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Morphological Characterization of High Grain Iron and Zinc Concentration Bio-fortified lentil (*Lens culinaris Medikus subsp. culinaris*) in Nepal

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Abstract

Lentil (Lens culinaris Medikus subsp. culinaris) is the wonderful, most popular & preferred food legume of Nepalese people and commonly grown in the rice and maize based cropping system. Morphological characters have been frequently used in order to know the diversity in germplasm collections. The main objectives of the study were to characterize the bio-fortified lentil accessions using morphological markers and select the DUS accession for using targeted the best utilization in hybridization program. Plant breeders can use these morphological 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 color, testa color, testa pattern and cotyledon colors can also be used for testing hybridity and keeping genetic purity at genetic level. 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. Out of 25 lentil accessions, 18 accessions were observed as erect and compact growth habit, 22 accessions had green stem color, 21 accessions had dense leaf pubescence and dark foliage, 7 accessions had prominent tendril, 3 accessions had white blue veins flower and 4 accessions had large seeded accession.

Keywords: Lentil; Characterization; Morphological markers; Qualitative traits

Introduction

Lentil (*Lens culinaris Medikus subsp. culinaris*) is a diploid (2x = 2n =14 chromosomes) autogamous annual species with a haploid genome size of an estimated 4063Mbp [1]. Lentil is the wonderful, most popular & preferred food legume of Nepalese people and commonly grown in the rice and maize based cropping system. Globally, it is cultivated for its protein-rich grains in as many as 53 countries on 6.65 million ha area with annual production of 7.97 million tons with an average productivity around 1.19 ton/ha [2]. However, top ten countries, namely Canada, India, Turkey, Nepal, Australia, China, Iran, USA, Syria, and Ethiopia contribute about 95% of the global production. Its more than 85% lentil was produced in five specific regions including India, Nepal, and Bangladesh (32%); western Canada (29%); Turkey and northern Syria (18%); Australia (4%). In Nepal, lentil is the most important legumes which shares about 64% and 67% of the total legume area and production, respectively with productivity of around 1200kg/ha [3]. Swift cooking quality, tasty pink red cotyledons, high micronutrient contents and almost organic products make Nepalese lentil highly preferable to the lentil consumers and popular in the international market [4]. Bangladesh, Singapore, SriLanka, Germany, Korea, UK, Indonesia are the major export markets for Nepalese lentils [5]. 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 [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 in four province no. 2, 5, 7 and 1 and in top ten districts Dang, Rauthat, Kailali, Bardiaya, siraha, Bara, Banke, Nawalparasi, Saptari, Parsa which covers about 90% area. 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 [7-10]. First recorded detailed morphological descriptions of lentil landraces and species from Asia [11]. Morphological markers like growth habit, stem color, leaf pubescence, leaflet sizes, foliage color, flower ground color, no. of flowers per peduncle, pod pigmentation, seed sizes, seed coat color, seed coat pattern and cotyledon color, lodging susceptibility, no. of nodules/plant, cold susceptibility etc are important for testing hybridity segregates 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 days, India has witnessed the emergence of large and' highly competitive variety development programs. However, morphological descriptors of many cultivars are imprecise. Any characters/traits of the plants are governed by oligogenes or polygenes, the character produced by oligogenes are generally made of one or few genes with large, easily detectable effects

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Table 1: Source of collection and country of origin of 25 lentil accessions.

