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Morphological characterization of lentil (*Lens culinaris* Medikus.) Varieties based on six qualitative traits

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Abstract
The distinctiveness of candidate variety from already released varieties is an important criterion for granting the plant breeders right in the present era of intellectual property rights. A total of 48 lentil genotypes were grouped into distinct groups for each character based on different forms of morphological characters. Almost all the genotypes have orange cotyledon except PL 6 which has yellow cotyledon and only local accession IC 201738 had black testa colour. For plant type 29%, 65% and 6% genotypes were classified into erect, semi-spreading and spreading, respectively. Based on rust scoring 67% genotypes were resistant type and only 33% genotypes were susceptible type. Three different groups *i.e.,* light green, medium green and dark green were formed and 15%, 69% and 17% genotypes were present in different groups, respectively. 79% genotypes were mottled type and 21% were non-mottled type.

Keywords: Testa, Candidate variety, lentil, cotyledon, rust

1. Introduction
Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is a diploid (2*x*=2*n*= 14 chromosomes) autogamous annual species with a haploid genome size of an estimated 4063 Mbp (Arumuganathan and Earle, 1991) [1]. In India, lentil is locally known as "Masoor" and either whole grain or split seeds of lentil are used as "dhal" (dry, dehulled, split seed used for cooking). Globally, it is grown in about 4.52 million hectares area with an annual production of 4.83 million tonnes and with an average productivity around 1067 kg/ha. In India, lentil is the second most important *rabi* legume crop after chickpea. In India, it is cultivated on 1.47 million hectares area with an annual production of 1.04 million tonnes to with productivity around 705 kg/ha during 2014 (Agricultural Statistics Division, 2014-2015). Although, it is not a major crop but it is consumed for its high levels of protein, fibers, antioxidants and micronutrients including iron, zinc, selenium and vitamin (A and B complex), folate and β-carotene (Erskine and Sarker 2004) [6]. The crop has great significance in cereal-based cropping systems because of its nitrogen-fixing ability, early maturing and tolerance to drought. It is mainly grown as rainfed crop in Uttar Pradesh, Madhya Pradesh, Chattishgarh, Punjab, Haryana, Jharkhand, Bihar, Rajasthan, Uttarakhand, West Bengal, Jammu and Kashmir and some north eastern states.

Lentil is a slender, softly pubescent, annual herbaceous plant which had considerable range of morphological variations among its germplasm. Considerable variations among the characters for use in breeding and selection programmes have been reported (Malik *et al.* 1984, Ramgiry *et al.* 1989, Sarker and Erskine 2001, Tullu *et al.* 2001) [11, 14, 16, 22]. Barulina (1930) [3] first recorded detailed morphological descriptions of lentil landraces and species from Asia. Morphological markers like colour of stem, flower and foliage colour, plant habit, cotyledon and testa colour and testa pattern are important for testing hybridity and keeping genetic purity to be used in marker assisted selection. Targeted and more efficient utilization of germplasm by plant breeders can be achieved if the trait characteristics of accessions are known.

In recent decades, India has witnessed the emergence of large and' highly competitive variety development programmes. However, morphological descriptors of many cultivars are imprecise. Being signatory to the GATT (General Agreement on Trade and Tariff), Government of India has enacted its *Sui genesis* system called as "Protection of Plant Varieties and Farmer's Right act (PPV & FR) 2001". Under the act new varieties will be registered on DUS (Distinctness, Uniformity and Stability) testing for which documentation of diagnostic characters of the varieties are required. Moreover for maintenance of genetic purity of varieties during seed production and certification programme there is an urgent need of identification

and documentation of diagnostic features of released varieties with their accurate identification keys giving detailed description on comparative basis with clear-cut features of distinctness. Therefore, realizing the importance and necessity of varietal characterization, the present investigation has been undertaken to characterize the diverse lentil accessions on the basis of qualitative traits.

