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Agro-morphological traits assessment and polyphenols compounents analysis in some advanced lentil's lines (Lens culinaris M.)

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Abstract. Lentil (*Lens culinaris* Medik.) crop plays an important role in both the agriculture sustainability than in food security by providing nutrients to vulnerable people worldwide. However, climate change mainly during flowering and pods filling, impact negatively seed yield and quality. The aim of this study is to select lines with high productivity and good nutritional quality which will be integrated into breeding program of novel varieties development. Thus, current study undertakes the evaluation of 25 advanced lines using nine agro-morphological traits related to seed yields potential and yield components, and total phenolics (TP), total flavonoids (TF) and condensed tannin (CT). ANOVA analysis showed significant variability among lines for almost recorded parameters. The clustering of variability revealed five groups at 85% of similarity. The first cluster grouped earlier genotypes that produced high seeds size (1000-seed weight (41.9g)). The second cluster grouped the high yielding genotypes; while the third one, grouped lines with high polyphenols concentration TP (32.1mgEAG/100g fw), TF (165.5 µgEQ/100g fw) and CT (1010.1 µgEC/100g fw). The fourth cluster regrouped lines with high potential of seedlings (59.8%) and yield potential according to total pods number per plant(142), fertile pods plant^{-1} (111), number of seeds plant^{-1} (156), and weight of seeds plant^{-1}(4.30g). The last cluster included one line (LN-52) that was distinguished with its high performance for almost all recorded parameters. The selection of lines (LN-03, LN-62 and LN-52) is proposed to be used in lentil breeding programs.

Keywords: Lentil; Agro-Morphological Traits; Polyphenols; Morocco.

1 Introduction

Lentil (*Lens culinaris* Medik.) is one of the important food legumes and also known as the oldest cultivated crops [1]. It's always been a central part of Moroccan traditional diet system, as well as in most part of the world including South Asia, Europe, the Middle East and Africa [2]. Moroccan lentil production between 2008 and 2018 was respectively 9.380 and 31.7 tons [3]. Those statistics have been instable mainly due to climate variation. For that, the improvement of cultivated lentil comes to increase productivity under abiotic stress conditions in order to satisfy food

security.

Lentils are well known as a rich source of proteins 25.1%, carbohydrate 59%, fat 0.5%, minerals 2.1% and sufficient amount of vitamins [4]. Indeed, lentils are considered to be a good source of antioxidant due to their high amount of phytochemical compounds such as polyphenols in comparison to other common pulses [5,6]. A range of different phytochemicals such as alkaloids, glycosides, phytosterols, terpenoids, phenolics, flavonoids, tannins, carotenoids, tocopherols, phytic acids and saponins are found in seeds lentil [7,8]. These are found to have various disease preventive capacities, especially from oxidative damages caused by free radicals. These oxidative damaged are considered to be one of the major factors involved in the generation of several chronic diseases in humans such as cardiovascular diseases, disorders like Alzheimer's and Parkinson's diseases, cancer, and diabetes [9]. Therefore, the consumption of food rich in polyphenols leads to a better prevention of several chronic diseases [10].

The selection of promising lentil genotypes is based on agro-morphological traits and nutritional quality, which were always been considered as an important factor in any crop improvement program. Many lentil breeders have been successfully used those traits to classify and measure the pattern of phenotypic diversity [11-12,13].

The main objective of this study was to investigate yield, yield components and polyphenols concentrations of 25 advanced lines then compare them to Moroccan improved populations, varieties and landraces. The selection of specific advanced lines for their high productivity and good nutritional values will be used for lentil breeding programs.

2 Materials and Methods

2.1 Plant materials

The study was carried out at the National Institute of Agronomic Research, in Sidi el Aydi's station in Morocco, during 2017-2018 growing season. A collection of 25 advanced lines were compared to the 6 landraces, 6 improved population and 3 varieties. A total of 40 genotypes, was set up as a split-plot design with three replicates. The distribution information is shown in Table 1.

Table 1. Lentil collection studied.

ID	Name of Population	ID	Name of Population	ID	Name of Population	ID	Name of Population
LN-01	Advanced Line	LN-49	Advanced Line	LN-60	Advanced Line	PA-12	Improved population
LN-02	Advanced Line	LN-50	Advanced Line	LN-61	Advanced Line	LR-14	Landrace
LN-03	Advanced Line	LN-52	Advanced Line	LN-62	Advanced Line	LR-15	Landrace
LN-04	Advanced Line	LN-53	Advanced Line	LN-63	Advanced Line	LR-16	Landrace
LN-05	Advanced Line	LN-54	Advanced Line	LN-64	Advanced Line	LR-18	Landrace
LN-33	Advanced Line	LN-55	Advanced Line	PA-06	Improved population	LR-21	Landrace
LN-43	Advanced Line	LN-56	Advanced Line	PA-07	Improved population	LR-22	Landrace
LN-46	Advanced Line	LN-57	Advanced Line	PA-08	Improved population	Bak	Variety
LN-47	Advanced Line	LN-58	Advanced Line	PA-09	Improved population	Bch	Variety
LN-48	Advanced Line	LN-59	Advanced Line	PA-11	Improved population	Chk	Variety