Accession Code no	Accessions	Pedigree	Types of materials	Source/Origin
1	ILL-8006	ILL5888 x ILL5782	Breeding lines	Bangladesh
2	RL-6	ILL8008 x ILL5888	Nepal cross	Nepal
3	RL-12	Sindur x Khajura-2	Nepal cross	Nepal
4	ILL-7715	FLIP95-59L	Breeding lines	ICARDA
5	ILL-7164	PAK86591	Breeding lines	ICARDA
6	ILL-3490		Breeding lines	ICARDA
7	Khajura-2 (PL639)	L9-12xType 8	Released	GPPUAT, Pantanagar
8	Simal (LG7)	Land race from India	Released	India
9	Shital (ILL2580)	L1278	Released	ICARDA/India
10	Sagun (ILL6829)	ILL4907(Pakistan)x ILL4605 (Argentine)	Released	ICARDA
11	HUL-57	Mutant of HUL1	Cultivar	BHU, Banaras, India
12	LG-12	Local landrace from India	Cultivar	India
13	PL-4	UPL175x(PL184xP228)	Cultivar	GPPUAT, Pantanagar
14	RL-11	-	Nepal cross	Nepal
15	RL-4 (Khajura-3)	ILL6037xILL8007	Released	Nepal
16	ILL-2712	-	Breeding lines	ICARDA
17	Black Masuro	Local landrace from Ramechap, Nepal	Released	Nepal
18	RL-79	99S95-2-1	Nepal cross	Nepal
19	ILL-6467	ILL4605xILL2582	Breeding lines	ICARDA
20	ILL-7979	FLIP1996-47L	Breeding lines	ICARDA
21	ILL-6819	FLIP88-461	Breeding lines	ICARDA
22	ILL-7723 (Khajura-4)	Sel89503	Released	ICARDA/Pakistan
23	WBL-77	Mutant of BR25	Cultivar	RAU, Dholi, India
24	ILL-4605	ILL5888x ILL5782	Cultivar	USA
25	RL-49	NR9901-1-17	Nepal cross	Nepal

which causes show distinct classes and are known as the qualitative characters or oligogenic traits such as stem pigmentation color, flower color, cotyledon color etc. Likely in polygenes character governed by several genes with small individual effects usually additive which are highly influenced by the environments and they don't show any clear cut classes called as quantitative characters or plygenic traits like plant height, days to flower, days to maturity, grain yield, hundred seed weight etc. Morphological characters have been frequently used in order to know the diversity in germplasm collections [10,12-20]. Considering all these facts, the main objectives of the study was to characterize the bio-fortified lentil accessions using morphological markers and select the DUS accession for using targeted the best utilization in hybridization program.

Materials and Methods

The present study was conducted with a comprised of 25 different lentil accessions, which has a high concentration of grain Iron and Zinc, among which six lines were released varieties, 7 advanced breeding lines, 5 cultivars and five Nepalese cross lines (Table 1). Sources of accessions were from the International Center for Agricultural Research in Dry Areas (ICARDA), Shuttle breeding and SAARC trial (Bangladesh Agricultural Research Institute, Indian Institute of Pulses Research) and National Agriculture Genetic Resources Centre (Gene Bank), Nepal. These accessions were originated from SAARC countries -14 lines (Nepal-7, India-6, Bangladesh-1) and 11 ICARDA breeding lines were used for this study. A total of 25 lentil accessions were evaluated in winter season of 2016/17 in alpha lattice design (5x5) with three-replication. The unit plot size consisted of six rows of 2m length with row to row and plant to plant spacing of 25cm and continues, respectively. The recommended packages of practice were strictly followed for raising a good crop. Accessions were evaluated for 18 morphological descriptors viz. growth habit, stem color, leaf pubescence, leaflet sizes, foliage color, tendril length, flower ground color, no. of flowers per peduncle, pod pigmentation, seed sizes, seed coat color, seed coat pattern, cotyledon color, lodging susceptibility, no. of nodules/plant, cold susceptibility score, stemphylium blight score. These characters were recorded as per descriptors of IBPGR. The observations were recorded in five plants in each plot at specified stages of crop growth period when the characters under study had full of expression. Traits like Growth habit, stem color, leaf pubescence, leaflet sizes, foliage color, flower ground color, flowers per peduncle were recorded during the flower bud stage while disease scoring was done at pod formation stage. Other observations like pod pigmentation, seed sizes, seed coat color, seed coat pattern, cotyledon color were observed visually after harvesting the seeds at maturity.