Materials and Methods

The present investigation was conducted with 48 lentil accessions, which were released varieties, advance breeding lines and local collections (Table 1). The genotypes were obtained from Norman E. Borlaug Crop Research Centre, G.B. P. U. A & T. Pantnagar and all are maintained at G. B. P. U. A. T, Pantnagar (Table 1). The 48 lentil genotypes were evaluated in *rabi* season of 2015-16 in a randomized block designs (RBD) with three replications. The unit plot size consisted 3 rows of 4m length with row to row and plant to plant spacing of 22.5 cm and 4 -5 cm, respectively. The recommended package of practices was strictly followed for raising a good crop. Genotypes were evaluated for 6 morphological descriptors *viz.*, plant type, foliage colour, testa clour, testa mottling, cotyledon colour and rust score. These characters were recorded as per DUS guidelines of PPV&FR (Singh *et al.* 2006) [19]. The observations were recorded on 5 plants in each replication at specified stages of crop growth period when the characters under study had full expression. Intensity of the green colour of foliage and plant type was recorded at flower bud stage whereas, rust disease score was done at pod formation stage by following Khare *et. al.* (1993) [9]. Observation on seed characters like, testa colour, testa pattern and cotyledon colour were observed visually after harvesting the seeds at maturity.

Results and Discussion

Six different qualitative characters of 48 lentil genotypes were studied which are important morphological markers for keeping genetic purity. Adequate amount of variation was present among the 48 genotypes for six important characters under study. The 48 genotypes under study were classified into discrete groups under 6 major categories representing 6 qualitative characters *viz.* plant type, foliage colour, testa colour, testa mottling, cotyledon colour and rust score (Table 1). On the basis of present preliminary characterization, these genotypes were grouped into different categories and may be used as reference cultivars. They were classified into separate groups under each character as shown in Table 2 on the basis of national guidelines for DUS testing in lentil (Singh *et al.*, 2006) [19].

Varieties have been categorized as erect, semi-spreading and spreading based on growth habit. Out of 48 genotypes 31 genotypes were fall in semi-spreading group and 14 genotypes were erect type and 3 were of spreading type. It supports the observations of Thakur and Bajpai (1993) [21], and Singh and Singh (1994) [20]. This trait is monogenically governed with dominant expression of the tall erect plant habit (Ladizinksky, 1979) [10]. Erect and spreading plant types were extreme expressions for this trait and these are more stable but semi-spreading expression may be altered with environmental fluctuations.

Among the morphological traits observed during field evaluation, the foliage colour may be considered as distinguishing, stable and uniform trait for DUS characterization. Lentil germplasms have been categorized as light green, dark green and medium dark green and genotypes under study contains all three form of foliage colour. Medium green foliage was more common among the genotypes under study, as 33 genotypes had medium dark green foliage. Only seven genotypes observed with light green foliage and rest 8 genotypes were of dark green foliage type. On the basis of results obtained, it could be emphasized that morphological characters play a very important role and they do contribute towards genotype divergence in lentil. Light green colour foliage turns yellow during maturity and leaves drop down during crop maturation. In contrast, dark green foliage persist for long and stay green during advanced stage of crop growth and resulted in high seed yield with fully matured seed. Stay green character of dark green foliage is desirable and genotypes with dark green foliage should be developed for high yield potential. Similar observations were also reported by Mishra *et al.* (2001) [13]. Hoque *et al* (2002) [7] reported that foliage colour is controlled by single gene with dominance of dark green foliage over light green foliage.

Expression of seed characters like, testa colour, testa pattern and cotyledon colour are uniform and stable. Therefore, these traits are better criteria for distinguishing the lentil genotypes. Three classes were formed on the basis of testa colour *i.e.*, grey, brown and black. Among 48 genotypes under study 26 genotypes had brown testa colour, 21 genotypes had grey testa colour and only single local collection (IC 201738) observed with black colour testa. Only two types of classes were formed based on testa mottling *i.e.*, presence of mottling and absence of testa mottling. Out of 48 genotypes, testa mottling was absent only on 10 genotypes and on rest of the genotypes the mottling was present. Testa colour and testa mottling are found to be the most stable and uniform traits for certification of genetic purity of genotypes at seed level as their expressions are least influenced by environment. Hence, these two traits in combination can be utilized to characterize the lentil accessions.

Visual assessment for cotyledon colour was done one month after harvesting the seeds and can be categorized in three different classes as per National DUS guidelines *i.e.*, yellow, olive green and orange. Genotypes under study were classified into two category *i.e.*, yellow, and orange. Almost all genotypes had orange colour cotyledon except the genotype (PL 6) which had yellow cotyledon. Sharma and Emami (2002) [17]; Bakhsh *et al.* (2013) [2]; Singh *et al.* (2014) [18] and Dixit *et al.* (2011) [5] showed that cotyledon colour was controlled by single gene hence, trait is less influenced by the environment. Therefore, it can be utilized for distinguishing the lentil genotypes. The intensity of cotyledon colour is affected with storage condition and time so; this trait may be useful only for varietal protection before entering into active seed multiplication chain.

