

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

3
4
5
6

7 **Abstract**

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

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

25 **Introduction**

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

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

Commented [RV1]: and

Commented [RV2]: Use 'and' in text citation

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

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

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

58 Method and Material

59 An experiment was conducted at Research Farm, Banda University of Agriculture and
60 Technology, Banda during the *Rabi* season of 2022-2023. The Research ~~is located~~ was
61 conducted between latitudes 24°53'–25°55' N and longitudes 80°07'–81°34' E. Its climate is
62 semi-arid, with hot summers and cold winters. The monsoon season typically begins in mid-
63 June and lasts until the end of September, bringing an average rainfall of 800-900 mm. About
64 80% of this rainfall comes from the southwest monsoon. Relative humidity peaks at around
65 85% in August and drops to its lowest in April. Meteorological data for the crop season is
66 presented in Table 3.1. The soil at the experimental site is clay loam with a pH of 8.40, low
67 organic content (0.33%), and limited nitrogen and phosphorus. The experimental material
68 ~~was consisting-~~ consisted of twenty-eight lentil genotypes and these materials were grown in
69 a Randomized Block Design with three replications for DUS (Distinctness, Uniformity, and
70 Stability) characterization. Data were recorded at the stage of maximum trait expression, in
71 accordance with the DUS guidelines of the PPV & FR Act, 2007.

72 **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

73

74 **Result and Discussion**

75 The present investigation of all lentil genotypes ~~were~~was evaluated for thirteen
76 morphological traits, with two traits found to be monomorphic. All entries exhibited leaf
77 pubescence, a finding consistent with Choudhury *et al.* (2014). The plant height for all
78 genotypes was recorded at less than 40 cm, classifying them as dwarf (PPV & FRA, 2007).
79 For Distinctness, Uniformity, and Stability (DUS) characterization, the intensity of green

80 foliage colour was identified as a distinctive, consistent, and stable trait. The genotypes were
 81 categorized into light green, medium green, and dark green. Medium green foliage was the
 82 most common, observed in 46.43% of the genotypes, while 35.71% showed light green and
 83 17.86% had dark green foliage. According to Choudhary *et al.* (2017), dark green plants
 84 persist longer and remain green during advanced crop stages, whereas light green plants
 85 mature and turn brown, a pattern also noted in the field. Hoque *et al.* (2002) reported that one
 86 gene controls leaf colour, with dark green predominating over light green. 85.71 % genotypes
 87 exhibited orange cotyledons except for 14.28 % genotype, which had yellow cotyledons.
 88 Additionally, 32.14% of the genotypes showed stem anthocyanin, while it was absent in
 89 67.86%. ~~Of the~~Among the genotypes, 39% genotypes were erect, with the remaining being
 90 semi-erect (32%) and horizontal (29%). All the 28 genotypes displayed pubescence and
 91 medium flowering (60-80 days). Regarding leaflet size, 42.86% had large leaflets, 32.14%
 92 had medium-sized leaflets, and 25.00% had small leaflets. Violet flower colour was observed
 93 in 64.29% of genotypes, with 21.43% showing white and 14.29% having blue petals. Plant
 94 height was short in 64.29% of genotypes, with the remaining 35.71% showing medium
 95 height. For 100-seed weight, genotypes were categorized as very large-seeded (>3.0 g), large-
 96 seeded (2.6-3.0 g), and small-seeded (<2.0 g). The distribution was 42.86% medium, 32.14%
 97 small, and 25% large-seeded. Seed testa colour, mottling, and cotyledon colour were
 98 measured post-harvest. Testa colour and mottling were found to be the most stable and
 99 consistent features for genetic purity verification (Choudhary *et al.*, 2017). Grey seed testa
 100 colour was observed in 53.57% of entries, brown in 28.57%, and green in 17.85%. Twenty-
 101 four genotypes exhibited testa mottling, while four (590/8461/2-4-S1, 010596134-2-S1-R1,
 102 20095-83193-16M-S2, and 20095-968501-S5) did not. Vandenberg and Slinkard (1990)
 103 reported that seed coat colour is controlled by two dominant genes at independent loci. A
 104 dominant gene at one locus produces gray colour, double dominant genes produce brown
 105 colour, and double recessive genes produce green colour. The trait of seed testa mottling is
 106 governed by multiple alleles at a single locus. 14.28 % genotypes showed yellow cotyledon
 107 colour, while the others had orange cotyledons.