Results

A total of eighteen different qualitative and quantitative traits of 25 lentil accessions were studied which are important morphological markers for keeping distinguish uniform and stable lines. Adequate amount of variation was present among the 25 accessions for eighteen important traits under study. The 25 accessions under study were classified into discrete groups under 18 major categories representing 14 qualitative characters viz. growth habit, stem color, leaf pubescence, leaflet sizes, foliage color, flower ground color, no. of flowers per peduncle, pod pigmentation, seed sizes, seed coat color, seed coat pattern and cotyledon color and 4 quantitative characters viz. lodging susceptibility, no. of nodules/plant, Inactive nodules, cold susceptibility (Table 2). On the basis of present preliminary characterization, these genotypes were grouped into different categories and may be used as a reference accession. They were classified into separate groups under each character as shown in Table 3.1 and Table 3.2.

Growth habit

On the basis of IBPGR descriptors for DUS testing in lentil, accessions have been categorized as Erect and Compact and semi-spreading based on the growth habit. Out of 25 accessions; 18 accessions were fall in erect and compact group and 7 accessions were semi-spreading type.

Stem color

Stem color is an important morphological character, varied from green to purple. Green stem color was found in three accessions and the rest 22 accessions were with purple stem. Most of the accessions originated from ICARDA were found with purple stem while the green stem color of other accessions was originated from India and Nepal.

Table 2: Qualitative and Quantitative traits used in the characterization of lentil accessions from 'Lentil Descriptors' by IBPGR (1985).

Traits	Description
Growth habit	Qualitative
Stem color	Qualitative
Leaf pubescence	Qualitative
Leaflet sizes	Qualitative
Foliage color	Qualitative
Tendril length	Qualitative
Flower ground color	Qualitative
No. of flowers per peduncle	Qualitative
Pod pigmentation	Qualitative
Seed sizes	Qualitative
Seed coat color	Qualitative
Seed coat pattern	Qualitative
Cotyledon color	Qualitative
Lodging susceptibility	Quantitative
No. of nodules /plant	Quantitative
Inactive nodules	Quantitative
Cold susceptibility	Quantitative
Stemphlium blight	Qualitative

Leaf pubescence

Leaf pubescence is also typical character containing in the lentil leaves which has distinguished the varietal characters. In general, it is categorized into three group's i.e. dense pubescence, slight pubescence and absent pubescence. Dense pubescence was found in 21 accessions likely slight pubescence was found in three accessions and no pubescence or glabrous was found in 1 accession.

Leaflet sizes

Leaflet sizes are another important key morphological character; it is generally categorized into three groups' small, medium and large leaflets sizes. Most of the lentil accessions were found small leaflets mainly originated from ICARDA. 13 different accessions had found small leaflets, while 9 accessions had large leaflet sizes and three accessions were medium leaflets sizes out of 25 accessions.

Foliage color

Among the morphological traits observed during field evaluation, the foliage colour may be considered as distinguishing, stable and uniform trait for DUS characterization. Lentil accessions have been categorized as light green and dark green and accessions under study contains both two form of foliage color. Dark green foliage was more common among the accessions under study, as 21 accessions had dark green foliage. Only four accessions observed with light green foliage type.

Tendril lengths

During crop growth and at maturity tendril keeps the plants intermingled and the canopy upright. This allows less seed loss in mechanical harvest. Depending upon the tendril lengths it may be prominent with long tendril or rudimentary with short or no tendril. Prominent tendril was found in 7 accessions and rudimentary with 18 accessions.

Flower color

Flower colors are the most important morphological traits which helps to distinguish, uniform and stable traits of any accessions. In general, there are broadly two groups of flower color pertaining in lentil i.e. blue and white with blue veins. In our study, white with blue veins flowers were observed in 3 accessions only while blue flowers which are governed by one gene were found in 22 accessions. Basically this trait is very important trait for testing hybridity segregates in crossing program and a key trait to keeping varietal purity at final level of seed production.