Lentil rust is one of the major foliar disease which causes substantial yield losses in lentil. Pantnagar is a hot spot for lentil rust and the symptoms of rust are visible on all plant parts. Hence, rust scoring of genotypes under study were done at field level and on the basis of rust disease scale genotypes under study were classified into two groups *i.e.*, resistant and susceptible. The resistant group consisted of 32 genotypes out of 48 and rest 16 genotypes showed susceptibility for rust. While developing new varieties resistance to rust should be considered and monogenic dominant control of lentil rust was reported by many researchers, Saha *et al.* (2010) [15]; Mekonnen *et al.* (2014) [12] and Dikshit *et al.* (2016) [4]. Hence, transfer of rust resistance is easy and characterization based on rust resistance will helpful for protection under PVP & FR legislation.

Table 1: Characterization of lentil varieties/advance breeding lines based on six qualitative characters

S. No.	Genotypes	Rust Scoring	Plant Type	Foliage Colour	Testa Colour	Testa Mottling	Cotyledon Colour
1.	PL406	7	3	3	4	1	3
2.	PL 639	3	3	3	2	3	3
3.	PL 4	7	3	3	2	3	3
4.	PL 5	3	3	3	2	3	3
5.	PL 6	3	1	1	2	1	1
6.	PL 7	3	3	3	4	1	3
7.	PL 8	3	3	3	4	3	3
8.	PL 9	3	3	1	2	3	3
9.	PL 153	5	1	1	4	3	3
10.	DPL 15	3	1	2	4	3	3
11.	DPL 58	3	3	1	2	3	3
12.	DPL 62	3	3	2	4	1	3
13.	FLIP 96-51	5	3	2	2	3	3
14.	IC 201627	3	3	2	4	3	3
15.	IC 201648	3	1	2	4	3	3
16.	IC 201675	3	5	2	2	3	3
17.	IC 207709	3	1	2	2	3	3
18.	IC201738	3	3	2	5	3	3
19.	ICC 279032	5	3	2	2	3	3
20.	IPL 406	3	3	2	4	1	3
21.	K 75	5	3	2	2	3	3
22.	KLS 218	3	3	2	2	3	3
23.	L 4147	5	3	2	4	1	3
24.	L 4148	5	1	1	2	1	3
25.	L 4188	5	3	1	4	3	3
26.	LH 84-8	3	1	3	4	3	3
27.	LL 864	3	1	2	2	1	3
28.	LL 875	5	3	2	4	3	3
29.	LL 931	3	3	1	4	3	3
30.	PL 029	3	1	2	2	3	3
31.	PL 164	3	1	2	4	3	3
32.	PL 210	3	1	2	4	3	3
33.	PL 213	3	3	2	4	3	3
34.	PL 218	5	3	1	4	3	3
35.	PL 220	3	1	2	4	3	3
36.	PL 221	5	3	2	4	1	3
37.	PL 225	3	3	2	4	3	3
38.	PL 227	5	1	2	4	3	3
39.	PL 239	5	3	2	2	3	3
40.	PL 242	5	3	2	2	3	3
41.	PL 244	3	3	2	2	3	3
42.	PL017	3	5	2	2	3	3
43.	PL028	3	5	2	4	1	3
44.	PL038	3	3	2	4	3	3
45.	PL046	5	3	2	2	3	3
46.	PL056	5	3	2	2	3	3
47.	PL 015	3	1	2	4	3	3
48.	PL 234	3	3	2	4	3	3
States of characteristics according to national test Guideline		1-3 Resistant 5-9 Susceptible	1- Erect, 3- Semi-erect 5- Spreading	1- Light Green 2-Medium Green, 3- Dark Green	2- Grey 4- Brown 5- Black	1- Absent 3- Present	1- Yellow 3- Orange

Table 2: Classification of 48 lentil genotypes into distinct groups based on six qualitative characters

