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Study of Relationships of Leaf Relative Water Content, Cell Membrane Stability and Duration of Growth Period with Grain Yield of Lentil under Rain-Fed and Irrigated Conditions

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Abstract: Drought is one of the most important abiotic stresses that limit crop production in arid and semi-arid regions of the world. Lentil (*Lens culinaris* L.), a valuable legume crop, is produced mainly as 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, cultivars and a local genotype at the Ardabil research center for agriculture and natural resources, Ardabil, Iran. Experimental design was a Randomized Complete Block Design (RCBD) with three replications under both rain-fed and irrigated conditions. Combined ANOVA analysis procedure showed significant differences among all the evaluated traits. Significant differences between characters revealed that there was 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 conditions, but cell membrane leakage (electrical conductivity) increased. Correlation coefficients showed strongly positive relation between grain yield with RWC (r = + 0.98**), strongly negative and non significant with 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 were not a strong relation between yield and calculated traits for drought tolerance except RWC. This character therefore, could be effective in evaluation of drought tolerance and identification of high yielding genotypes (ILL 6031, ILL 9893 and ILL 8095).

Key words: RWC, CMS, drought stress, grain yield, lentil, relationship

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 mm is considered as 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 Setter, 2003). Lentil (*Lens culinaris* L.) is traditionally grown as a rain-fed crop under various cropping systems that often suffer from intermittent and terminal drought (Mishra *et al*., 2007). Lentil contains large amounts of proteins and has the ability to fix atmospheric nitrogen symbiotically with certain bacteria and thus contribute greatly to soil fertility (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 great importance (Anjam *et al*., 2005; Soltani *et al*., 2001). Sometimes, relationship between leaf water potential (Ψ_w) and RWC of leaves is used for evaluate water deficit magnitude in the plant tissues and cells and predicting tissues resistant to desiccation resulted from water deficit (Ferrat and Lovatt, 1999; Khan *et al*., 2007). It seems that tissues, which 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 *et al*., 1988). Saneoka *et al*. (2004) reported that RWC in lentil

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genotypes under drought stress is lower than non stress conditions. Drought stress damages the plasma membrane so cell content percolates to the outside. Magnitude of this damage can be determined via ionic secretion measurement (Ferrat and Lovatt, 1999; Khan *et al*., 2007). Saneoka *et al.* (2004) in lentil 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 to identify the relationship between RWC, Electrical Conductivity (EC), CMS and growth period and determination of suitable factors including the high yield achievement in lentil genotypes under drought stress and non stress conditions in Ardabil.

MATERIALS AND METHODS

In order to study, the relationship between RWC, EC, CMS and growth period with grain yield in lentil genotypes under well-watered and stress conditions, 11 cultivars, promising genotypes and one genotype, which was selected from local genotypes of Ardabil, compared at Agricultural Research Station in Ardabil, Iran. The region has been placed in semi-arid and cold zone (Alt 1350 m, 48°20′E and 38°15′N). Soil type was clay-loam with pH about 7.7 and depth of 70 cm. The experiments were conducted in non drought (irrigated) and drought (rain-fed) conditions in randomized complete block design with three replications. Lentil seed were sown at a depth of 4-6 cm and a density of 200 seed m^{-2} in 4 m long rows, 4 rows $plot^{-1}$, 25 cm between rows, on 13 April 2005. Plots in no drought conditions were irrigated as required (2 irrigations), while no irrigation was performed in plots of stress. During growth period and after harvesting the following traits were measured.

Leaf RWC: To determine the RWC at 50% flowering stage (after drought stress), selected young and fully expanded leaf samples in each cultivar and replication were taken in plastic pockets and carried to the laboratory, immediately. Then, determinations of the fresh weight of the leaves were performed in distilled water for 24 h in refrigerator (about 5°C). After 24 h passed, turgger weight of the leaves was recorded. In order to determine the dry weight of the leaves, samples were taken in the oven for 48 h at 70°C. RWC of the leaves was calculated as follows (Dhopte and Manuel, 2002):

$$RWC = \frac{W_f - W_d}{W_t - W_d} \times 100$$

Where:
RWC = RWC of leaves (%)
W_f = Fresh weight of leaves (g)
W_d = Dry weight of leaves (g)
W_t = Turger weight of leaves (g)

Leaf electrical conductivity: To measure the Electrical Conductivity (EC) of leaves at the end of flowering period (moisture stress) in all of replications, 20 punched leaf disks were prepared from young leaves randomly, then put into 20 mL distilled water. Afterwards, these samples carried to the refrigerator (about 5°C) and after 24 h, electrical conductivity of leaves were read using sensitive EC meter. Eventually, EC of distilled water (as control) were subtracted from these rates and the EC of leaves were obtained.

Growth period length (maturity): In order to determine the number of days from planting to ripening, when one third of plant down was yellowish and color of pods became yellow/green, ripening time was recorded.

Grain yield: Grain yield was harvested after deleting 0.5 m from end of two middle growing lines and full deleting of two around lines, from each plot that was 1.5 m^2 area then, grain yield was weighed using sensitive balance scale with 0.01 g accuracy and eventually was changed into the kg ha^{-1} unit.