Number of flowers per peduncle

Number of flowers per peduncle is also a morphological trait which helps to distinguish the variety in minute level. It is categorized into two groups i.e. two flowers per peduncle and three flowers per peduncle. In our study, majority of the accessions i.e. 17 accessions had two flowers per peduncle while three flowers per peduncle was found in eight accessions.

Pod pigmentation

In general, there are two types of pod pigmentation i.e. absent or present. Pod pigmentation observation were observed during the well matured pods or harvesting stage. In our study, all accessions had absent any type of pod pigmentation.

Table 3.1: Morphological Characterization of lentil accessions based on qualitative traits as per prescribed in IBPGR.

SN	Accessions	Growth Habit	Stem Color	Leaf pubescence	Leaflet sizes	Foliage color	Tendril length	Flower ground color	No. of flowers per peduncle	Pod pigmentation	Seed size	Seed coat color	Seed Coat pattern
1	ILL-8006	Semi-spreading	Purple	Dense	Medium	Dark Green	Rudimentary	Blue	3	Absent	Medium	Dotted	Brown
2	RL-6	Erect and Compact	Purple	Dense	Small	Dark Green	Rudimentary	Blue	2	Absent	Small	Dotted	Brown
3	RL-12	Semi-spreading	Purple	Dense	Small	Dark Green	Rudimentary	Blue	3	Absent	Small	Dotted	Brown
4	ILL-7715	Erect and Compact	Purple	Dense	Small	Dark Green	Rudimentary	Blue	2	Absent	Small	Dotted	Brown
5	ILL-7164	Erect and Compact	Purple	Dense	Small	Dark Green	Rudimentary	Blue	3	Absent	Small	Dotted	Brown
6	ILL-3490	Erect and Compact	Purple	Dense	Small	Dark Green	Rudimentary	Blue	3	Absent	Small	Dotted	Brown
7	Khajura-2	Erect and Compact	Purple	Dense	Small	Dark Green	Rudimentary	Blue	2	Absent	Medium	Dotted	Brown
8	Simal	Semi-spreading	Purple	Dense	Small	Dark Green	Rudimentary	Blue	2	Absent	Small	Dotted	Brown
9	Shital	Semi-spreading	Purple	Dense	Small	Dark Green	Rudimentary	Blue	2	Absent	Small	Dotted	Brown
10	Sagun	Erect and Compact	Purple	Dense	Small	Dark Green	Rudimentary	Blue	3	Absent	Medium	Dotted	Brown
11	HUL-57	Erect and Compact	Purple	Dense	Large	Dark Green	Rudimentary	Blue	2	Absent	Medium	Dotted	Brown
12	LG-12	Erect and Compact	Purple	Slight	Large	Dark Green	Rudimentary	Blue	2	Absent	Medium	Dotted	Grey
13	PL-4	Erect and Compact	Green	Dense	Medium	Dark Green	Rudimentary	Blue	2	Absent	Large	Dotted	Brown
14	RL-11	Semi-spreading	Purple	Dense	Small	Dark Green	Prominent	Blue	3	Absent	Medium	Dotted	Brown
15	RL-4	Erect and Compact	Green	Dense	Small	Dark Green	Rudimentary	Blue	2	Absent	Medium	Dotted	Brown
16	ILL-2712	Erect and Compact	Purple	Dense	Medium	Dark Green	Rudimentary	Blue	2	Absent	Medium	Dotted	Brown
17	Black Masuro	Erect and Compact	Purple	Sparse slight	Large	Dark Green	Rudimentary	Blue	2	Absent	Medium	Absent	Black
18	RL-79	Semi-spreading	Purple	Slight	Small	Light Green	Prominent	White with blue vein	2	Absent	Medium	Marbled	Grey
19	ILL-6467	Erect and Compact	Purple	Dense	Large	Dark Green	Prominent	White with blue vein	3	Absent	Small	Marbled	Brown
20	ILL-7979	Erect and Compact	Purple	Dense	Large	Dark Green	Rudimentary	Blue	2	Absent	Medium	Spotted	Grey
21	ILL-6819	Semi-spreading	Purple	Dense	Large	Dark Green	Rudimentary	Blue	2	Absent	Small	Dotted	Brown
22	ILL-7723	Erect and Compact	Purple	Dense	Small	Light Green	Prominent	White with blue vein	3	Absent	Large	Marbled	Grey
23	WBL-77	Erect and Compact	Purple	Dense	Large	Dark Green	Prominent	Blue	2	Absent	Small	Dotted	Brown
24	ILL-4605	Erect and Compact	Purple	Dense	Large	Light Green	Prominent	Blue	2	Absent	Large	Marbled	Grey
25	RL-49	Erect and Compact	Green	Slight	Large	Light Green	Prominent	Blue	2	Absent	Large	Marbled	Grey