Commented [RV3]: Reframe this sentence

Commented [RV4]: Reference?

108

109 **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-S2, 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-S2, KM-1, 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, 20095-83193-16M-S2, 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

Formatted Table

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

110



Fig 1: Foliage intensity of green colour

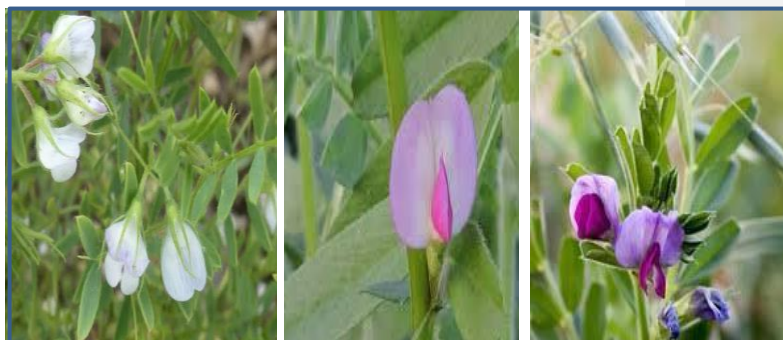


Fig 2: Plant growth habit



Fig 3: Leaf Pubescence

119



121

122

123

124

125

126

Fig 4: Stem: Anthocyanin
colouration

Fig 5: Flower Colour



12

12

13

13

13

13

134

Fig 6: Seed Testa Colour

135

1

1

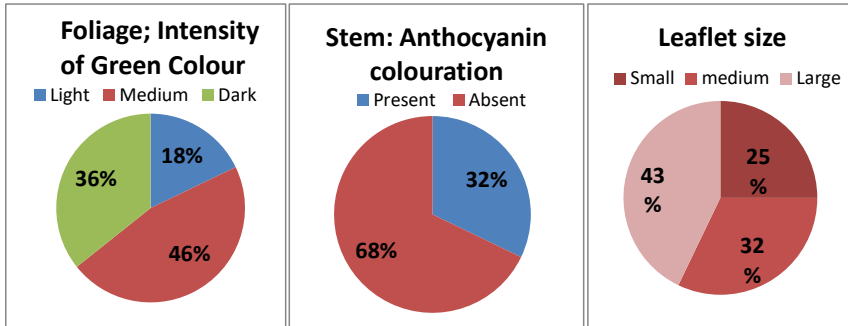
1



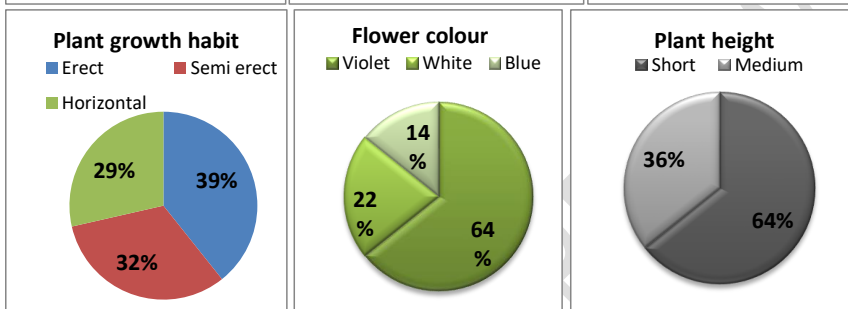
Fig 7: Seed Testa mottling

Fig 8: Cotyledon Colour

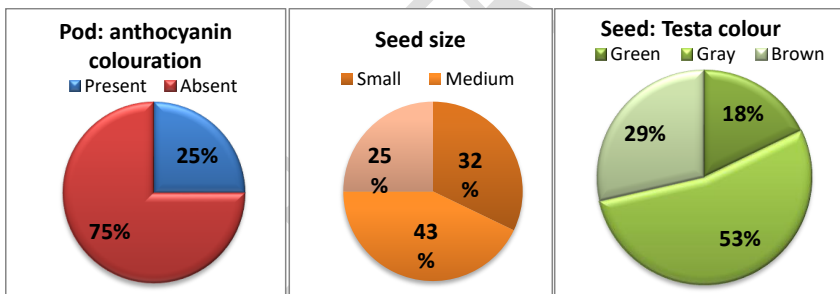
139



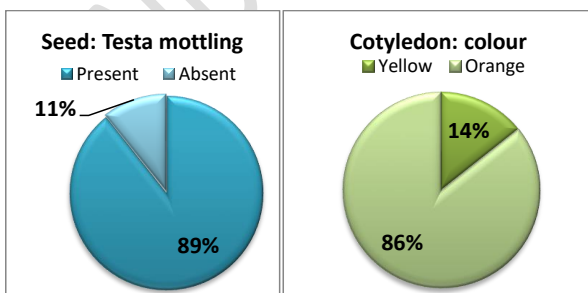
140



141



142



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

144 **Conclusion**

145

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

157 **References**

158 Kumar, J., Basu, P. S., Srivastava, E., Chaturvedi, S. K., Nadarajan, N., & Kumar, S. (2012).
159 Phenotyping of traits imparting drought tolerance in lentil. *Crop and Pasture Science*, 63(6),
160 547-554.

161
