Evaluation of a lentil collection (*Lens culinaris* Medik) using morphological traits and digital phenotyping

Evaluación de una colección de lentejas (*Lens culinaris* Medik) utilizando caracteres morfológicos y fenotipado digital

María Andrea Espósito [1, 2], Ileana Gatti [2, 3], Carolina Julieta Bermejo [2], Enrique Luis Cointry [2]

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Abstract

The objective of this work was to evaluate 81 lentil cultivars using morphological traits and seed characteristics by digital phenotyping. Caliber (C) and the color traits luminosity (L), color coordinates a and b, and color index (CI) were measured and analyzed with appropriate software. Additionally, also yield (Y), plant height (PH) and days to flowering (DF) were measured. Highly significant differences between cultivars were found for all traits, while high broad sense heritability (H^2B) for C (97%), CI (94%), a (93%) and L and b (83%) were found, indicating high genetic variability for these traits. Digital phenotyping showed to be a powerful tool for germplasm characterization along with field evaluation of agronomical traits. Principal Component Analysis and Cluster Analysis allows de identification of differentiated groups of cultivars with similar characteristics, leading to a more efficient use of the germplasm available as commercial cultivars or as parents in a breeding program. Among these groups, group 1 had 32 cultivars with highest C and group 2 had 21 cultivars with higher Y.

Keywords
lentil • digital phenotyping • morphological characterization

1 EEA INTA Oliveros. Ruta Nacional 11. Km 353. C. P. 2206. mesposi@unr.edu.ar
2 Universidad Nacional de Rosario. Facultad Ciencias Agrarias. Cátedra Mejoramiento Vegetal y Producción de Semillas. IICAR-CONICET. Campo Experimental. Villarino s/n. Zavalla. C.C. 14 (S2125ZAA).
3 Consejo de Investigaciones UNR (CIUNR). Universidad Nacional de Rosario.

Resumen

El objetivo de este trabajo fue evaluar 81 cultivares de lenteja usando caracteres morfológicos y características de semilla utilizando fenotipado digital. El Calibre (C) y los caracteres Luminosidad (L), las coordenadas de color a y b, y el índice de color (IC) fueron medidos y analizados con un software apropiado; también fueron medidos el rendimiento (Y), altura de planta (PH) y los días a floración (DF). Se encontraron diferencias altamente significativas entre cultivares para todos los caracteres y se obtuvieron elevados valores de heredabilidad en sentido amplio (H^2B) para las variables C (97%), IC (94%), a (93%) y L y b (83%) indicando la presencia de alta variabilidad genética. El fenotipado digital mostró ser una poderosa herramienta para la caracterización de germoplasma junto con la evaluación a campo de caracteres agronómicos. El Análisis de Componentes Principales y el análisis de agrupamiento permitieron la identificación de diferentes grupos de cultivares con características similares lo que conduce a un uso más eficiente del germoplasma disponible como cultivares comerciales o como parentales en un programa de mejoramiento genético. Entre estos grupos, el grupo 1 tuvo 32 cultivares con mayor C y el grupo 2 tuvo 21 cultivares con mayor Y.

Palabras clave
lenteja • fenotipado digital • caracterización morfológica

Introduction

Lentil (*Lens culinaris Medik. ssp. culinaris*) is one of the most ancient crops in history (McVicar *et al.*, 2005). This cool season pulse, used in human nutrition as whole grain or flour, is an excellent source of dietary fiber, protein, healthy fat, carbohydrates and a range of micronutrients (Thavarajah *et al.*, 2011). Its high levels of low digestible carbohydrates reduces glycemic response in humans (Siva *et al.*, 2017) and its high fiber content gives it strong satiating properties, resulting in lower food intake (Faris *et al.*, 2013). This pulse is also a significant dietary source of a plethora of vitamins including folate, thiamin (B1) and riboflavin (B2) and relatively high levels of Mg, P, Ca and S (16).

All these characteristics make lentil a fundamental dietary component in low-income population and developing countries, as it is a substitute to proteins from livestock and fisheries (7) and have beneficial effects on human health (9).

The main consumer countries are those from Asia, north of Africa, Western Europe and part of Latin America. There are several market classes based on consumer preference, seed size and color. According to seed size the classes can be extra small (29-32 g/1000 seeds), small (33-45 g/1000 seeds), medium (51-52 g/1000 seeds) and large (55-73 g/1000 seeds). According to seed coat color they can be classified in green, brown, gray and purple or black; with cotyledon colors ranging from yellow to red and green (8).

In Argentina, lentil is cultivated mostly in the central area (south of the province of Santa Fe and north of the province of Buenos Aires), where is an important rainfed crop during the winter season (2). The main problem for growers is the lack of available cultivars, as only two commercial varieties are used in the present.