Seed sizes

Seed sizes are broadly classified into three group i.e. small, medium and large seeded lentil. In our study, 10 accessions were grouped into small seed size, 11 lentil accessions had medium seed sizes and 4 accessions in large seed sizes. Lentil seed sizes are grouped into two types: macrosperma, or large seeded varieties and microsperma, or small seeded varieties. Macrosperma is found mainly in the Mediterranean region and the New World (seed size ranging from 6 to 9 mm in diameter and yellow cotyledons with little or no pigmentation) and microsperma (2 to 6 mm with red orange or yellow cotyledons) is found on the Indian subcontinent, Near East and East Africa, respectively, are known [32,33]. The first one includes the Chilean or yellow cotyledon types while the latter includes the small seeded Persian or red cotyledon lentils [34].

Seed coat colour, seed coat pattern and cotyledon color

Expression of seed characters like, seed coat color, seed coat pattern and cotyledon color are uniform and stable. Therefore, these traits are better criteria for distinguishing the lentil accessions. Three classes were formed on the basis of seed coat color i.e., grey, brown and black. Among 25 accessions under study 18 accessions had brown seed coat color, 6 accessions had grey seed coat color and only single local collection observed with black color seed coat. On the basis of

Table 3.2: Morphological Characterization of lentil accessions based on qualitative traits as per prescribed in IBPGR.

Entry	Accessions	Cotyledon color	Lodging susceptibility	No. of Nodules / plant	Inactive Nodules	Cold susceptibility (1-9 scale)	Stemphylium blight (1-9 scale)
1	ILL-8006	Red	High	10.5	4	5	3
2	RL-6	Red	High	21.5	4.5	5	5
3	RL-12	Red	High	10	3	5	4
4	ILL-7715	Red	High	19	4.5	7	3
5	ILL-7164	Red	High	20.5	6.5	5	3
6	ILL-3490	Red	High	13	3.5	5	3
7	Khajura-2	Red	High	20.5	4.5	5	3
8	Simal	Red	Medium	3.5	2	7	3
9	Shital	Red	High	16	4.5	5	7
10	Sagun	Red	High	21.5	7.5	5	1
11	HUL-57	Red	High	10.5	4	1	3
12	LG-12	Red	High	24.5	7.5	1	3
13	PL-4	Red	High	17	7	5	3
14	RL-11	Red	High	11	4	1	3
15	RL-4	Red	High	21.5	7.5	5	3
16	ILL-2712	Red	High	17.5	4.5	1	3
17	Black Masuro	Red	High	12.5	4.5	7	1
18	RL-79	Red	High	11.5	4	1	1
19	ILL-6467	Red	High	15	5	1	1
20	ILL-7979	Red	High	15	6	1	1
21	ILL-6819	Red	High	27	9.5	1	3
22	ILL-7723	Red	Medium	16.5	6	1	3
23	WBL-77	Red	Medium	10.5	3.5	1	3
24	ILL-4605	Red	High	17	5	1	2
25	RL-49	Red	High	22	6.5	1	3

seed coat pattern, four types of classes were formed based on spotted, dotted, marbled and absent. Out of 25 accessions, 19 accessions had dotted, five accessions had marbled, 1 accession spotted and 1 accession absent.

