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Evaluation of Early Stage Traits as an Indicator of Genetic Variation in Winter Lentil

Abstract

Lentil is one of the oldest cultured crops with cereals which are cultivated in the wide geography of the world due to its high adaptability, quality nutritional value, important usage areas and economic value. Morphological, physiological and biochemical parameters during the early vegetative stage are an important indicator of adaptation to environmental conditions. This study was carried out to investigate the variation on some agronomic and physiological traits of 49 lentil genotypes during the early growth stage. Although there were significant differences in terms of plant height and total chlorophyll content, differences between genotypes for plant dry and fresh weight, stem diameter and length of epicotyl were statistically found insignificant. Plant height, plant fresh weight, plant dry weight, length of epicotyl, stem diameter and total chlorophyll content varied between 2.53-4.78 cm, 0.17-0.39 g, 0.04-0.13 g, 1.53-3.00 cm, 0.72-1.05 cm and 28.4-38.2%, respectively. As a result, it was determined that observations taken 30 days after emergence are not sufficient to constitute genetic variation and use as an early prediction criteria field conditions. The longer-term and comprehensive studies should be carried out to form genetic variation and interpret the results in field conditions.

Keywords
Adaptation, Early prediction, Genetic Variation, *Lens culinaris*, SPAD-502

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is one of the most important legume crops. Due to its valuable seeds for human food and animal feed, the lentil has been cultivated almost all over the World. The seed and straw of lentil are rich in starch, protein, dietary fiber and many micronutrients including Zn, β-carotene and Fe (Li and Ganjyal, 2017). Moreover, lentil is a substantial part of crop rotation systems due to the ability of biological nitrogen fixation that contributes to sustainability in agriculture (Erskine et al., 2018). The other main factors that make lentil important are the tap-root system effectively processes the different layers of the soil (Razavi et al., 2016), high adaptability to different environmental conditions (Abbas et al., 2019), beneficial microorganism population in the rhizosphere area like other legumes (Brahmaprakash et al., 2017) and economically valuable crop. According to FAO (2019), almost 7.6 million tonnes of lentils were produced on more than 6.1 million hectares area in 2019.

The main factor affecting plant growth, yield components and seed quality are water and nutrient availability, organic matter, adaptation of genotype to environmental conditions and stress in grain legumes (Hampton et al., 2016; Uttam et al., 2018; Ceritoglu and Erman, 2020; Ceritoglu et al., 2020; İpekeşen and Biçer, 2021). Genetic traits of genotype have an important role in adaptation. There are many environmental stress factors threatening lentil cultivation such as drought, chilling, cold and high temperature and salinity. Early detection of various problems may help to predict adaptation, growth period and crop yield (Al-Gaadi et al., 2016). Because it is of vital importance for producers to detect some problems at an early stage. There are different methods used for early prediction such as estimation of yield using climate parameters (Prasad et al., 2006), remote and proximal sensing (Campbell and Wynne, 2011), collecting and analysis of data by drones (Joyce et al., 2019). Although these high technology methods could be helpful to early estimation, they are too expensive and required expertise. Therefore, we wondered whether an analysis of early stage parameters, which is based on morphological and physiological observations and has no high economic cost, could provide information about genetic variation and plant adaptation. This study was laid out to understand whether early vegetative stage parameters of different lentil genotypes could give an idea about adaptability.

MATERIAL and METHODS

The study was carried out at the location, 41°52' east longitude and 37°55' north latitude, in Siirt University during the 2019 winter season. The altitude of the location is 586 m. The location of the study area was determined by Google Earth. The 48 nursery and elite nursery lines with one local check (Fırat-87) of lentil that have different phenology, origin and seed mass, were sown on 22 November 2019. The used lines have a wide genetic variation and are crossed on improvement in earliness and drought tolerance by ICARDA. The cv. Fırat-87 was obtained from GAP International Agricultural Research and Training Center (GAPUTAEM). Pedigree information of used materials was given in Table 1.