To solve this inconvenient, a breeding program is being carried out in the National University of Rosario, with the objective of obtaining new cultivars with higher yield and suitability for the different export markets.

A first step in any breeding program is to evaluate the variability of the germplasm available in the working collection using traits with agronomical importance. The characterization of traits whit high heritability and the evaluation of traits with low heritability and highly influenced by the environment can determine the utilization of this germplasm (3, 4).

The aim of the present work was to evaluate the genetic variability of a lentil collection for agronomical traits and seed characteristics (size and color) for later selection of accessions for commercial use or as parents in the breeding program. Seed traits where evaluated using digital phenotyping, performed by non-destructive, automated and image-based technology that offers an objective and quantitative method for estimation of morphological parameters as color and size.

Materials and Methods

Plant material and experimental design

Eighty one accessions of a working collection (table 1, page 4) were sowed in July of 2016, in plots of 3 m long and 3 rows 0.25 apart (approximately 200 plants) at the Experimental Field of the College of Agricultural Sciences, Rosario National University, located in Zavalla (33°1' S and 60°53' W) in a complete randomized design with three replications. The harvest was done manually.

Traits analyzed

The analyzed variables were days to 50% of flowering (DF); plant height (PH), (cm from the root, in 20 plants per plot) and yield (Y grams per plot). Color traits and seed caliber (C) were measured on two-dimensional digital images of 600 dpi taken on a Samsung CLX 3300 scanner of samples of 50 seeds per repetition and analyzed using Tomato Analyzer (TA) software (12).

The color traits were the coordinates *a* and *b*, and the psychometric index of lightness L from the CieLab system of color where:

- Coordinate *a* indicates the greenness-redness of the color (-*a* is green, +*a* is red) and varies between -128 and 128.
- Coordinate *b* indicates blueness-yellowness of the color (-*b* is blue, +*b* is yellow) and varies between -128 and 128.
- Parameter L is an approximate measurement of luminosity, the property according to which each color can be considered as equivalent to a member of the greyscale between black and white.

With these color parameters, a color index (CI) was calculated as: CI = (1.000 x a) / (L x b).

Statistical analysis

An ANOVA between cultivars and a comparison of means using the Fisher's least significant difference test (LSD) (14) were performed.

Table 1. Name and country of origin of the evaluated cultivars.

Tabla 1. Nombre y país de origen de los cultivares evaluados.

Cultivar	Country	Cultivar	Country
1A	Lebanon	25A	Lebanon
1R	Lebanon	26A	Lebanon
2A	Lebanon	26R	Lebanon
3A	Lebanon	27A	Lebanon
3R	Lebanon	27R	Lebanon
4A	Lebanon	28A	Lebanon
4R	Lebanon	28R	Lebanon
5A	Lebanon	29A	Lebanon
6R	Lebanon	29R	Lebanon
7A	Lebanon	30A	Lebanon
7R	Lebanon	30R	Lebanon
8A	Lebanon	31R	Lebanon
9A	Lebanon	32A	Lebanon
9R	Lebanon	32R	Lebanon
10A	Lebanon	33A	Lebanon
10R	Lebanon	33R	Lebanon
11A	Lebanon	34A	Lebanon
12A	Lebanon	34R	Lebanon
12R	Lebanon	35A	Lebanon
13R	Lebanon	35R	Lebanon
14A	Lebanon	A1062	Argentina
14R	Lebanon	A1145	Argentina
15A	Lebanon	B1051	Argentina
15R	Lebanon	B1052	Argentina
16A	Lebanon	B1053	Argentina
16R	Lebanon	B1054	Argentina
17A	Lebanon	B1055	Argentina
17R	Lebanon	B1056	Argentina
18A	Lebanon	B1151	Argentina
18R	Lebanon	B1153	Argentina
19A	Lebanon	B1156	Argentina
19R	Lebanon	B1157	Argentina
20A	Lebanon	B1181	Argentina
20R	Lebanon	B1182	Argentina
21A	Lebanon	NAILE	Canada
22A	Lebanon	PARDINA	Spain
22R	Lebanon	ROSE TOWN	Canada
23A	Lebanon	SEL 133	Argentina
23R	Lebanon	SEL 205	Argentina
24A	Lebanon	SILVINA	Argentina
24R	Lebanon		

Broad sense heritability was calculated as

$$\mathbf{H^2B = \sigma^2 g/(\sigma^2 g+\sigma^2 e)}$$

where:
σ^2g = represents genotypic variance
σ^2e = represents the environmental variance.

Finally, a Principal Component analysis (PC) and a Cluster analysis using average linkage method with Euclidean distances were performed in order to identify groups of cultivars with similar characteristics.

