Fig 1: Foliage intensity of green colour

Fig 2: Plant growth habit

Fig 3: Leaf Pubescence

Fig 4: Stem: Anthocyanin colouration

Fig 5: Flower Colour

Fig 6: Seed Testa Colour

Fig 7: Seed Testa mottling

Fig 8: Cotyledon Colour

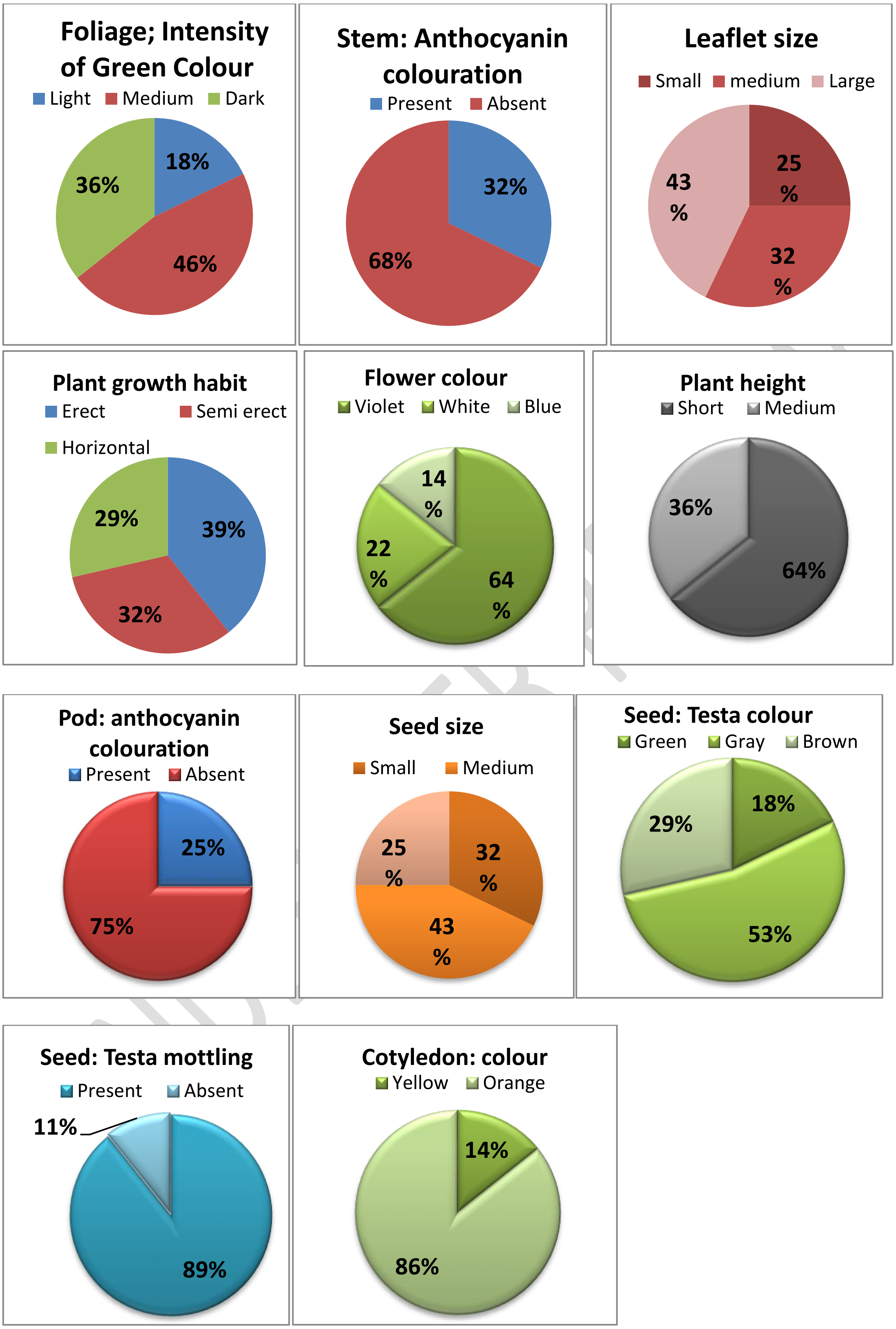

Fig 9: Frequency distribution of 28 genotypes based on DUS characteristics

Conclusion

The present investigation entitled "DUS Characterization of Lentil (*Lens Culinaris* L.) Genotypes" for all characters. Morphological markers are essential tools for breeders in identifying parental material with desired traits. This study uncovered significant genetic variation in agro-morphological traits among lentil genotypes. In accordance with the PPV & FR Act of 2007, the findings are valuable not only for the registration of new varieties but also for a deeper understanding of varietal classification and clustering. The data gathered from the characterization of lentil genotypes provide an important resource for ongoing research. These insights pave the way for their application in various areas such as selection, molecular breeding, and trait discovery, enhancing future breeding programs and contributing to the development of improved lentil varieties. Overall, the study's outcomes have broad implications for lentil research, facilitating both practical and scientific advancements.

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