Table 1. Description of pedigree of lentil (*Lens culinaris* Medik. subsp. culinaris) genotypes used in the study

GID	Designation	CROSS
21151	ILL2245	ILL2245
35	ILL4605	ILL4605
37	Fırat-87	Local check
3819	x2011s_118 _ 12	FLIP97-29LXFLIP97-33L
3713	x2011s_119 _ 25	FLIP97-29LXFLIP97-33L
3673	x2011s_119 _ 26	FLIP97-29LXFLIP97-33L
3699	x2011s_123 _ 42	ILL1712XILL10072
3662	x2011s_161 _ 1	ILL8008XILL8010
3836	x2011s_169 _ 32	ILL8008XL-4147
3684	x2011s_171 _ 7	Barimusor- 6xL-7713
3653	x2011s_172 _ 34	ILL7723XADA'A
3840	x2011s_183 _ 16	ILL7115XILL2585
3842	x2011s_192 _ 45	ILL10749XILL7979
3658	x2011s_204 _ 23	ILL10750X33108
3679	x2011s_204 _ 30	ILL10750X33108
3664	x2011s_221 _ 5	ILL10750XILL1959
3844	x2011s_35 _ 36	ILL10731XILL4637
3710	x2011s_55 _ 22	ILL4605XL-4147
3678	x2011s_55 _ 9	ILL4605XL-4147
3659	x2011s_60 _ 48	L-4147XILL4649
3829	x2011s_63 _ 9	ILL3796XILL4605
3828	x2011s_75 _ 17	ILL7978XILL5888
3837	x2011s_97 _ 17	ILL5883XILL10750
3759	x2011s91_76 _ 4	ILL4605XILL5597
3726	x2013_126 _ 8	FLIP97-34LXFLIP97-33L
87	ILL8006	ILL8006
3705	x2011s_11 _ 12	ILL4605XBARIMASUR-6
3703	x2011s_110 _ 23	ILL8007XILL759
3689	x2011s_129 _ 13	FLIP96-49LXFLIP97-33L
3701	x2011s_130 _ 1	ILL4402XILL7979
3805	x2011s_139 _ 4	ILL4402XILL7950
3687	x2011s_171 _ 13	Barimusor- 6xL-7713
3695	x2011s_171 _ 2	Barimusor- 6xL-7713
3696	x2011s_203 _ 2	ILL10749XILL3597
3664	x2011s_221 _ 5	ILL10750XILL1959
3690	x2011s_226 _ 6	ILL10800XILL4419
3649	x2011s_59 _ 20	L-4147XILL4649
3839	x2011s_60 _ 28	L-4147XILL4649
3697	x2011s_72 _ 54	ILL7978XILL7537
3771	x2011s133_119 _ 15	FLIP97-29LXFLIP97-33L
3750	x2011s17_20 _ 3	BARIMASUR-6XLIRL-22-46-1-1-1-0
3780	x2011s242_230 _ 3	ILL10801XILL2711
3761	x2011s91_77 _ 6	ILL4605XILL5597
3744	x2013_126 _ 54	FLIP97-34LXFLIP97-33L
3743	x2013_171 _ 17	Barimusor- 6xL-7713
3737	x2013_19 _ 16	BARIMASUR-6XLIRL-21-50-1-1-1-0
3715	x2013_20 _ 7	BARIMASUR-6XLIRL-22-46-1-1-1-0
3716	x2013_21 _ 2	BARIMASUR-6XLIRL-22-46-1-1-1-0

(GID: Germplasm id)

The soil samples that were taken from 0-20 cm depth before sowing time was analyzed in the Central Laboratory of Siirt University. It was composed of medium-deep, low in organic matter, enough in potassium, low in soluble phosphorus, limy and mild saline. The pH was light alkaline near neutral. The texture was determined as clay loam (Saraçoğlu et al., 2014). Investigated traits of the soil were given in Table 2.

Table 2. Chemical composition of the soil taken from the study area

Depth (cm)	Texture	pH (1:1)	EC (dS/m)	Lime ($CaCO_3$) (%)	Organic Matter (%)	Phosphorus (P_2O_5) (kg da^{-1})	Potassium (K_2O) (kg da^{-1})
0-20	Clay-loam	7.60	6.56	9.4	1.1	1.56	162

The terrestrial climate prevails in the region. Although the average temperatures showed similarity with long-term values relative humidity and total precipitation were determined fewer than long-term throughout the vegetation period. The climatic data showed that lentil seedlings were subjected to drought stress during the first month of the growing season compared with previous seasons. Climatic data were given in Table 3.

Table 3. Some climatic values during the vegetation period

Vegetation Period	Average Temperature (°C)		Relative humidity (%)		Total precipitation (mm)	
	2019	Long-term	2019	Long-term	2019	Long-term
November	11.9	10.6	50.2	62.7	51.4	74.3
December	7.5	5.1	75.0	72.5	75.8	90.6
Mean	9.7	7.9	62.6	67.6		
Total					127.2	164.9