Cotyledon color

Visual assessment for cotyledon color was done one month after harvesting the seeds and can be categorized in three different classes as per IBPGR DUS guidelines i.e. Red, Yellow, Green, Black. Genotypes under study were classified into two categories i.e. yellow, and red. Almost all genotypes had found red color cotyledons.

Number of nodules per plant

Number of nodules per plant determines the capacity of biological nitrogen fixation by the lentil variety in the soil. It can be assumed that higher the number of nodules will have more nitrogen fixation which supply to the plants itself and provide more yield and also high protein content in the seed. Generally, there are categorized in three groups of nodules per plant i.e. very high, fair and poor. In our study, 8 accessions had the very high nodules per plant, 16 accessions had fair nodules per plant and 1 accession had poor nodules per plant.

Stemphylium blight disease

Lentil stemphylium blight is one of the major foliar diseases which cause substantial yield losses in lentil. In Nepal it was first reported during 1993. In Bangladesh and India estimated yield losses of 62% (Bakr 1991, Erksine and Sarker 1997). Nepalgunj is a hot spot for lentil stemphylium and the symptoms of stemphylium are visible on all plant parts. Hence, stemphylium scoring of accessions under study were done at field level and on the basis of stemphylium disease score accessions on a 1-9 scale under study were classified into four groups i.e. highly resistant, resistant, moderately resistant and moderately susceptible. Disease score was recorded before flowering and after flowering as followed by the Chen, 2007. Highly resistant group consisted of 6 accessions, 17 accessions were resistant out of 25, and rest 1 accession was moderately resistant and 1 accession showed moderately susceptibility for stemphylium blight.

Fusarium wilt disease

Likewise, fusarium wilt was also serious disease in low land growing lentils where it is prevailing soil moisture stress. Based on the scale disease (1-9 score) contrary same as stemphylium blight. In our study, 4 accessions were found highly resistant to fusarium wilt disease, 16 accessions were resistant and 5 accessions were moderately resistant.

Lodging susceptibility

Lodging susceptibility is in fact quantitative trait that determined

Table 4: Classification of 25 lentil accessions into distinct groups based on qualitative characters.