All statistical analysis were made using the software InfoStat for Windows (1).

Results and discussion

Genetic variability

Mean value, standard deviation (SD), F value for the ANOVA analysis, Fisher's least significant difference value (LSD) and broad sense heritability (H^2B) for all the traits are shown in table 2, page 5-6; table 3, page 7-8. All traits presented highly significant differences between cultivars ($p<0.001$), and broad sense heritability varied between 0.33 for DF to 0.97 for C, demonstrating the existence of genetic variability suitable for selection purposes. Bermejo *et al.* (2012) in the evaluation of 28 lentil RIL's; Lázaro *et al.* (2001) in a working collection of Spanish materials and Erskine *et al.* (1998) in collections from ICARDA (International Center for Agricultural Research in the Dry Areas) found similar values of H^2B.

Regarding mean values, for DF, cultivars 7A, Rose Town and Pardina were late (104 and 105 DF) while cultivar 34A was the earliest (57 DF).

Table 2. Mean, standard deviation (SD), LSD value, F value and broad sense heritability (H^2B) for days to flowering (DF), plant height (PH), yield (Y) and seed caliber (C).

Tabla 2. Media, desviación estándar (SD), valor de LSD, valor de F y Heredabilidad en sentido amplio (H^2B) para los caracteres días a floración (DF), altura de planta (PH), rendimiento (Y) y calibre de grano (C).

	DF		PH (cm)		Y (g)		C (mm)	
Cultivar	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
10A	99.5	6.4	29.0	4.2	123.0	38.2	7	0.00
10R	98.0	8.5	28.0	5.7	72.5	29.0	5	0.01
11A	98.0	8.5	29.0	9.9	173.5	13.4	7	0.01
12A	98.0	8.5	33.0	5.7	116.5	72.8	7	0.00
12R	98.0	8.5	25.0	2.8	185.0	131.5	5	0.01
13R	94.0	2.8	34.5	6.4	86.0	48.1	6	0.01
14A	101.0	7.1	36.0	5.7	97.0	1.4	7	0.01
14R	95.5	0.7	26.0	2.8	54.5	57.3	6	0.00
15A	93.5	3.5	31.5	5.0	138.5	14.9	7	0.00
15R	92.5	5.0	28.5	2.1	98.5	12.0	6	0.01
16A	88.0	11.3	24.5	0.7	206.5	41.7	6	0.00
16R	98.0	2.8	21.0	4.2	66.0	33.9	6	0.00
17A	82.0	2.8	28.5	5.0	88.0	46.7	8	0.01
17R	88.0	5.7	26.0	1.4	58.5	23.3	6	0.01
18A	98.0	2.8	25.5	3.5	100.5	14.9	6	0.01
18R	86.5	3.5	32.0	9.9	66.0	18.4	5	0.01
19A	95.0	4.2	34.0	5.7	64.5	47.4	7	0.01
19R	90.0	2.8	29.5	3.5	67.5	24.8	5	0.01
1A	101.5	0.7	29.5	0.7	69.0	12.7	7	0.00
1R	87.5	0.7	29.5	0.7	43.5	5.0	6	0.02
20A	98.5	2.1	30.0	4.2	49.0	26.9	7	0.01
20R	94.5	0.7	31.5	0.7	81.0	49.5	6	0.01
21A	97.5	0.7	34.5	0.7	126.5	2.1	7	0.00
22A	87.5	20.5	32.5	0.7	101.0	1.4	7	0.04
22R	95.5	0.7	29.5	0.7	90.5	5.0	6	0.01
23A	87.5	0.7	29.5	0.7	105.0	9.9	7	0.01
23R	91.5	0.7	39.5	0.7	117.5	6.4	6	0.02
24A	93.5	0.7	26.5	2.1	104.0	19.8	7	0.01
24R	99.0	1.4	32.0	11.3	77.5	3.5	6	0.01
25A	97.5	0.7	28.0	1.4	136.5	44.6	7	0.04
26A	94.0	1.4	26.0	1.4	92.5	20.5	7	0.01
26R	87.5	0.7	27.5	0.7	114.5	2.1	6	0.02
27A	100.5	0.7	29.0	7.1	143.0	72.1	7	0.00
27R	90.5	0.7	31.0	1.4	80.5	30.4	6	0.03
28A	91.5	0.7	37.0	2.8	31.5	31.8	6	0.01
28R	94.5	0.7	28.5	0.7	94.5	5.0	4	0.00
29A	102.0	5.7	31.0	7.1	156.5	9.2	7	0.00
29R	92.0	1.4	28.5	5.0	99.5	47.4	6	0.00
2A	102.5	0.7	31.0	1.4	162.0	45.3	7	0.01
30A	92.0	1.4	32.5	3.5	86.0	0.0	7	0.01
30R	91.5	0.7	30.5	3.5	175.5	21.9	6	0.00
31R	76.5	29.0	28.0	2.8	175.0	56.6	6	0.02

Table 2 (cont.). Mean, standard deviation (SD), LSD value, F value and broad sense heritability (H^2B) for days to flowering (DF), plant height (PH), yield (Y) and seed caliber (C).