The research was conducted in a completely randomized block design (CRBD) with three replications. Each plot was formed as 1 m wide and 2 m length. The 5 rows were placed in each plot and inter-row spacing was determined as 25 cm (Kraska et al. 2020). The 80 kg ha- seed was sown per plot (Tepe et al., 2005). Also, 140 kg DAP ha^{-1} was applied with sowing under the seed drill (Dona et al. 2020). There was no need to weed management due to the short vegetation period. The study was carried out in rainfed conditions and concluded 30 days after emergence (DAE). Plant height (PH), fresh weight (FW), dry weight (DW), length of epicotyl (LE), stem diameter (SD), and total chlorophyll content (TCC) were investigated in the study. Ten plants were randomly collected from each plot for observations. The TCC was determined in the field before collecting samples. The LE was measured with a millimetric ruler. The aboveground biomass was weighed for FW in laboratory conditions. Then, the samples were separately put into a blotter, and placed into the incubator at 70°C for 48 hours (Soysal et al., 2020). After incubation, the DW was recorded. The SD was calculated at 1 cm above the soil surface by an electronic caliper (Mitutoyo 500-182-30 digital caliper, Co. Ltd., Japan) (Verbree et al., 2015). The TCC was measured by a portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd., Japan) on the upper fully expanded leaf (Dong et al., 2019).

The normality of data was controlled by the Shapiro-Wilk test. Data were calculated by analysis of variance in JUMP 5.0.1. software according to the CRBD. The results were grouped according to the TUKEY test.

RESULT and DISCUSSION

The forty-eight lines, improved by ICARDA, and one local check was investigated in terms of some agronomic and physiological traits at the early vegetative stage in field condition. According to the results, there were some significant differences (<0.05) on the PH and TCC among genotypes while there was not any significant difference in terms of the other traits (Table 4).

Table 4. Analysis of variance belonging to investigated parameters

Source of variation	DF	Plant height MS	Plant height F prob.	Fresh weight MS	Fresh weight F prob.	Dry weight MS	Dry weight F prob.
Genotypes	48	0.603	*	0.005	ns	0.0008	ns
		Length of epicotyl		Stem diameter		Total chlorophyll content	
	48	0.262	ns	0.011	ns	11.004	*

(MS: means of the square, ns: Non-significant, DF: Degree of freedom, *: <0.05)

Table 5. Some early vegetative stage parameters of different lentil genotypes

GID	Plant height	Fresh weight	Dry weight	Length of epicotyl	Stem diameter	Total chlrophyll content
21151	3.47 b-k	0.24	0,04	2,22	0.94	32.4 c-j
35	4.27 a-d	0.35	0.13	2.23	1.04	33.2 a-j
37	3.10 e-k	0.26	0.05	2.38	0.90	31.7 c-j
3819	3.97 a-i	0.23	0.07	2.53	0.89	31.5 c-j
3713	3.10 e-k	0.25	0.07	1.63	1.00	34.2 a-h
3673	3.85 a-j	0.32	0.08	1.53	0.86	34.0 a-h
3699	3.47 b-k	0.26	0.07	1.85	0.92	33.7 a-i
3662	3.62 b-k	0.27	0.09	2.38	0.95	31.7 c-j
3836	3.23 d-k	0.17	0.04	2.45	0.96	33.4 a-i
3684	4.41 ab	0.26	0.09	2.30	0.99	37.7 ab
3653	3.47 b-k	0.26	0.07	2.80	0.99	35.6 a-e
3840	3.89 a-i	0.32	0.10	1.93	0.95	36.2 a-d
3842	3.97 a-h	0.29	0.07	2.58	0.99	34.4 a-h
3658	3.94 a-h	0.29	0.10	2.25	0.93	32.4 c-j
3679	3.44 b-k	0.30	0.08	2.10	0.88	33.0 a-j
3664	3.69 b-j	0.24	0.08	2.45	0.88	30.3 g-j
3844	3.50 b-k	0.31	0.10	1.87	0.90	32.6 b-j
3710	2.89 g-k	0.20	0.07	2.22	0.73	33.1 a-j
3678	3.10 e-k	0.24	0.10	2.65	0.82	33.5 a-i
3659	3.57 b-k	0.19	0.05	2.48	0.86	34.8 a-g
3829	3.34 b-k	0.25	0.07	2.45	0.91	33.2 a-j
3828	3.57 b-k	0.22	0.05	2.51	0.72	29.1 hij
3837	3.82 a-j	0.28	0.05	1.92	0.97	31.8 c-j
3759	4.32 a-d	0.39	0.09	2.18	1.05	34.1 a-h
3726	3.98 a-h	0.27	0.05	2.63	0.89	34.1 a-h
87	2.53 k	0.19	0.05	2.68	0.97	32.3 c-j
3705	3.35 b-k	0.23	0.06	2.42	0.93	35.4 a-e
3703	2.80 i-k	0.20	0.04	3.00	0.79	30.7 e-j
3689	4.78 a	0.34	0.10	2.15	0.97	28.5 j
3701	4.38 abc	0.26	0.06	1.81	0.95	32.9 a-j
3805	2.76 j-k	0.23	0.09	2.35	0.90	34.0 a-h
3687	3.75 a-j	0.31	0.07	2.27	0.95	33.1 a-j
3695	3.48 b-k	0.29	0.10	2.36	0.87	34.8 a-g
3696	3.03 fk	0.20	0.05	2.75	0.85	38.2 a
3664	3.69 b-j	0.24	0.08	2.45	0.91	33.2 a-j
3690	3.93 a-h	0.24	0.11	2.88	0.87	35.1 a-f
3649	2.79 i-k	0.21	0.08	1.63	0.89	30.7 d-j
3839	2.57 k	0.21	0.06	2.55	0.94	29.4 hij
3697	4.11 a-f	0.25	0.07	2.00	0.91	35.1 a-f
3771	4.46 ab	0.35	0.10	2.27	1.06	29.8 hij
3750	4.21 a-e	0.29	0.08	2.26	0.95	34.2 a-h
3780	3.77 a-j	0.27	0.09	2.32	0.97	33.2 a-j
3761	4.75 a	0.35	0.12	2.60	0.89	35.6 a-e
3744	3.93 a-h	0.26	0.08	2.36	0.97	35.8 a-e
3743	3.51 b-k	0.25	0.09	2.98	0.98	36.6 abc
3737	2.79 i-k	0.24	0.07	2.53	0.99	32.8 b-j
3715	4.05 a-g	0.32	0.08	2.99	1.01	35.4 a-e
3716	4.03 a-g	0.27	0.08	2.10	0.93	28.4 j
Mean	3.63	0.26	0.08	2.34	0.92	33.2
TUKEY	0.92*	0.05	0.02	0.02	0.001	2.21*
VK	9.5	10.1	8.2	8.3	9.7	1.3