2.2 Agro-morphological traits

Agro-morphological traits were recorded for six randomly plants for each genotype. A total of nine traits data were measured on seedlings rate (%), days to flowering, number of fertile nodes plant^{-1}, number of pods plant^{-1}, number of fertile pods plant^{-1}, number of seeds plant^{-1}, weight of seeds plant^{-1} (g), 1000-seed weight (g) and yield potential (q/ha).

2.3 Polyphenol compounds

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1) Preparation of extract

Seed samples were ground (1 g each) and homogenized with a 40ml of solution containing 50% of acetone and 50% of distilled water, the samples were kept for 4 hours to complete extraction with stirring every 30 min. Samples were then centrifuged at 3500 rpm for 10 min. The supernatants were filtered through a Whatman No.1 filter paper. The extracts obtained, were stored at -20 ^{o}C for further analysis.

2) Determination of total phenolics (TP)

The total phenolic contents were determined as reported in Singleton and Rossi (1965) method [14], using the Folin–Ciocalteu phenol reagent. The mixtures were read at 765 nm against reagent blank. Total phenolic content was calculated by establishing standard curve using different concentrations of gallic acid. The results were expressed as mg of gallic acid equivalents(GAE)/g of fresh weight (fw) of the product.

3) Determination of total flavonoids (TF)

The total flavonoid content of lentil extracts was determined according to Herald, Gadgil, & Tilley, (2012) method [15]. The absorbance was read at 490 nm. TF was expressed as µg quercetin equivalents (µg QE)/g FW.

4) Determination of condensed tannin (CT)

Tannin content was determined by acidified vanillin assay reported to Downey & Hanlin, (2010) method [16]. The absorbance was recorded at 550 nm and the result was expressed as µg catechin equivalents (µg CAE)/g FW.

2.4 Statistical Analysis.

Analysis of variance (ANOVA) was conducted and differences were considered significant at $p<0.05$. A hierarchical cluster was built on the base of similarity and dissimilarity among the 40 lentil genotypes for 12 traits, the correlation was calculated using the JMP statistical software (SAS Institute, 2004).

3 Results and Discussion

3.1 Agro-morphological traits

The analysis of variance showed highly significant difference among the tested

genotypes for almost measured traits (Table 2). The average of seedlings rate was 57% with low variation among evaluated genotypes. The genotypes earliness was slightly varied (3.3%), indicating selection impact for earliness to be able to escape drought of lentil growing season. However, significant difference was observed mainly for earliness (91-106), weight of seeds plant^{-1} (0.15-11.5g), seed yield (10.3-33.7q/ha), number of fertile pods plant^{-1} (15.0-305.0) and number of seeds plant^{-1} (21.0-347.0). The differences might be attributed to genotype potential, environment conditions and to the interaction of genotype x environment as reported by Singh et al (2006), Neupane et al (2013), Dugassa et al (2014) & Erskine et al (2016) [17,18,19 & 2]. The higher value of coefficient of variation was found for almost traits, such as weight of seeds plant^{-1} (CV= 55.5%), number of fertile pods plant^{-1} (CV= 43.3), number of seeds plant^{-1} (CV= 40.8%), number of pods plant^{-1} (CV= 38.7%), and number of fertile nodes plant^{-1} (CV= 34.0%). These traits are highly affected by the environmental conditions [20].

Table 2. Analysis of variance for different agro-morphological traits in lentil.

Variables	Mean ± E.M	Range	F-value	CV%
Seedlings rate (%)	56.9±4.96	37.7- 74.6	2.37***	8.7
Days to flowering	100.3±3.26	91.0-106.0	2.13**	3.3
Number of fertile nodes plant^{-1}	60 ±22.2	18.0 -199.0	2.83***	37.0
Number of pods plant^{-1}	120±46.6	24.0 -346.0	2.48***	38.7
Number of fertile pods plant^{-1}	94±40.9	15.0- 305.0	2.36***	43.3
Number of seeds plant^{-1}	133±54.4	21.0-347.0	2.33***	40.8
Weight of seeds plant^{-1} (g)	3.64±2.02	0.15- 11.50	2.00**	55.5
1000-seed weight (g)	32.1±3.31	21.8 -48.0	9.94***	10.3
Yield (q/ha)	19.4±4.49	10.3- 33.7	6.57**	23.2