Tabla 2 (cont.). Media, desviación estándar (SD), valor de LSD, valor de F y Heredabilidad en sentido amplio (H^2B) para los caracteres días a floración (DF), altura de planta (PH), rendimiento (Y) y calibre de grano (C).

	DF		PH (cm)		Y (g)		C (mm)	
Cultivar	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
10A	99.5	6.4	29.0	4.2	123.0	38.2	7	0.00
10R	98.0	8.5	28.0	5.7	72.5	29.0	5	0.01
11A	98.0	8.5	29.0	9.9	173.5	13.4	7	0.01
12A	98.0	8.5	33.0	5.7	116.5	72.8	7	0.00
12R	98.0	8.5	25.0	2.8	185.0	131.5	5	0.01
13R	94.0	2.8	34.5	6.4	86.0	48.1	6	0.01
14A	101.0	7.1	36.0	5.7	97.0	1.4	7	0.01
14R	95.5	0.7	26.0	2.8	54.5	57.3	6	0.00
15A	93.5	3.5	31.5	5.0	138.5	14.9	7	0.00
15R	92.5	5.0	28.5	2.1	98.5	12.0	6	0.01
16A	88.0	11.3	24.5	0.7	206.5	41.7	6	0.00
16R	98.0	2.8	21.0	4.2	66.0	33.9	6	0.00
17A	82.0	2.8	28.5	5.0	88.0	46.7	8	0.01
17R	88.0	5.7	26.0	1.4	58.5	23.3	6	0.01
18A	98.0	2.8	25.5	3.5	100.5	14.9	6	0.01
18R	86.5	3.5	32.0	9.9	66.0	18.4	5	0.01
19A	95.0	4.2	34.0	5.7	64.5	47.4	7	0.01
19R	90.0	2.8	29.5	3.5	67.5	24.8	5	0.01
1A	101.5	0.7	29.5	0.7	69.0	12.7	7	0.00
1R	87.5	0.7	29.5	0.7	43.5	5.0	6	0.02
20A	98.5	2.1	30.0	4.2	49.0	26.9	7	0.01
20R	94.5	0.7	31.5	0.7	81.0	49.5	6	0.01
21A	97.5	0.7	34.5	0.7	126.5	2.1	7	0.00
22A	87.5	20.5	32.5	0.7	101.0	1.4	7	0.04
22R	95.5	0.7	29.5	0.7	90.5	5.0	6	0.01
23A	87.5	0.7	29.5	0.7	105.0	9.9	7	0.01
23R	91.5	0.7	39.5	0.7	117.5	6.4	6	0.02
24A	93.5	0.7	26.5	2.1	104.0	19.8	7	0.01
24R	99.0	1.4	32.0	11.3	77.5	3.5	6	0.01
25A	97.5	0.7	28.0	1.4	136.5	44.6	7	0.04
26A	94.0	1.4	26.0	1.4	92.5	20.5	7	0.01
26R	87.5	0.7	27.5	0.7	114.5	2.1	6	0.02
27A	100.5	0.7	29.0	7.1	143.0	72.1	7	0.00
27R	90.5	0.7	31.0	1.4	80.5	30.4	6	0.03
28A	91.5	0.7	37.0	2.8	31.5	31.8	6	0.01
28R	94.5	0.7	28.5	0.7	94.5	5.0	4	0.00
29A	102.0	5.7	31.0	7.1	156.5	9.2	7	0.00
29R	92.0	1.4	28.5	5.0	99.5	47.4	6	0.00
2A	102.5	0.7	31.0	1.4	162.0	45.3	7	0.01
30A	92.0	1.4	32.5	3.5	86.0	0.0	7	0.01
30R	91.5	0.7	30.5	3.5	175.5	21.9	6	0.00
31R	76.5	29.0	28.0	2.8	175.0	56.6	6	0.02

*** significant with $p<0.001$. / *** diferencias significativas con $p<0,001$.

Table 3. Mean, standard deviation (SD), LSD value, F value and broad sense heritability (H^2B) for L, *a* and *b* coordinates of color and color index (CI).

Tabla 3. Media, desviación estándar (SD), valor de LSD, valor de F y Heredabilidad en sentido amplio (H^2B) para los caracteres L, las coordenadas de color a y b y el índice de color (CI).