162 Food and Agriculture Organization of the United Nations, FAOSTAT. (n.d.). Retrieved from
163 <http://www.fao.org/faostat/en/#QCL> (Accessed 04 August 2019).

164
165 Erskine, W., & Sarker, A. (2004). Lentil: Evolutionary relationships among cultivated and
166 wild species. *Genetic Resources and Crop Evolution*, 51(5), 643-653.

167
168 Ladizinsky, G. (1979). The origin of lentil and its wild genepool. *Euphytica*, 28(1), 179-187.

169
170 Ferguson, M. E., & Robertson, L. D. (1996). Morphological and cytogenetic characterization
171 of *Lens orientalis*, the wild progenitor of lentil. *Theoretical and Applied Genetics*, 92(1), 81-
172 88.

173
174 Cubero, J. I. (1981). Origin, taxonomy, and domestication. In *Lentils* (pp. 15-38). C.A.B.
175 International.

176
177 Food and Agriculture Organization of the United Nations (FAO). (2022). Lentil production
178 statistics.

179
180 UPOV. (2019). General introduction to the examination of distinctness, uniformity, and
181 stability (DUS) and the development of harmonized descriptions of new varieties of plants.

182
183 Kumar, J., Sharma, S., Singh, M., & Kaur, P. (2022). Molecular marker technologies for
184 lentil breeding: Applications in DUS testing. *Journal of Crop Science and Biotechnology*,
185 25(1), 123-134. <https://doi.org/10.1007/s12892-022-00123-8>

