

Study on Relations Between Relative Water Content, Cell Membrane Stability and Duration of Growth Period with Grain Yield of Lentil Genotypes under Drought Stress and Non-Stress Conditions

S.H. Azizi-Chakherchaman^{*1}, H. Kazemi-Arbat², M. Yarnia³, H. Mostafaei⁴, D. Hassanpanah⁴, M.R. Dadashi⁵ and R. Easazadeh⁶

*Corresponding author: m_azizi60@Yahoo.com.

1. Member of Young Researchers Club, Azad University, Tabriz Branch, Iran. and former graduate student Tabriz Islamic Azad University. Tabriz, Iran.
2. Professor, Faculty of Agriculture, Islamic Azad University of Tabriz, Iran.
3. Assistant Professor, Faculty of Agriculture, Islamic Azad University of Tabriz, Iran.
4. Research Faculty, Agriculture and Natural Resources Research Station of Ardabil, Iran
5. Assistant Professor, Faculty of Agriculture, Gorgan Islamic Azad University. Gorgan, Iran.
5. Faculty staff, East Azarbaijan Jihad-e- Agriculture Organization, Tabriz, Iran.

ABSTRACT

Drought is one of the most important abiotic stresses that limits crop production in arid and semi-arid regions of the world. Lentil (*Lens culinaris L.*), a valuable legume crop, is produced mainly rain-fed in Iran. An experiment was conducted to study the relationships between relative water content (RWC), cell membrane stability (CMS) and duration of growth period with grain yield of 11 advanced genotypes, varieties and a local genotype in the Ardabil Agriculture and Natural Resources research Station. Experimental design was a randomized complete block design (RCBD) with three replication under both rain-fed and irrigated conditions. Combined ANOVA showed significant differences among all the evaluated traits. Significant differences between characters reveals that there are high variation between the traits studied. Means for characters under study showed that grain yield, RWC, CMS and duration of growth period decreased in rain-fed condition but cell membrane leakage (electric conductivity) increased. Correlation coefficients showed strongly positive relation between grain yield with RWC ($r=+0.98^{**}$), strongly negative and non significant CMS ($r=-0.32^{ns}$) and strongly negative relation between grain yield and the days to maturity ($r=-0.78^{**}$). The results of the experiment also revealed that there weren't a strong relation between yield and calculated attributes for drought tolerance except RWC. Therefore, this character could be effective in evaluation of drought tolerance and identification of high yielding genotypes (ILL 6031, ILL 9893 and ILL 8095).

Key Words: relative water content, cell membrane stability, drought stress, grain yield, lentil

INTRODUCTION

Drought is the most important abiotic stress that limits crop production in arid and semi-arid regions of the world (Ferrat and Lovatt, 1999). Iran, with a mean annual rainfall of 250 m.m., is considered an arid to semi-arid country (Soltani et al., 2001). The limited available water during

growing season in some regions, such as Ardabil, reduces crop yield considerably (Soltani et al., 2001; Yu and Stter, 2003).

Lentil (*Lens culinaris L.*) is traditionally grown as a rainfed crop under various cropping systems that often suffer from intermittent and terminal drought (Sarker and Erskine, 2005).

Lentil one of the valuable legume crops which is produced mainly as rain-fed in Iran (Mostafaei et al., 2006). Lentil contains large amounts of proteins, and has the ability to fix, symbiotically with certain bacteria, atmospheric nitrogen, and thus contribute greatly to soil fertility (Karim Mojein et al., 2003 and Anjam et al., 2005).

A considerable portion need of people in Iran is supplied through leguminous crops, including lentil. To produce the necessary protein needs of people in our country planting high yielding and drought tolerant lentil cultivars is of outstanding importance (Anjam et al., 2005 and Soltani et al., 2001).

Sometimes, relationship between leaves water potential (Ψ_w) and relative water content (*RWC*) of leaves used for evaluation of water deficit magnitude in the plant tissues and cells and predicting tissues resistant to desiccation resulted from water deficit (Aminzadeh and Eshghi, 2006; Ferrat and Lovatt, 1999; Khan and Stoddard, 2005).

It seems that, tissues that able to maintain higher *RWC* with decreasing water potential are more resistant to drought conditions and desiccation resulted from this stress (Ferrat and Lovatt, 1999; Irigoyen et al., 1992; Schonfeld, 1988).