	L		a		b		CI	
Cultivar	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
10A	55.2	1.1	9.6	0.6	20.7	0.6	8.4	0.1
10R	53.4	1.6	15.0	0.2	23.2	0.0	12.2	0.6
11A	52.3	1.4	8.6	0.7	19.2	0.1	8.6	1.0
12A	52.9	1.5	10.2	0.5	20.8	0.3	9.3	0.6
12R	58.2	1.3	10.0	0.8	22.5	0.8	7.7	1.1
13R	50.4	1.4	16.5	0.2	22.0	0.6	14.8	0.6
14A	54.7	0.9	10.0	0.6	21.1	0.5	8.6	0.5
14R	47.1	0.7	11.3	0.5	17.3	0.1	13.9	0.9
15A	46.1	0.8	11.2	0.3	17.7	0.1	13.8	0.5
15R	47.8	0.2	11.8	0.3	20.5	0.6	12.1	0.0
16A	54.3	0.1	8.0	0.8	19.6	0.1	7.6	0.7
16R	54.3	0.1	8.0	0.8	19.6	0.1	7.6	0.7
17A	51.7	2.3	9.9	0.9	19.0	0.3	10.1	1.2
17R	44.8	2.4	12.8	1.0	19.6	0.4	15.4	3.0
18A	51.3	0.3	3.8	0.2	16.7	0.2	4.4	0.3
18R	63.4	18.1	7.0	3.3	15.1	7.1	7.6	2.2
19A	53.1	1.0	10.3	0.1	20.5	0.1	9.4	0.2
19R	40.9	0.8	15.1	0.6	21.7	0.1	17.1	1.0
1A	53.8	2.1	9.2	0.8	20.4	0.4	8.4	0.9
1R	46.8	0.7	9.1	0.2	13.6	0.2	14.4	0.7
20A	53.3	0.4	10.5	0.1	20.1	0.3	9.8	0.0
20R	55.9	0.8	5.3	0.2	18.3	0.2	5.2	0.0
21A	53.0	0.3	8.6	0.4	20.0	0.3	8.1	0.2
22A	49.0	0.8	11.2	0.8	19.6	0.6	11.7	0.6
22R	44.7	2.2	12.6	1.1	19.5	0.4	14.5	1.7
23A	54.8	1.1	7.4	0.0	19.7	0.1	6.9	0.2
23R	54.6	1.5	8.1	0.3	18.2	0.5	8.1	0.7
24A	55.8	0.8	7.2	0.8	19.5	0.3	6.6	0.7
24R	45.9	0.7	9.3	0.2	14.2	0.1	14.5	0.6
25A	52.8	0.8	8.2	0.2	19.4	0.3	8.0	0.2
26A	55.0	1.0	6.3	0.3	18.6	0.5	6.1	0.0
26R	44.6	0.9	12.4	0.1	19.9	0.3	14.0	0.3
27A	53.9	0.7	9.1	0.3	18.7	0.2	9.0	0.1
27R	54.0	1.6	8.5	1.6	19.1	0.7	8.2	1.5
28A	47.1	1.1	12.1	1.0	19.4	0.7	13.2	0.9
28R	45.2	0.5	8.6	0.1	15.3	0.1	12.5	0.3
29A	48.7	1.7	11.6	0.9	19.5	0.1	12.3	1.3
29R	49.1	1.6	11.5	1.2	20.9	0.4	11.2	1.3
2A	50.1	0.7	3.7	0.2	16.0	0.1	4.6	0.2
30A	49.0	0.2	11.3	0.2	19.3	0.1	11.9	0.1
30R	44.5	1.2	11.5	0.6	19.3	0.9	13.5	1.6
31R	53.3	1.4	8.7	0.7	20.3	0.1	8.1	0.9

*** significant with p<0.001. / *** diferencias significativas con p<0,001.

Table 3. (cont.). Mean, standard deviation (SD), LSD value, F value and broad sense heritability (H^2B) for L, *a* and *b* coordinates of color and color index (CI).

Tabla 3. (cont.). Media, desviación estándar (SD), valor de LSD, valor de F y Heredabilidad en sentido amplio (H^2B) para los caracteres L, las coordenadas de color a y b y el índice de color (CI).

