Characterization of interspecific derivatives of lentil using DUS guidelines

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ABSTRACT

The distinctness of a candidate variety from previously released varieties is a major criterion for granting of plant breeders' rights. At the same time, there is a need to maintain the genetic purity of varieties during seed production and certification programmes. Twenty nine lentil recombinant inbred lines (RILs) were assessed at two locations along with two checks viz Markanday and Vipasha for their distinctness, uniformity and stability (DUS) under Protection of Plant Varieties and Farmer's Rights (PPV & FR) Act 2001. All these genotypes were categorized based on three quantitative and ten qualitative descriptors. The variability in the expression of these distinguishing traits was observed at seed and plant levels. Seed size, seed colour, foliage colour, anthocyanin colouration on stem, leaflet size, plant growth habit and plant height were found to be the most useful characters for sound classification of genotypes and utilization in strengthening of lentil breeding programmes.

Keywords: Characterization; DUS; genotypes; lentil; RILs

INTRODUCTION

Lentil (*Lens culinaris* Medik) is a self-pollinating true diploid (2n = 2x = 14) grain legume which shows less than 0.80 per cent of natural cross-pollination (Wilson and Law 1972). Flowers of a lentil plant are complete having a typical structure of family Leguminosae and sub-family Papilionaceae (Muehlbauer et al 1980). Lentils are included in the cool season group of pulses and are a rich source of energy, carbohydrates, vitamins, iron, potassium and zinc (Tickoo et al 2005). With about 26 per cent protein content, lentils provide the third highest amount of protein from any plant-based food after soybeans and hemp.

In the recent decades, India has witnessed the emergence of large and highly competitive varietal development programmes. This makes it necessary to identify and document the diagnostic features of varieties with their accurate identification keys giving detailed description on comparative basis with clear cut features of distinctness. The Government of India has enacted its sui genesis system called as Protection of Plant Varieties and Farmer's Rights (PPV & FR) Act 2001 (Gosavi 2004) and designated plant breeder's rights (PBR). Under these rights, all the new varieties are registered on the basis of DUS (distinctness, uniformity and stability) testing for which characterization of genotypes for diagnostic traits is fundamental. Therefore, realizing the importance of varietal characterization, the present investigations were undertaken to characterize the lentil genotypes.

MATERIAL and METHODS

The present investigations were conducted with 29 identified promising recombinant inbred lines (RILs) of lentil which had been derived from two crosses viz Cross 1: ILL8006 (*Lens culinaris*) × ILWL 62 (*L orientalis*) and Cross 2: ILL10829 *(L culinaris*) × ILWL 30 (*L ervoides*) (Table 1). These genotypes

Table 1. List of descriptors used for the characterization of 31 lentil genotypes

DUS character	State	Stage of observation
Foliage: Intensity of green colour	Light, medium, dark	Flower bud stage
Stem anthocyanin colouration	Absent, present	50% flowering
Time of flowering (days)	Early (<60), medium (60-80), late (>80)	50% of the plants with at least one open flower
Leaf pubescence	Absent, present	50% flowering
Leaflet size	Small, medium, large	-do-
Plant growth habit	Erect (<30°), semi-erect (30-60°), horizontal (>60°)	-do-
Flower colour of standard	White, pink, blue, violet, others (specify)	-do-
Plant height (cm)	Short (40), medium (40-60), long (40-60)	Harvest maturity
Pod anthocyanin colouration	Absent, present	Fully developed green pod
Seed size (100-seed weight) (g)	Small (<2), medium (2-2.5), large (2.6-3.0), very large (>3)	Mature seed
Seed testa colour	Green, grey, pink, brown, black	-do-
Seed testa mottling	Absent, present	-do-
Cotyledon colour	Yellow, olive green, orange	-do-

were procured from National Bureau of Plant Genetic Resources (NBPGR), Shimla, Himachal Pradesh. Seeds of 22 RILs from Cross 1 and 7 RILs from Cross 2 were sown in randomized bock design with three replications each on 13 November 2019 at Pulse Research Station, Berthin, Dist Bilaspur, Himachal Pradesh and 23 November 2019 at the Department of Seed Science and Technology, CSK HPKV, Palampur, Dist Kangra, Himachal Pradesh along with two standard checks viz Markanday and Vipasha. A singe plot consisted of 3 rows of 3 m length with row to row and plant to plant spacing of 25 cm and 10 cm respectively. The observations on 13 descriptors were recorded on 5 plants in each replication at specified stages of crop growth as per the national DUS testing guidelines on lentil (Anon 2007).