Traits		Name of accessions	No. of Accessions
Stemphylium blight	1(No infection)	Sagun, Black Masuro, RL-79, ILL-6467, ILL-7979, ILL-4605	6
	3(Resistant)	ILL-2712, ILL-6819, ILL-7723, ILL-8006, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, HUL-57, LG-12, PL-4, RL-11, RL-4, WBL-77, RL-49, RL-12	17
	5(Moderately Resistant)	RL-6	1
	7(Moderately Susceptible)	Shital	1
Fusarium wilt	1(No infection)	LG-12, Black Masuro, ILL-6467, WBL-77	4
	3(Resistant)	RL-6, RL-12, ILL-7715, ILL-7164, Shital, Sagun, HUL-57, PL-4, RL-11, RL-4, RL-79, ILL-7979, ILL-6819, ILL-7723, ILL-4605, RL-49	16
	5(Moderately Resistant)	ILL-8006, ILL-3490, Khajura-2, Simal, ILL-2712	5
Growth habit	Erect and Compact	RL-6, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Sagun, HUL-57, LG-12, PL-4, RL-4, ILL-2712, Black Masuro, ILL-6467, ILL-7979, ILL-7723, WBL-77, ILL-4605, RL-49	18
	Semi-spreading	ILL-8006, RL-12, Simal, Shital, RL-11, RL-79, ILL-6819	7
Stem color	Green	PL-4, RL-4, RL-49	3
	Purple	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, RL-11, ILL-2712, Black Masuro, RL-79, ILL-6467, ILL-7979, ILL-6819, ILL-7723, WBL-77, ILL-4605	22
Leaf pubescence	Absent	Black Masuro	1
	Slight	LG-12, RL-79, RL-49	3
	Dense	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, PL-4, RL-11, RL-4, ILL-2712, ILL-6467, ILL-7979, ILL-6819, ILL-7723, WBL-77, ILL-4605	21
Leaflet sizes	Small	RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, RL-11, RL-4, RL-79, ILL-7723	13
	Medium	ILL-8006, PL-4, ILL-2712	3
	Large	HUL-57, LG-12, Black Masuro, ILL-6467, ILL-7979, ILL-6819, WBL-77, ILL-4605, RL-49	9
Foliage color	Dark Green	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, PL-4, RL-11, RL-4, ILL-2712, Black Masuro, ILL-6467, ILL-7979, ILL-6819, WBL-77	21
	Light Green	RL-79, ILL-7723, ILL-4605, RL-49	4
Tendril length	Rudimentary	ILL-7979, ILL-6819, ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, PL-4, RL-4,ILL-2712, Black Masuro	18
	Prominenet	RL-11, RL-79, ILL-6467, ILL-7723, WBL-77, ILL-4605, RL-49	7
Flower color	Blue(One gene)	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, PL-4, RL-11, RL-4, ILL-2712, Black Masuro, ILL-7979, ILL-6819, WBL-77, ILL-4605, RL-49	22
	White with blue veins	RL-79, ILL-7723, ILL-6467	3
No. of flowers per peduncle	2 flowers per peduncle	RL-6, ILL-7715, Khajura-2, Simal, Shital, HUL-57, LG-12, PL-4, RL-4, ILL-2712, Black Masuro, RL-79, ILL-7979, ILL-6819, WBL-77, ILL-4605, RL-49	17
	3 flowers per peduncle	ILL-8006, RL-12, ILL-7164, ILL-3490, Sagun, RL-11, ILL-6467, ILL-7723	8
Pod pigmentation	Absent	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164 ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, PL-4, RL-11, RL-4,ILL-2712, Black Masuro, RL-79, ILL-6467, ILL-7979, ILL-6819, ILL-7723, WBL-77, ILL-4605, RL-49	25
Seed sizes	Small	RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Simal, Shital, ILL-6467, ILL-6819, WBL-77	10
	Medium	ILL-8006, Khajura-2, Sagun, HUL-57, LG-12,RL-11, RL-4, ILL-2712, Black Masuro, RL-79, ILL-7979	11
	Large	PL-4, ILL-7723, ILL-4605, RL-49	4
Seed coat pattern	Spotted	ILL 7979	1
	Dotted	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, PL-4, RL-11, RL-4, ILL-2712, ILL-6819, WBL-77, RL-79	19
	Marbled	RL-79, ILL-6467, ILL-7723, ILL-4605, RL-49	5
	Absent	Black Masuro	1
Seed Coat color	Grey	LG-12, RL-79, ILL-7979, ILL-7723, ILL-4605, RL-49	6
	Brown	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, PL-4, RL-11, RL-4, ILL-2712, ILL-6467, ILL-6819, WBL-77	18
	Black	Black Masuro	1