Characters		Name of Genotypes	No. of Genotypes
Rust Score	Resistant	PL 639, PL 6, PL 7, PL 8, PL 9, DPL 15, DPL 62, IC 201627, IC 201648, IC 201675, IC 207709, IC201738, KLS 218, LH 84-8, LL 864, LL 931, PL 029, PL 164, PL 210, PL 213, PL 220, PL 225, PL 244, PL017, PL028, PL038, PL 015, PL 234, PL 5, DPL 58, IPL 406, L 4147	32
	Susceptible	PL 4, PL 153, FLIP 96-51, ICC 279032, K 75, L 4148, L 4188, LL 875, PL 218, PL 221, PL 227, PL 239, PL 242, PL046, PL056, PL 406	16
Plant Type	Erect	PL 6, PL 220, PL 210, PL 164, PL 029, LL 864, LH 84-8, L 4148, IC 207709, IC 201648, DPL 15, PL 153, PL 227, PL	14
	Semi-Spreading	PL 234, PL406, PL056, PL046, PL038, PL 9, PL 8, PL 7, PL 639, PL 5, PL 4, PL 244, PL 242, PL 239, PL 225, PL 218, PL 213, LL 931, LL 875, L 4147, L 4188, KLS 218, FLIP 96-51, PL 221, K 75, IPL 406, ICC 279032, IC201738, IC 201627, DPL 62, DPL 58	31

	Spreading	PL017, PL028, IC 201675	3
Foliage Colour	Light Green	PL 6, PL 153, DPL 58, L 4148, L 4188, LL 931, PL 218	7
	Medium Green	DPL 15, DPL 62, FLIP 96-51, IC 201627, IC 201648, IC 201675, IC 207709, IC201738, ICC 279032, IPL 406, K 75, KLS 218, L 4147, LL 864, LL 875, PL 029, PL 164, PL 210, PL 213, PL 220, PL 221, PL 225, PL 227, PL 239, PL 242, PL 244, PL017, PL028, PL038, PL046, PL056, PL 015, PL 234	33
	Dark Green	PL406, PL 639, PL 4, PL 5, PL 8, LH 84-8, PL 7, PL 9	8
Testa Colour	Black	IC 201738	1
	Brown	PL 7, PL 8, PL 153, DPL 15, DPL 62, IC 201627, IC 201648, IPL 406, L 4147, L 4188, LH 84-8, LL 875, LL 931, PL 164, PL 210, PL 213, PL 218, PL 220, PL 221, PL 225, PL 227, PL028, PL038, PL 015, PL 234	26
	Grey	PL 639, PL 4, PL 5, PL 6, PL 9, DPL 58, FLIP 96-51, IC 201675, IC 207709, ICC 279032, K 75, KLS 218, L 4148, LL 864, PL 029, PL 239, PL 242, PL 244, PL017, PL046, PL056	21
Cotyledon Colour	Orange (Pink)	PL406, PL 639, PL 4, PL 5, PL 7, PL 8, PL 9, DPL 15, DPL 58, DPL 62, FLIP 96-51, IC 201627, IC 201648, IC 201675, IC 207709, IC201738, ICC 279032, IPL 406, KLS 218, L 4148, L 4188, LH 84-8, LL 875, LL 931, PL 164, PL 210, PL 213, PL 218, PL 220, PL 221, PL 225, PL 227, PL 242, PL 244, PL017, PL028, PL038, PL046, PL056, PL 234, PL 153, K 75, L 4147, LL 864, PL 029, PL 239, PL 015	47
	Yellow	PL 6	1
Testa Mottling	Present	PL406, PL 639, PL 4, PL 5, PL 8, PL 9, PL 153, DPL 15, DPL 58, FLIP 96-51, IC 201627, IC 201648, IC 201675, IC 207709, IC201738, ICC 279032, K 75, KLS 218, L 4188, LH 84-8, LL 875, LL 931, PL 029, PL 164, PL 210, PL 213, PL 218, PL 220, PL 225, PL 227, PL 239, PL 242, PL 244, PL017, PL038, PL046, PL056, PL 015, PL 234	38
	Absent	PL 6, PL 7, DPL 62, IPL 406, L 4147, L 4148, LL 864, PL 221, PL028	10

Conclusion

In conclusion, it can be said that plant breeders can use these genetic variations to make decision regarding the choice for selecting superior genotypes for improvement or to be utilized as parents for the development of future cultivars through hybridization. Furthermore, important morphological markers like, plant type, foliage colour, testa colour, testa pattern and cotyledon colours can also be used for testing hybridity and keeping genetic purity at genetic level. The information obtained will be useful in DUS characterization of lentil varieties in the event of implementation of PVP & FR Act, 2001. The information obtained by the identification keys at seed and plant levels may be useful for discrimination and verification of varieties, hybridity testing and maintenance of genetic purity at genetic level during seed production and certification programme.

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