RESULTS and DISCUSSION

Three quantitative and ten qualitative characters were studied for classifying 31 lentil genotypes into discrete groups using DUS guidelines (Table 2). Adequate variability was present among the genotypes for eight characters viz intensity of green colour in foliage, stem and pod anthocyanin colouration, leaflet size, plant growth habit, plant height, seed size and seed testa colour.

On the basis of intensity of green colour in foliage, the genotypes were classified as light, medium and dark. Hoque et al (2002) reported that foliage colour is controlled by a single gene with dominance of dark green foliage. Medium green colour was most common among the genotypes as this group included 15 RILs and both the checks. Four RILs showed light green foliage colour and rest eight RILs had dark green foliage.

Anthocyanins are naturally occurring pigments which impart blue, red or purple pigment to plant parts. Ladizinsky (1979) reported anthocyanin pigmentation as a monogenically controlled trait and its presence or absence is considered as a distinct character. At flower bud stage, the genotypes were observed for the presence of anthocyanin on their stems. Similarly, at green pod stage, the presence or absence of anthocyanin colouration on pods was considered as a distinct character. Reddish-purple spots appeared on the pods due to the presence of anthocyanin. C1 RIL 2, C1 RIL 51 and C2 RIL 80 along with both the checks showed the presence of anthocyanin on their stem while presence of anthocyanin colouration on pods was observed in C1 RIL 2, C1 RIL 49, C1 RIL 51, C1 RIL 63, C1 RIL 71 and C2 RIL 79. The obtained results validate the observations reported previously by Anwar and Bhatti (1986) and Lazaro et al (2001) in lentil. Stage specific nature of anthocynin colouration makes these characters to be less likely used for characterization.

Time of flowering was one of the traits with less variability. At both the locations, all RILs and both the checks were categorized as late flowering as they

Table 2. Details of the groups of lentil genotypes based on DUS characterization

Genotype	A	B	C	D	E	F	G	H	I	J	K	L	M
C1 RIL 2	Medium	Present	Late	Present	Small	Horizontal	Violet	Short	Present	Small	Grey	Present	Orange
C1 RIL 8	Dark	Absent	Late	Present	Medium	Horizontal	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 9	Dark	Absent	Late	Present	Small	Semi-erect	Violet	Short	Absent	Small	Brown	Present	Orange
C1 RIL 10	Medium	Absent	Late	Present	Large	Erect	Violet	Medium	Absent	Large	Grey	Present	Orange
C1 RIL 12	Dark	Absent	Late	Present	Medium	Semi-erect	Violet	Medium	Absent	Medium	Brown	Present	Orange
C1 RIL 20	Medium	Absent	Late	Present	Medium	Erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 21	Dark	Absent	Late	Present	Medium	Erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 24	Dark	Absent	Late	Present	Large	Semi-erect	Violet	Short	Absent	Large	Grey	Present	Orange
C1 RIL 43	Light	Absent	Late	Present	Medium	Erect	Violet	Medium	Absent	Large	Grey	Present	Orange
C1 RIL 49	Medium	Absent	Late	Present	Medium	Semi-erect	Violet	Short	Present	Medium	Brown	Present	Orange
C1 RIL 51	Dark	Present	Late	Present	Small	Semi-erect	Violet	Short	Present	Small	Grey	Present	Orange
C1 RIL 53	Dark	Absent	Late	Present	Medium	Semi-erect	Violet	Short	Absent	Small	Grey	Present	Orange
C1 RIL 57	Medium	Absent	Late	Present	Medium	Erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 58	Medium	Absent	Late	Present	Large	Semi-erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 62	Medium	Absent	Late	Present	Large	Semi-erect	Violet	Short	Absent	Small	Grey	Present	Orange
C1 RIL 63	Dark	Absent	Late	Present	Small	Semi-erect	Violet	Short	Present	Medium	Brown	Present	Orange
C1 RIL 64	Medium	Absent	Late	Present	Medium	Semi-erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 65	Medium	Absent	Late	Present	Large	Semi-erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 70	Medium	Absent	Late	Present	Large	Semi-erect	Violet	Short	Absent	Medium	Brown	Present	Orange
C1 RIL 71	Dark	Absent	Late	Present	Medium	Semi-erect	Violet	Short	Present	Small	Grey	Present	Orange
C1 RIL 97	Light	Absent	Late	Present	Medium	Erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C1 RIL 98	Light	Absent	Late	Present	Medium	Semi-erect	Violet	Short	Absent	Large	Brown	Present	Orange
C2 RIL 72	Medium	Absent	Late	Present	Large	Erect	Violet	Short	Absent	Medium	Grey	Present	Orange
C2 RIL 75	Medium	Absent	Late	Present	Large	Horizontal	Violet	Short	Absent	Medium	Brown	Present	Orange
C2 RIL 76	Medium	Absent	Late	Present	Medium	Semi-erect	Violet	Short	Absent	Medium	Brown	Present	Orange
C2 RIL 77	Light	Absent	Late	Present	Medium	Erect	Violet	Medium	Absent	Large	Grey	Present	Orange
C2 RIL 79	Medium	Absent	Late	Present	Small	Erect	Violet	Short	Present	Medium	Grey	Present	Orange
C2 RIL 80	Medium	Present	Late	Present	Medium	Semi-erect	Violet	Short	Absent	Medium	Brown	Present	Orange
C2 RIL 6	Dark	Absent	Late	Present	Small	Erect	Violet	Short	Absent	Medium	Grey	Present	Orange
Markanday	Medium	Present	Late	Present	Large	Semi-erect	White	Short	Absent	Large	Pink	Absent	Orange
Vipasha	Medium	Present	Late	Present	Large	Semi-erect	White	Short	Absent	Medium	Pink	Absent	Orange