	L		a		b		CI	
Cultivar	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
10A	55.2	1.1	9.6	0.6	20.7	0.6	8.4	0.1
10R	53.4	1.6	15.0	0.2	23.2	0.0	12.2	0.6
11A	52.3	1.4	8.6	0.7	19.2	0.1	8.6	1.0
12A	52.9	1.5	10.2	0.5	20.8	0.3	9.3	0.6
12R	58.2	1.3	10.0	0.8	22.5	0.8	7.7	1.1
13R	50.4	1.4	16.5	0.2	22.0	0.6	14.8	0.6
14A	54.7	0.9	10.0	0.6	21.1	0.5	8.6	0.5
14R	47.1	0.7	11.3	0.5	17.3	0.1	13.9	0.9
15A	46.1	0.8	11.2	0.3	17.7	0.1	13.8	0.5
15R	47.8	0.2	11.8	0.3	20.5	0.6	12.1	0.0
16A	54.3	0.1	8.0	0.8	19.6	0.1	7.6	0.7
16R	54.3	0.1	8.0	0.8	19.6	0.1	7.6	0.7
17A	51.7	2.3	9.9	0.9	19.0	0.3	10.1	1.2
17R	44.8	2.4	12.8	1.0	19.6	0.4	15.4	3.0
18A	51.3	0.3	3.8	0.2	16.7	0.2	4.4	0.3
18R	63.4	18.1	7.0	3.3	15.1	7.1	7.6	2.2
19A	53.1	1.0	10.3	0.1	20.5	0.1	9.4	0.2
19R	40.9	0.8	15.1	0.6	21.7	0.1	17.1	1.0
1A	53.8	2.1	9.2	0.8	20.4	0.4	8.4	0.9
1R	46.8	0.7	9.1	0.2	13.6	0.2	14.4	0.7
20A	53.3	0.4	10.5	0.1	20.1	0.3	9.8	0.0
20R	55.9	0.8	5.3	0.2	18.3	0.2	5.2	0.0
21A	53.0	0.3	8.6	0.4	20.0	0.3	8.1	0.2
22A	49.0	0.8	11.2	0.8	19.6	0.6	11.7	0.6
22R	44.7	2.2	12.6	1.1	19.5	0.4	14.5	1.7
23A	54.8	1.1	7.4	0.0	19.7	0.1	6.9	0.2
23R	54.6	1.5	8.1	0.3	18.2	0.5	8.1	0.7
24A	55.8	0.8	7.2	0.8	19.5	0.3	6.6	0.7
24R	45.9	0.7	9.3	0.2	14.2	0.1	14.5	0.6
25A	52.8	0.8	8.2	0.2	19.4	0.3	8.0	0.2
26A	55.0	1.0	6.3	0.3	18.6	0.5	6.1	0.0
26R	44.6	0.9	12.4	0.1	19.9	0.3	14.0	0.3
27A	53.9	0.7	9.1	0.3	18.7	0.2	9.0	0.1
27R	54.0	1.6	8.5	1.6	19.1	0.7	8.2	1.5
28A	47.1	1.1	12.1	1.0	19.4	0.7	13.2	0.9
28R	45.2	0.5	8.6	0.1	15.3	0.1	12.5	0.3
29A	48.7	1.7	11.6	0.9	19.5	0.1	12.3	1.3
29R	49.1	1.6	11.5	1.2	20.9	0.4	11.2	1.3
2A	50.1	0.7	3.7	0.2	16.0	0.1	4.6	0.2
30A	49.0	0.2	11.3	0.2	19.3	0.1	11.9	0.1
30R	44.5	1.2	11.5	0.6	19.3	0.9	13.5	1.6
31R	53.3	1.4	8.7	0.7	20.3	0.1	8.1	0.9

*** significant with $p<0.001$. / *** diferencias significativas con $p<0,001$.

In PH, cultivar 4A was the tallest (44 cm) while B1053 was the shortest, with only 20.5 cm of plant height. Cultivars B1157 and B1051 had the best yielding with 363 g plot^{-1}and 317 g plot^{-1} respectively, and cultivar SEL was the poorest with 15.5 g plot^{-1}.

Digital phenotyping showed that cultivars 17A, 22A, 19A and 30A had larger seeds with calibers of 7.5 mm, 7.4 mm, 7.4 mm and 7.3 mm respectively, while B1181, B1182 and 28R had the smallest seeds, with calibers ranging from 0.44 to 0.45. Color parameter L was high for cultivar 18R (63.43) and low for B1182 (36.40).

The *a* coordinate of color showed the highest values for 13R and 19R (16.45 and 15.10 respectively) meaning that these two cultivars are material for greater reddish color, while 35A, 18A and 2A showed the least (4.81, 3.79 and 3.70 respectively).

The *b* coordinate denotes the greenish color and was high for 10R and 12R, (23.22 and 22.45 respectively) and low for Pardina (13.26). When the color index (CI) was analyzed, cultivars B1182 (24.11) and B1181 (23.53) had the highest values, while 18A (4.43) was the lowest.