Cotyledon color (Red, Yellow, Green, Black)	Red	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164 ILL-3490, Khajura-2, Simal, Shital, Sagun, HUL-57, LG-12, PL-4, RL-11, RL-4,ILL-2712, Black Masuro, RL-79, ILL-6467, ILL-7979, ILL-6819, ILL-7723, WBL-77, ILL-4605, RL-49	25
Lodging susceptibility	None		
	Low		
	Medium	Simal, ILL-7723, WBL-77	3
	High	ILL-8006, RL-6, RL-12, ILL-7715, ILL-7164, ILL-3490, Khajura-2, Shital, Sagun, HUL-57, LG-12, PL-4, RL-11, RL-4, ILL-2712, Black Masuro, RL-79, ILL-6467, ILL-7979, ILL-6819, ILL-4605, RL-49	22
No. of Nodules /plant	Very good(>20-30)	ILL-6819, LG-12, RL-49, RL-6, Sagun, RL-4, ILL-7164, Khajura-2	8
	Fair(10-20)	ILL-7715, ILL-2712, PL-4, ILL-4605, ILL-7723, Shital, ILL-6467, ILL-7979, ILL-3490, Black Masuro, RL-79, RL-11, ILL-8006, HUL-57, WBL-77, RL-12	16
	Poor(<10)	Simal	1
Cold susceptibility (1-9 scale)	Resistant	HUL-57, LG-12, RL-11, ILL-2712, RL-79, ILL-6467, ILL-7979, ILL-6819, ILL-7723, WBL-77, ILL-4605, RL-49	12
	Moderately resistant	ILL-8006, RL-6, RL-12, ILL-7164, ILL-3490, Khajura-2, Shital, Sagun, PL-4, RL-4	10
	Susceptible	ILL-7715,Simal, Black Masuro	3

by the environmental influences, planting time and soil fertility. This is quantified into three group i.e. low, medium and high susceptibility. Most of the accessions i.e. 22 accessions were high lodging susceptibility and three accessions had medium susceptibility.

Cold susceptibility

Cold susceptibility is also showed the symptoms during the seedling stage of cold climate which causes turns into reddish brown plant foliage observed in the field. In fact, reddish foliage means the symptoms of phosphorus deficiency particularly seen in those areas where more calcium carbonate adheres in the soil. In mid and high hills can also be encountered in the lentil field. Cold susceptibility is categorized in three classes, in our study, 12 accessions were found resistant to cold climate, 10 accessions were moderately resistant and three accessions were found susceptible to the cold climate.

Discussion

Growth habit supports the observations of [21], and [22]. This trait is monogenically governed with dominant expression of the tall erect plant habit [23]. 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.

It is reported that based on the leaf pubescences, any accession can be identified either it is insect resistant or susceptible. Sometimes its pubescence works as the barrier to direct invade the crops.

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 color 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 [24]. It was reported that foliage color is controlled by single gene with dominance of dark green foliage over light green foliage [25].

It can be assumed that the higher the number of flowers per peduncle means higher the numbers of pod setting and finally produces more yield than two flowers bearer. Testa colour and testa mottling are found to be the most stable and uniform traits for certification of genetic purity of genotypes at four level as their expressions are least influenced by environment. Hence, these two traits in combination can be utilized to characterize the lentil accessions.

Cotyledon colour was controlled by single gene hence; trait is less influenced by the environment [26-29]. 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. Cotyledon with red colour in lentil is preferred in Bangladesh, India, Nepal and other countries of Ethiopia, Eritrea, Sudan, Egypt, Turkey, Syria, etc. Likely yellow cotyledon lentil is preferred by the consumers of North Africa, Central Asia and Caucasus countries. Therefore, cotyledon colour might be a valuable trait for breeding purpose because of the consumers' preferences and its value chain in the international market.

It is said that in Root rot complex of lentil the main pathogens associated with plant mortality were *Fusarium oxysporum f.sp. Lentis* (62.0%), *Rhizoctonia bataticola* (25.2%) and *Sclerotium rolfsii* (9.8%) in India [30]. Screening of lentil breeding lines for resistance to fusarium wilt found that yield losses can range from 25-95 % depending on the variety [31]. It might be both soil and seed borne pathogen. At seedling stage, sudden drooping of the leaves followed by the leaves drying and the eventual death of the seedling. At reproductive stage drooping and wilting of the uppermost leaflets and finally become completely yellow and die.

Conclusion

Plant breeders can use these morphological 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 color, testa color, testa pattern and cotyledon colors can also be used for testing hybridity

and keeping genetic purity at genetic level. 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 program.

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