A= Foliage: intensity of green colour, B= Stem: anthocyanin colouration, C= Time of flowering, D= Leaf pubescence, E= Leaflet size, F= Plant growth habit, G= Flower colour of standard, H= Plant height, I= Pod anthocyanin colouration, J= Seed size, K= Seed testa colour, L= Seed testa mottling, M= Cotyledon colour

took more than 80 days to show 50 per cent blooming. The average days taken by the genotypes to show 50 per cent flowering ranged from 94-104 days at Berthin and 98-133 days at Palampur. All the RILs flowered earlier than both the checks at both the locations. Another trait with no variability was leaf pubescence. Indian lentils are characterized by dense pubescence on their leaflets and similar results were observed in this study as all the genotypes were pubescent. Earlier Lazaro et al (2001) observed the presence of pubescent as well as non-pubescent genotypes in their study on lentil. Not much variation was observed among the genotypes for the colour of standard as it was observed to be violet in all the RILs. Only the checks had white standard colour. Pink, blue or any other standard colour was completely absent.

The RILs showed high variation for leaflet size (length) on the basis of which they were categorized into small, medium and large. C1 RIL 2, C1 RIL 9, C1 RIL 51, C1 RIL 63, C2 RIL 79 and C2 RIL 6 had small sized leaflets. Fifteen RILs had medium and ten genotypes including both the checks had large sized leaflets.

There was wide variation among the RILs for plant growth habit on the basis of which they were categorized as erect, semi-erect and horizontal. This trait is monogenically governed with dominant expression of erect habit (Ladizinsky 1979). Maximum number of genotypes fell under the semi-erect group with 16 RILs and both the checks followed by ten RILs in erect group and three in horizontal group.

The height of plants was measured at harvest maturity. At Berthin, minimum mean plant height was observed for C1 RIL 8 (23.5 cm) and maximum for RIL C1 43 (43.06 cm). Similar results were obtained at Palampur but the mean heights of plants were less as compared to that at Berthin. At Berthin, C1 RIL 10, C1 RIL 12, C1 RIL 43 and C2 RIL 77 were medium in height and all other RILs including the checks were short. None of the RILs was long in height. At Palampur, all the RILs and both the checks were observed to be short supporting the fact that DUS characterization should only be conducted in areas best suited for a particular crop.