Cluster Analysis

Cluster analysis (figure 1, page 10), showed that cultivars conformed six groups with differential traits. This analysis allows the identification of cultivars with convenient characteristics, as seed size. A comparison of mean values of each group (table 4, page 11) using the Fisher's least significant difference test (LSD) showed that Group 1 had 32 cultivars with high C; group 2 had 21 cultivars with higher Y but lower CI. Group 3 had 17 cultivars with high values for coordinates *a* and *b*; group 4 included only one cultivar (18R) with the highest L; group 5 had 4 cultivars with lower C and coordinate *b*; and group 6, with 6 cultivars, had the cultivars with higher DF and CI but lower C and L.

Principal Component Analysis

Principal component analysis showed that two principal components explained 58% of the variation in the data set (PC1, 40% and PC2, 18%) and with the addition of a third component the proportion of variation explained reached 73% (PC3, 15%).

However, when the first two components are plotted against each other (figure 2, page 11) the cultivars conform 4 clearly differentiated groups. PC1 was associated with C, L, *a*, *b* and CI while PC2 was associated with DF, PH and Y.

In figure 2 (page 11), points represent lentil cultivars and vectors represent analyzed traits.

The perpendicular projection of the points on the vectors indicates the relative position of that cultivar against the others for that particular trait, having the highest values those cultivars in the positive direction of the vector, while the angle between vectors shows the correlation among traits. In this case, cultivars B1181 and B1182 had the highest values of CI and cultivar B1051 had the highest yield. Correlations shows that traits CI and *a*, DF and PH, C and L, and Y and *b* have positive correlations, while CI and a have *a* negative correlation with L.

There is a clear concordance between groups obtained by Cluster Analysis and by Principal Component Analysis. Groups 2 and 6 are separated groups, and were conformed with the same cultivars in both analyses. Groups 1 and 2 in one hand, and groups 3 and 5 on the other, conform two different groups in the Principal Component analysis. The selected materials, as parents for a breeding program, were those from group 2, given their higher yields and shorter cycle, and those from group 1, with higher caliber.