****=significant at P < 0.001 and **=significant at P < 0.01*
E.M: Average deviation; CV: Coefficient of variation

3.2 Phenolic compounds

High significant differences (P <0.001) in polyphenols were found among all studied genotypes (Table 3). The average phenols value (25.1 mg GAE/100g fw) was in accordance with Kalogeropoulos, (2010) study [21]. Whereas, flavonoids content (153.2 µg EQ/100g fw) were lower than that reported previously (221 µg EQ/100g fw) in Xu B et al, (2011) and Zia-ur-Rehman et al, (2005) studies [22, 23]. The condensed tannin content (CT) measured for evaluated genotypes was ranged from 500.5 to 1547.8 (882.9 mean) (µg Ec/100g fw). This result is in accordance with this indicated by Shamshir et al, (2018) 870 (µg Ec/100g fw) [24].

Table 3. Analysis of variance for polyphenols in lentil.

Variables	Mean ± E.M	Range	F-value	CV%
Phenols *(mg EAG/100g) fw*	25.1±1.90	13.2- 48.8	46.6***	7.5
Flavonoids *(µg EQ/100g) fw*	153.2±32.7	62.3-347.5	7.79***	21.3
Condensed tannin *(µg EC/100g) fw*	882.9±10.5	500.5-1547.8	1757.5***	1.2

****=significant at P < 0.001*

E.M: Average deviation; CV: Coefficient of variation

3.3 Correlation among agro-morphological and phenolic compounds data

Correlation test showed a significant positive correlation between Weight of seeds plant^{-1} and Number of pods plant^{-1} (r=0.696***), Number of seeds plant^{-1} (r= 0.809***), Number of fertile nodes plant^{-1} (r=0.350***) and 1000-seed weight (r= 0.256***). Whereas there were a high and significant correlation between Number of fertile pods plant^{-1} and Number of fertile nodes plant^{-1} (r=0.513***), Number of pods plant^{-1} (r=0.909***), Number of seeds plant^{-1} (r=0.814**) and Weight of seeds plant^{-1} (r= 0.751***). Number of pods plant^{-1} was positively correlated with Number of seeds plant^{-1} (r=0.777***) and Number of fertile nodes plant^{-1} (r=0.584***). As well as, high significant correlation was detected between Number of seeds per plant and Number of fertile nodes (r= 0.480***). Similar results were also reported by Shamshir et al, 2018 [24].

3.4 The clustering pattern and distribution

Based on the agro-morphological and nutrients trait, genotypes were grouped into five clusters at 85% of similarity (Fig.1). Cluster II and III grouped the highest number of genotypes (14 and 13 respectively). Genotypes grouped in the second cluster have a high yield potential (19.76 q/ha) ranged from 14.9q/ha (LN-55) to 25.1q/ha (LN-62) (Table 4, Table 5). While the third cluster grouped genotypes with high concentration of polyphenols (32.10 mg EAG/100g fw), of flavonoids (165.5µg EQ/100g fw) and of tannins (1010.1µg EC/100g fw)**.** The fourth cluster grouped genotypes characterized with high yield potential according to the Number of pods plant^{-1} (142 mean), Number of fertile pods plant^{-1} (111 mean), Number of seeds plant^{-1} (156 mean) and Weight of seeds plant^{-1} (4.30 g mean). Cluster I represent two advanced lines LN-01 and LN-05 which are characterized by earliness, Number of fertile nodes (66 mean) and high 1000-seed weight (41.85 g mean). While, the advance line LN-52 (Cluster V) was distinguished with high yield performance and high seed quality.

Table 4. Distribution of 40 genotypes of lentil in 5 cluster.

Clusters	No of Genotypes	% of Populations	Genotypes
I	2	100% LN	LN-01, LN-05
II	14	65% LN	LN-47, LN-48, LN-49, LN-50, LN-54, LN-55, LN-56, LN-57, LN-62,
		21% PA	PA-08, PA-09, PA-11,
		14% LR	LR-15, LR-18
III	13	69% LN	LN-33, LN-43, LN-46, LN-58, LN-59, LN-60, LN-61, LN-63, LN-64,
		8% LR	LR-22,
		23% VAR	Bak, Bch, Chk
IV	10	40% LN	LN-02, LN-03, LN-04, LN-53,
		30% PA	PA-06, PA-07, PA-12,
		30% LR	LR-14, LR-16, LR-21
V	1	100% LN	LN-52

Table 5. Means, minimum and maximum for all traits for 5 clusters in lentils.