Four DUS descriptors were observed on harvested seeds. On the basis of seed size, the genotypes were classified as small (<2 g), medium (2-2.5 g), large (2.6-3.0 g) and very large (>3 g) and there was considerable variability for seed size among the genotypes. The average 100-seed weight ranged from 1.79-2.81 g at Berthin and 1.77-2.77 g at Palampur. Seeds of lentil genotypes from both the locations showed similar classification. Small and large categories included six genotypes each and all other genotypes had medium seed size.

Seed testa colour is another distinguishing character controlled by two pairs of genes (Wilson and Hudson 1979). The RILs were categorized as brown, grey and pink on the basis of their seed coat colouration. Maximum number of RILs had grey coloured seed coat followed by brown and pink seed coat colour including eight and two genotypes respectively. Seeds of a particular lentil RILs harvested from both the locations showed the same testa colour. Testa mottling was found to be present in all the RILs and absent in both the checks. Similar results have been reported in lentil by Wilson and Hudson (1979) and Thakur and Bajpai (1993). Cotyledon colour showed the least amount of variability with the presence of only orange cotyledons.

CONCLUSION

It was concluded that out of seed size, seed coat colour, cotyledon colour and testa mottling, only seed size and seed colour were found to be helpful in characterization as cotyledon colour and testa mottling were unable to distinguish the RILs due to lack of variability. At plant level, foliage colour, anthocyanin colouration on stem, leaflet size, plant growth habit and plant height were the most useful characters which provided a sound classification system for lentil genotypes. Plant height showed varied observations at both the locations. The use of anthocyanin colouration on pods as a distinguishing character is limited as it is highly stage specific trait and can only be observed only for few days during green pod stage. Time of flowering, leaf pubescence and colour of standard showed no variability and were, therefore, the least useful characters in DUS characterization. Plant breeders may use this germplasm for the release of new lentil varieties or utilize the genotypes with desirable traits as parents in future lentil improvement programmes.

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REFERENCES

Anonymous 2007. Guidelines for the conduct of tests for distinctness, uniformity and stability on lentil (*Lens culinaris* Medik). Protection of Plant Varieties and Farmers' Rights Authority (PPV & FRA), Government of India, Registrar, on behalf of the Chairperson, PPV & FR Authority, New Delhi, India.

Anwar R and Bhatti MS 1986. Exploration and collection of natural genetic variability of lentil in Punjab-Pakistan. Lens Newsletter **13(2):** 3-5.

Gosavi S 2004. Characterization of lentil varieties. MSc (Agric) Thesis, Jawaharlal Nehru Krishi Vishvavidyalaya, Jabalpur, Madhya Pradesh, India.

Hoque ME, Mishra SK, Kumar Y, Kumar R, Tomar SMS and Sharma B 2002. Inheritance and linkage of leaf colour and plant pubescence in lentil (*Lens culinaris* Medik). Indian Journal of Genetics and Plant Breeding **62(2):** 140-142.

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

Lazaro A, Ruiz M, de la Rosa L and Martin I 2001. Relationship between agro-morphological characters and climatic parameters in Spanish landraces of lentil (*Lens culinaris* Medik). Genetic Resources and Crop Evolution **48:** 239-249.

Muehlbauer FJ, Slinkard AE and Wilson VE 1980. Lentil. In: Hybridization of crop plants (WR Fehr and HH Hadley, eds). American Society of Agronomy, Inc and Crop Science Society of America, Inc, pp 417-426.

Thakur HK and Bajpai GC 1993. Characterization of lentil germplasm for phenological and yield characters. Indian Journal of Pulses Research **6(1):** 89-91.

Tickoo JL, Sharma B, Mishra SK and Dikshit HK 2005. Lentil (*Lens culinaris*) in India: present status and future perspectives. Indian Journal of Agricultural Sciences **75(9):** 539-567.

Wilson VE and Hudson LW 1979. Inheritance of lentil seedcoat mottle. Journal of Hereditary **70(1):** 83-84.

Wilson VE and Law AG 1972. Natural crossing in *Lens esculenta* Moench. Journal of the American Society for Horticultural Science **97(1):** 142-143.