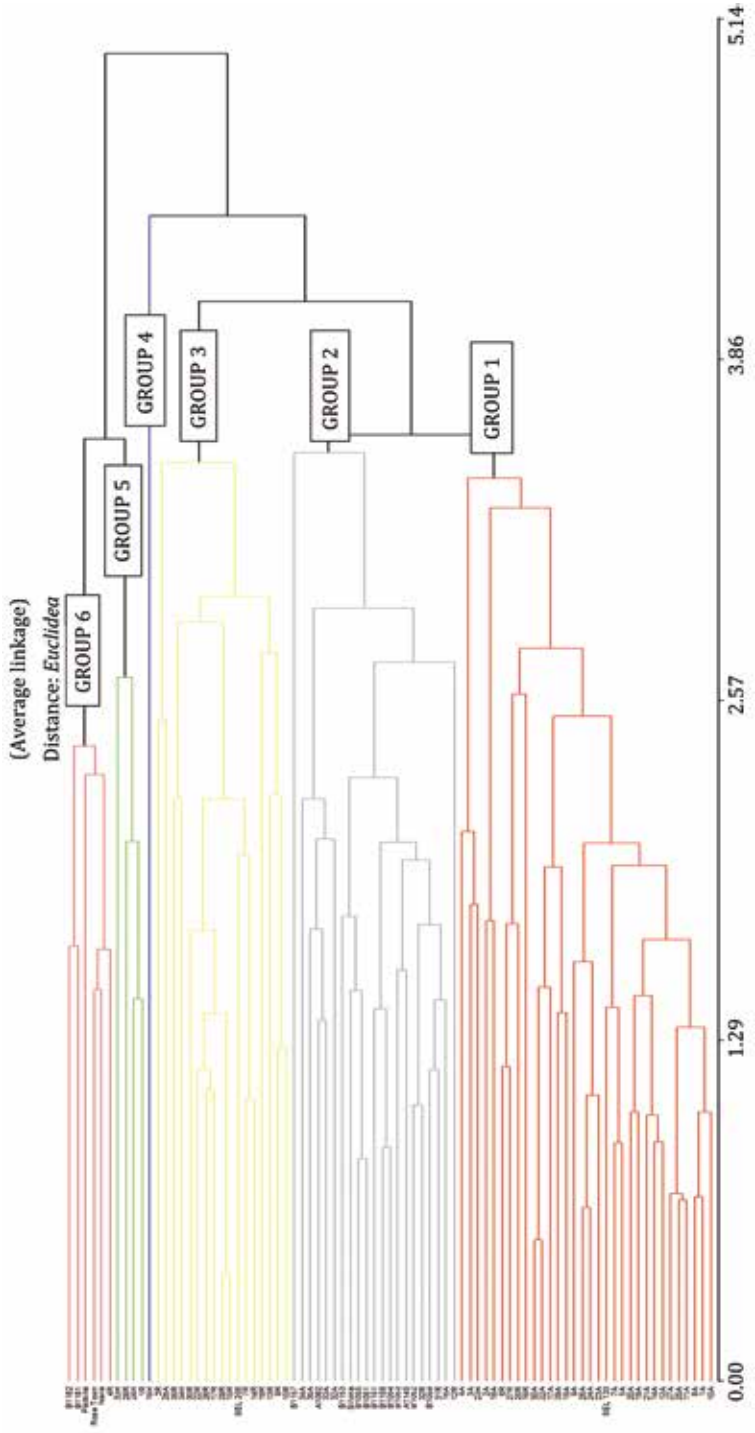

Figure 1. Cluster Analysis.
Figura 1. Análisis de conglomerados.

Table 4. Fisher's least significant difference test between group's means in the Cluster Analysis.

Tabla 4. Prueba de la mínima diferencia significativa de Fisher entre las medias de los grupos del Análisis de conglomerados.

	Traits							
Group	**DF**	**PH**	**Y**	**C**	**L**	**a**	**b**	**CI**
1	95.84a	20.83a	100.95b	0.67a	53.21c	8.42b	19.28b	8.23c
2	80.6b	32.0a	189.83a	0.63b	54.61b	6.97b	20.47a	7.35d
3	101.83a	29.06a	66.0b	0.56c	46.86d	12.86a	20.23a	13.08b
4	86.5ab	28.75a	84.97b	0.54cd	63.43a	8.68b	15.43c	7.60cd
5	90.91a	25.14a	76.5b	0.52d	46.10d	8.08b	15.06c	13.54b
6	88.88a	27.5a	124.50b	0.50d	38.93e	12.05a	14.65c	20.52a

Means with a common letter don't differ (p<0.05). / Medias con una letra común no son significativamente diferentes (p>0,05).

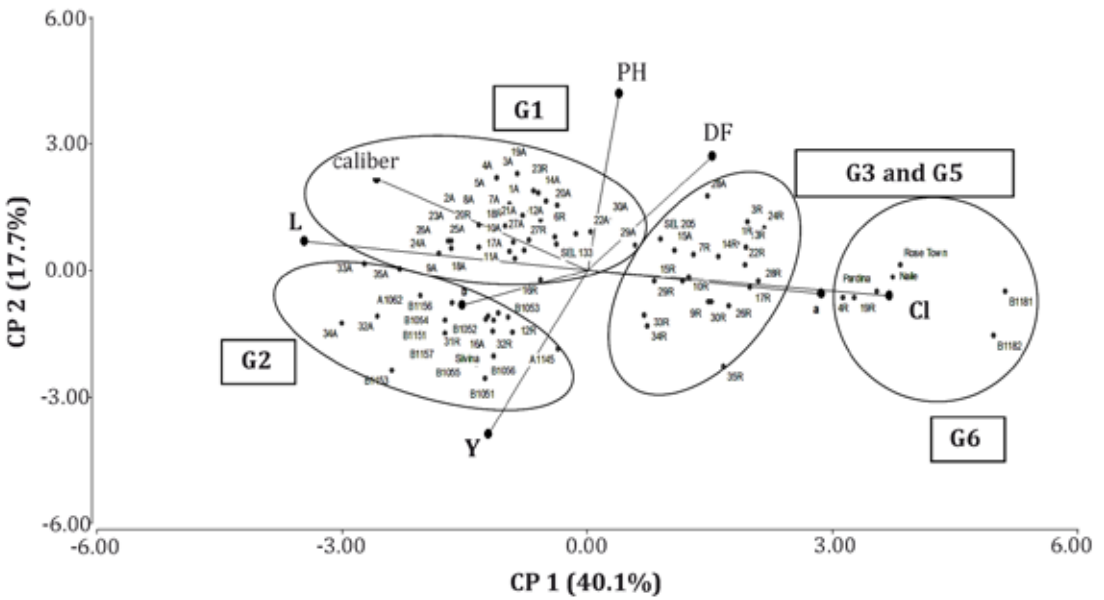

Figure 2. Biplot of the first two Principal Components. / **Figura 2.** Biplot de las dos primeras Componentes Principales.

Conclusions

Digital phenotyping showed to be a powerful tool for germplasm characterization along with field evaluation of agronomical traits. Principal Component Analysis and Cluster Analysis the identification of differentiated groups of cultivars with similar characteristics, leading to a more efficient use of the germplasm available.

Preliminary evaluation of the set of cultivars presented in this study demonstrate the existence of high phenotypic and genotypic diversity for different traits, showing their potential commercial or breeding value.

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