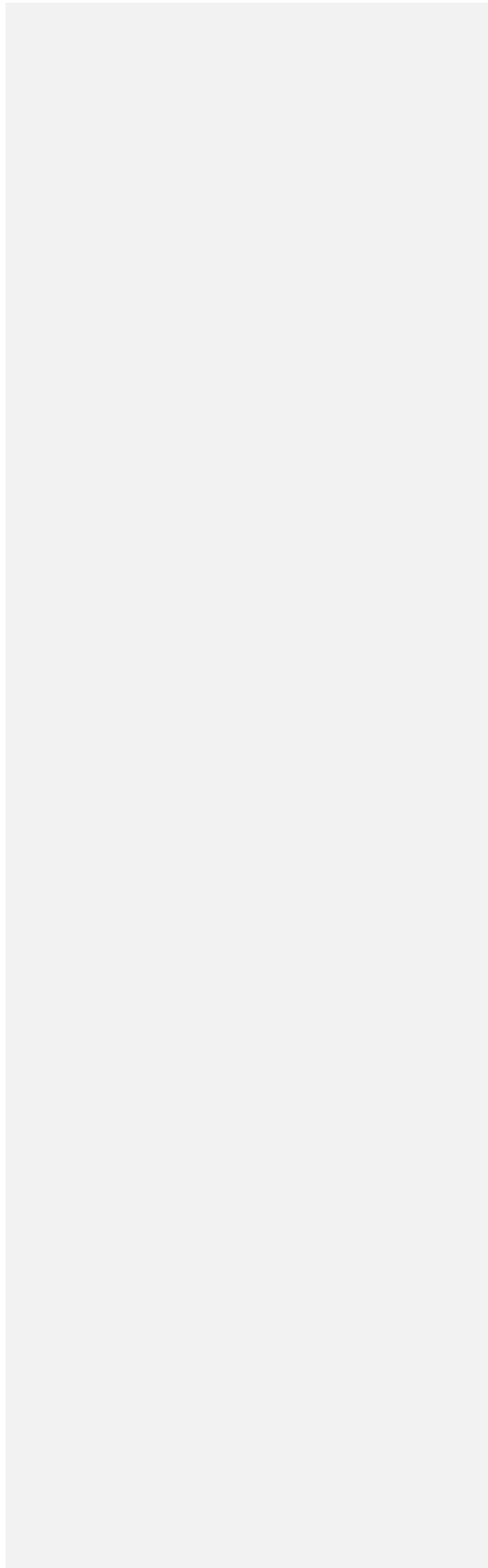
186

187
188 Food and Agriculture Organization of the United Nations (FAO). (2020). Lentil production
189 statistics.
190
191 FAOSTAT. (2019). Food and Agriculture Organization Corporate Statistical Database.
192
193 Singh, R., Sharma, B., & Kaur, P. (2021). Advances in lentil production in India. *Journal of*
194 *Crop Improvement*, 35(3), 347-359. <https://doi.org/10.1080/15427528.2021.1887834>
195
196 Choudhury, B., Saha, R., & Bhattacharya, S. (2014). Morphological characterization and
197 genetic stability in lentil. *Journal of Crop Improvement*, 28(2), 185-202.
198 <https://doi.org/10.1080/15427528.2014.897893>
199
200 Choudhary, A., Singh, M., & Sharma, R. (2017). Evaluation of lentil genotypes for
201 morphological traits and their implications for DUS characterization. *Journal of Agricultural*
202 *Science*, 9(4), 55-72. <https://doi.org/10.1017/S2040921120170024>
203
204 Hoque, M. S., Rezaul, M., & Iqbal, M. (2002). Genetic control of leaf color in lentil.
205 *Theoretical and Applied Genetics*, 104(5), 831-837. <https://doi.org/10.1007/s001220100750>
206
207 Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA). (2007).
208 Guidelines for DUS testing in lentil.
209
210 Vandenberg, A., & Slinkard, A. E. (1990). Seed coat color and testa mottling in lentil:
211 Genetic control and inheritance. *Canadian Journal of Plant Science*, 70(2), 605-611.
212 <https://doi.org/10.4141/cjps90-081>
213
214
215
216
217
218
219
220
221
222
223
224

Formatted: Swedish (Sweden)

225
226
227
228
229
230
231
232
233
234
235
236
237
238
239
240
241
242
243
244
245
246

UNDER PEER REVIEW



UNDER PEER REVIEW