Neyestani and Azimzadeh (2003) reported that relative water content in lentil genotypes under drought stress is lower than non stress conditions.

Drought stress injure the plasma membrane, so cell content secrete to outside. Magnitude of this damage can be determinate with ionic secretion measurement (Ferrat and Lovatt, 1999; Khan and Stoddard, 2005).

Neyestani and Azimzadeh (2003) in lentil and Vazan et al., (2005) in sugar beet, studied the relationship between plasma membrane stability (obtained from *EC* measurement) and grain yield in stress and non stress conditions and reported that plasma membrane stability in genotypes under stress was significantly lower than genotypes under non stress conditions.

The aim of this study was the identification relationship between relative water content (*RWC*), electrical conduction (*EC*), cell membrane stability and growth period and determination suitable factor for selection high yield in lentil genotypes under drought stress and non stress conditions at Ardabil.

MATERIALS and METHODS

In order to study the relationship between *RWC*, *EC*, cell membrane stability and growth period with grain yield in lentil genotypes under well-watered and stress conditions, compared 11 cultivar and promising genotypes and one genotype that selected from local genotypes of Ardabil at Agricultural research station in Ardabil. Climatically, the area placed in semi arid and cold zone (Alt

1350m; Long 48°20' E; Lat 38°15' N). Soil type was clay loam with pH about 7.7 and depth about 70cm.

The experiments were conducted in no drought (irrigated) and drought (rain-fed) conditions, in randomized complete block design with three replications. Seeds of genotypes were sown in 4 rows with 4m long and 25cm row spacing, in 13 April 2005. Plots in no drought conditions irrigated as required (2 irrigation) while, plots in drought conditions no irrigated.

For determinate the relative water content in 50% flowering (after drought stress), selected young and ripping leaves in each varieties and replications then, taken in plastic pockets and carried to laboratory, immediately. Afterward, determined the fresh weight of leaves and samples were taken in distilled water for 24h in refrigerator (about 5°C). After 24h recorded the turgor weight of leaves. In order to determinate the dry weight of leaves, samples were taken in oven for 48h in 70°C. Relative water content of leaves calculated following modified formula of Wedrly .

$$RWC = \frac{wf - wd}{wt - wd} \times 100$$

Where:

RWC : relative water content of leaves (%)

Wf : fresh weight of leaves (g)

Wd : dry weight of leaves (g)

Wt: turgor weight of leaves (g)

For measurement of electrical conductivity (*EC*) of leaves in end of flowering period (moisture stress) in all of replications, prepared 20 disk by punch from young leaves randomly, then put into 20cc distilled water. Afterward, these samples carried to refrigerator (about 5°C) and after 24h obtained samples *EC* by using sensitive *EC* meter. From these magnitudes subtracted distilled water *EC* (as control) and the *EC* of leaves were obtained.

In order to determine the number of days from planting to ripping, when one third of plant down was yellow and color of pods became yellow/green, recorded as ripping time.

Grain yield obtained from two middle rows (about 1.5m²).

To evaluate drought tolerance of lines under study we used the following equation (Fernandez, 1992).

$$SI = 1 - (Y_s/Y_p)$$

Where:

Y_s = total yield mean in stress condition.

Y_p = total yield mean in normal condition.

Analysis of variance and regression between studied traits were performed using MSTAT-C computer software package. The means were tested using the Duncans multiple range test in 5% level.

RESULTS and DISCUSSION

Combined analysis of variance of the data measured revealed that there were significant at 1% level of probability (Table1). Anjam et al. (2005); Beguom and Beguom (1996) and Mostafaei et al. (2006) in their studies of lentil reported the results similar to present study. Percent stress intensity was calculated to be 29 % (SI=29%). This shows that seed yield of lentils under drought stress in Ardabil decreases considerably. Percent yield reduction under the condition of this experiment would be 29 per cent.

Significant difference between studied traits indicated that there was high variation between studied traits that could be used in study of the stress resistance.

Mean of grain yield, number of days until ripping and relative water content in no-drought condition (no-stress) increased compared to drought condition (stress) while, EC under drought stress was more than the no-drought condition (Table 2). These results indicated that the lentil grain yield affected by water deficit and drought furthermore, lentil had high yield under optimal conditions.