(GID: Germplasm id)

The lines of 3689 and 3761 had the longest PH whereas lines of 3839 and 37 were determined as the shortest. The highest TCC was observed in 3696 while the lowest one was seen in 3716 and 3689. The PH and TCC varied between 2.53-4.78 cm and 28.4-38.2%, respectively (Table 5).

The results indicated that statistically significant differences were determined in two traits throughout the early growth period in field conditions. The main factor of this situation is thought to be caused by environmental factors such as low temperature and water source. Because the experiment was established in November and average temperatures were between 7-11 °C (Table 3). So, the growth rate was negatively affected in all genotypes although there was a wide genetic variation. Also, the lentil has a slow growth rate in the vegetative growth period (Erman et al., 2008). Though significant differences were determined in the TCC, it did not affect the growth parameters. It can be considered that although chlorophyll content exhibit differences among cultivars, produced physiological energy can not use for morphological growth during the first month after emergence. Studies conducted in hydroponic systems (Singh et al., 2013) and pot trials (Abi-Ghanem et al., 2010) have shown that there are significant differences and variations between early stage parameters since the plant growth rate is high in studies conducted under controlled conditions. So, genetic variations can be observed clearly. Since our study was carried out in the field conditions during late autumn, the effect of environmental factors had a significant negative impact. Because low temperature restricts vegetative growth especially at the early growth period (Öktem et al., 2008).

The water content and dry matter accumulation did not change among genotypes. Low temperatures especially the night period causes restrict physiological and metabolic activities, thereby, water and nutrient uptake, cell division, morphological growth are reduced (Khan et al., 2017). Low temperatures lead to induce noteworthy changes in gene expression and lipid composition of biomembrane, thereby, many tropical or sub-tropical plants are damaged or killed at low temperatures which are lower than 10 °C (Sanghera et al., 2011; Niu and Xiang, 2018). However, winter legumes, cereals and some other crops have a vital strategy, which is described as vernalization, to cope with the cold conditions of the winter season (Chouard, 1960). The plants gain tolerance to cold conditions with vernalization response, therefore, they are stimulated for flowering. But, low temperatures noteworthy restrict physiological, biochemical, and morphological growth. These pieces of information may help to explain why there are no significant differences between lines in terms of morphological characteristics.

CONCLUSION

Some agronomic traits of lentil genotypes were investigated during the early vegetative stage to understand whether genetic variation can be observed. However, it was concluded that the observations taken 30 days after emergence in the field conditions were not enough for genetic variation. As a result, observations taken 30 days after emergence are not sufficient to constitute genetic variation and use as an early prediction criteria field conditions. The longer-term and comprehensive studies should be carried out to form genetic variation and interpret the results in field conditions.

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