Cluster		Seedlings Rate (%)	Days to flowering	Number of fertile nodes plant^{-1}	Number of pods plant^{-1}	Number of fertile pods plant^{-1}	Number of seeds plant^{-1}	Weight of Seeds plant^{-1} (g)	1000-seed weight (g)	Yield (q/ha)	TPC (mg EAG/100g) fw	TFC (µg EQ/100g) fw	CTC (µg EC/100g) fw
I	*Mean*	57,5	98	67	99	72	102	3,44	41,9	14,7	20,5	141,0	552,3
	Min	57,4	94	56	97	71	98	2,85	39,0	14,0	19,7	116,5	548,4
	Max	57,5	101	77	101	72	106	4,02	44,7	15,3	21,3	165,4	556,2
II	*Mean*	54,5	101	48	94	74	105	2,66	30,3	19,8	21,6	144,3	936,0
	Min	43,9	97	31	71	54	76	1,53	26,7	14,9	16,5	74,8	501,0
	Max	62,7	105	67	125	102	138	4,29	36,6	25,1	29,3	203,4	1544,0
III	*Mean*	56,9	99	66	130	103	142	3,90	30,9	19,3	32,1	165,5	1010,1
	Min	51,7	94	47	101	73	120	2,77	26,2	15,0	23,4	82,0	857,5
	Max	63,5	103	112	151	135	193	5,20	37,7	23,2	47,6	234,0	1304,3
IV	*Mean*	59,8	101	65	143	111	157	4,30	34,4	19,7	21,0	151,9	669,3
	Min	49,5	94	48	115	88	118	3,34	27,4	15,6	15,1	86,0	511,0
	Max	70,2	103	81	187	151	186	5,28	39,6	25,0	27,2	259,6	860,4
V	**LN-52**	59,0	100	85	197	154	239	7,71	29,6	21,2	34,9	154,3	1283,4

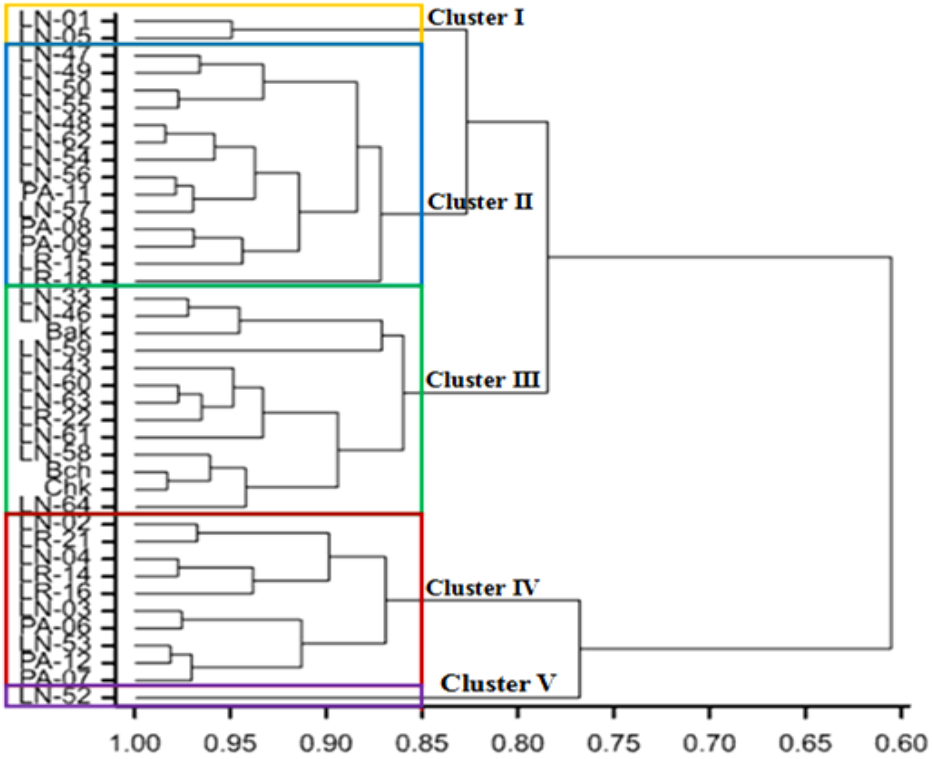

Figure 1. Dendrogram of 40 genotypes among all studies traits

4 Conclusion

Lentils play a crucial role in human nutrition, especially as a source of biologically active components such as polyphenols. The data provided from 40 genotypes based on agro-morphological and nutritional assessments showed an important basis for the improvement of lentil. Three advanced lines (LN-52, LN-03 and LN-62) were found promising for their earliness, high yield potential and high concentration of polyphenols components. These lines will be used to develop new productive varieties with high nutritional value and could be useful for genetic improvement.

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