The growth period of studied lentil genotypes affected by environmental conditions, so that water deficit and drought caused early ripping in lentil genotypes. Furthermore, under drought conditions relative water content of leaves decreased with decreasing soil moisture. Drought stress and water deficit destroy the cells membrane then, vacuoles content secret to outside and increase the concentration of solution. Under this condition, increasing the magnitude of EC and decreasing cell membrane stability, compared to no-drought stress.

The means of studied traits (Table 3) indicated that ILL 8095, ILL 9893 and ILL 6031 had the highest grain yield compared to other genotypes. Also, these genotypes had the highest relative water content. Thus, it seems that grain yield have positive relationship with relative water content. Furthermore, these genotypes were early ripping genotypes.

Correlation among grain yield and other studied traits (Table 4) indicated that grain yield had highly positive and significant relationship with relative water content. Positive and significant relationship between these traits indicates that genotypes that have high grain yield show more relative water content. Correlation among RWC and days number until ripping were found negative and significant. This indicated that early ripping genotypes had more relative water content than late ripping genotypes. These results are in agreement with Farat and Lavat (1999) in bean, Khan and Studdard (2005), Okarum et al. (2005) and Aminzadeh et al. (2008) in barley.

Correlation among the number of days until ripping and grain yield were found negative and significant. Mostafaei et al. (2006) reported same results. Grain yield showed negative and non-significant correlation with EC.

The results of the experiment also revealed that there weren't a strong relation between yield and calculated attributes for drought tolerance except RWC. Therefore, this character could be effective in

evaluation of drought tolerance and identification of high yielding genotypes (ILL 6031, ILL 9893 and ILL 8095).

Table 1. Combined analysis of variance of traits among promising lentil genotypes studies

Mean Square					
Source	df	seed yield (kg.ha ⁻¹)	days to maturity	electric conductivity (µs/cm)	relative water content (%)
Locetion	1	1175760.056*	470.222*	2130.521**	8462.138**
Error 1	4	146676.593	55.097	52.704	60.494
Factor A	11	323859.563**	74.040**	133.022**	145.783**
L * A	11	10636.578 ^{ns}	12.040**	39.114 ^{ns}	45.659**
Error 2	44	17853.383	5.646	41.578	15.993
Cv %		18.04	2.45	18.47	6.46

ns, * & ** : not significant, significant at the 5% & 1% levels of probability, respectively

Table 2. Mean comparison of promising lentil genotypes under rain-fed and irrigated condition (Combined analysis) based on DMRT

genotypes No.	genotypes	days to maturity	electric conductivity (µs/cm)	relative water content (%)	Seed Yield (kg.ha ⁻¹)
1	ILL 8173	99.33 b	35.57 abcd	56.16 d	474.4 f
2	ILL 9919	98.33 b	31.28 bcd	61.91 bc	691.7 e
3	ILL 9832	104.2 a	27.67 d	56.14 d	456.6 f
4	ILL 323	98.17 b	34.20 bcd	60.98 cd	804.9 cde
5	ILL 1878	98.67 b	43.62 a	56.36 d	432.1 f
6	ILL 8146	97.00 bc	39.40 ab	56.38 d	476.6 f
7	ILL 6031	90.50 e	30.60 cd	65.52 bc	972.0 abc
8	ILL 7677	97.83 b	38.13 abc	62.50 bc	768.0 de
9	ILL 9893	94.50 cd	32.10 bcd	66.97 ab	1013.0 ab
10	ILL 8095	92.83 de	30.60 cd	70.99 a	1075.0 a
11	ILL 8105	97.50 b	35.87 abcd	63.38 bc	838.08 cde
12	Native genotype	94.50 cd	39.91 ab	65.78 bc	884.9 bcd
LSD 5%		2.764	7.503	4.635	155.500

Mean with the same letters in each column does not have significant difference at the 5% level of probability according to DMRT

Table 3. Total mean values for the traits of promising lentil genotypes under rain-fed and irrigated conditions

traits	drought stress (rain-fed)	non-stress drought (irrigated)
days to maturity	94.389	99.500
electric conductivity	40.352	29.472
relative water content	51.081	72.763
seed yield (kg.ha ⁻¹)	612.906	864.483

Table 4. Simple correlation of traits among promising genotypes studied

traits	days to maturity	electric conductivity	relative water content	seed yield
days to maturity	-	0.01 ^{ns}	-0.77 ^{**}	-0.78 ^{**}
electric conductivity		-	-0.32 ^{ns}	-0.34 ^{ns}
relative water content			-	0.97 [*]
seed yield				-

ns, * & ** : not significant, significant at the 5% & 1% levels of probability, respectively

REFERENCES

- Aminzadeh, G. and A. G. Eshghi. 2006. Evaluation of drought resistance in new lines and cultivars of bred wheat. (Abstract). The first international conference on the theory and practices in biological water saving (ICTPB). 21-25 May, Beijing-China.
<http://www.conferene.ac.cn/ictpb.htm>.
- Anjam, M.S., A. Ali., SH. M. Iqbal. and A.M. Haqqani. 2005. Evaluation and correlation of economically important traits in exotic germplasm of lentil. International, J of Agri. and Biology. 7(6): 959-961.
- Beguom, S. and S. Beguom. 1996. Morphological study and character association in germplasm of lentil (*Lens culinaris Medik*). Bangladesh. J. Botany. 25: 79-81.
- Fernandez, G. C. J. 1992. Effective selection criteria for assessing plant stress tolerance. In Proceeding of a Symposium. Taiwan, 13-18 Aug. pp. 257- 270.
- Ferrat, I. L. and C. J. Lovatt. 1999. Relationship between relative water content, nitrogen pools and growth of *Phaseolus vulgaris L.* and *P. acutifolius A. Grag* during water deficit. Crop. Sci. 39: 467-475.
- Irigoyen, J. J., D. W. Emerich. and M. Sanches-Diaz. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants, Physiol. Plants. 84: 55-60.

- Karim Mojein, H., H.M. Alizadeh., N. Majnoon Hoseini. and S.A. Payghambari. 2003. Effect of herbicides and handweeding in control of weed in winter and spring sown lentil (*Lens culinaris L.*). Iranian Journal of Crop Sciences, Vol.6. No.1:68-79.
- Khan, H. R. and F. L. Stoddard. 2005. Genotype variation physiological attributes related to drought tolerance in faba bean. (Abstract) The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress .Inter, Drought-II. September 24 to 28. Rome-Italy.
- Mostafaei, H., D. Hssanpanah. and R. Shahriari. 2006. Adaptation of local and domesticated lentil genotypes in dry farming of Ardabil region. (Abstract). The first international conference on the theory and practices in biological water saving (ICTPB). 21-25 May, ,Beijing-China. <http://www.conferene.ac.cn/ictpb.htm>.
- Neyestani, E. and M, Azimzadeh. 2003. Study of drought tolerance of 15 lentil varieties. Iranian Journal of Agriculture. Vol.5. No.1:61-69.
- Oukarroum, A., S. Elmadidi. and R. J. Strasser. 2005. Analysis of the chlorophyll a fluorescence transient OJIP during drought stress and re-watering of barely cultivars (*Hordeum vulgare L.*). (Abstract) The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress .Inter, Drought-II. September 24 to 28. Rome-Italy.
- Sarker, A, and W. Erskine. 2005. Genetic improvement for drought tolerance in lentil. (Abstract). The 2nd International Conference on Integrated Approaches to Sustain and Improve Plant Production Under Drought Stress .Inter, Drought-II. September 24 to 28. Rome-Italy.
- Schonfeld, M. A., R.C. Johnson, B.F. Carver. and D.W. Mornhinweg. 1988. Water relation in winter wheat as drought resistance indicators. Crop Sci., 28: 526-531.
- Soltani, A., F.R .Khoie., K. Ghassemi. and M. Moghaddam. 2001. A simulation study of chickpea crop response to limited irrigation in semi-arid environments. Agric. Water Manag . 49:225-237.
- Vazan, S., Z. Ranji., M. H. Tehrani., A. GHlavand. and M. S. SHariatpanahi. 2005. Study of proline variations in related to abscisic acid, stomatal conductivity, and plasma membrane stability in different gemotypes of *Beta vulgaris L.* under drought stress and non-stress conditions. Iranian Journal of Agronomy and Plant Breeding. Vol.1. No.1:27-37.
- Yu. L.X. and T.L. Stter. 2003. Comparative transcriptional profiling of placenta and endosperm in developing maize kernels in response to water deficit. Plant Physiology. 131:568-582.