Stress Intensity (SI): To evaluate drought tolerance of genotypes under study, we used the following equation (Fernandez, 1992):

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

Where:
Y_s = Mean total yield in stress conditions
Y_p = Mean total yield in normal conditions

The whole data was analyzed using MSTATC software. Combined analysis of variance was done according to the expected value of mean squares software. Simple correlation of studied traits was measured. The means were tested using Duncan's multiple range tests at 5% level.

RESULTS

Grain yield: Simple and combined analysis of variance revealed the significant difference between genotypes in both rain-fed and irrigated environments at 1% probability level. In this experiment, drought stress intensity was 29% (SI = 0.29). This shows that lentil grain yield was decreased because of the water deficiency. Under this experiment conditions, the most grain yield loss under stress conditions compared with the control one, was >29%.

The means pertaining to the grain yield in Table 1 shows that ILL 8095, ILL 9893 and ILL 6031 genotypes were located in a, ab and abc groups with 1075, 1013 and 972 kg ha^{-1} yield and ILL 8173, ILL9832, ILL 1878 and ILL 8146 genotypes were located in group (a) with 474.4, 456.6, 432.1 and 476.6 kg ha^{-1} yield, respectively.

Total mean yield (Table 2), under irrigated conditions was 868.483 kg ha^{-1} and under rain-fed conditions was 612.906 kg ha^{-1}. Grzesiak *et al*. (1996) reported yield reduction under drought stress.

Duration of growth period: Results revealed the significant difference between genotypes as the number of day till maturity in both rain-fed and irrigated environments at 5% probability level.

The significant differences show that among studied genotypes there is sufficient variation. Mishra *et al.* (2007) have reported high variation as growth period length in lentil genotypes.

With regard to the mean comparisons of the number of day till maturity (Table 1), the ILL 9832 genotype with mean 104.2 days growth period, was the latest maturity genotype placed in group (a) and the ILL 6031 genotype with mean 90.5 day growth period was the earliest maturity genotype placed in group (e).

Total number of day till maturity under irrigated conditions was 99.500 and under rain-fed conditions was 94.389 (Table 2) and shown that growth period of studied lentil genotypes was affected by environment and water deficiency caused the genotypes become mature earlier.

The correlation among the traits such as the number of days till maturity with grain yield is negative and very significant (r = -0.78**) (Table 3).

RWC: There were significant differences at 5% probability level. It was cleared that differences among genotypes, environments (rain-fed and irrigated) and interaction effects of genotype x environment were significant at 1% probability level. These significant differences mean the phenotype variation among genotypes as RWC. So, the selection for this trait is possible. Costa-Fraca *et al.* (2000) reported that in combined variance analysis among genotypes and environment (rain-fed and irrigated) as RWC in lentil, there was significant variation, While Saneoka *et al.* (2004) reported no significant differences among lentil genotypes, but under environmental conditions (rain-fed and irrigated), there was significant differences at 5% probability level. According to these traits (Table 1) ILL 8095 genotype with 70.99% the maximum RWC, was placed in group (a) and ILL 8173, ILL 9832 and ILL 8146 genotypes with 56.16, 56.14, 56.36 and 56.38% were placed in other groups. Total RWC (Table 2) under irrigated conditions was 72.763 and in rain-fed conditions were 51.081. The results of this research were in accordance with the Ferrat and Lovatt (1999) on *Phaseolus vulgaris* L. and *P. acutifolius*, Irigoyen *et al.* (1992) on alfalfa, Oukarroum *et al.* (2005) on barley and Khan *et al.* (2007) on faba bean. Between RWC and grain yield there was positive and very significant correlation (r = +0.97**) (Table 3). Positive and very significant correlation means the genotypes that had high grain yield, included high RWC.

Table 1: Mean comparisons of promising lentil genotypes under rain-fed and irrigated conditions (Combined analysis) based on DMRT

Genotypes	Days to maturity	Electrical conductivity (μμos cm^{-1})	RWC (%)	Grain yield (%) (kg ha^{-1})
ILL 8173	99.33b	35.57abcd	56.16d	474.4f
ILL 9919	98.33b	31.28bcd	61.91bc	691.7e
ILL 9832	104.2a	27.67d	56.14d	456.6f
ILL 323	98.17b	34.20bcd	60.98cd	804.9cde
ILL 1878	98.67b	43.62a	56.36d	432.1f
ILL 8146	97.00bc	39.40ab	56.38d	476.6f
ILL 6031	90.50e	30.60cd	65.52bc	972.0abc
ILL 7677	97.83b	38.13abc	62.50bc	768.0de
ILL 9893	94.50cd	32.10bcd	66.97ab	1013.0ab
ILL 8095	92.83de	30.60cd	70.99a	1075.0a
ILL 8105	97.50b	35.87abcd	63.38bc	838.08cde
Native genotype	94.50cd	39.91ab	65.78bc	884.9bcd
LSD (5%)	2.764	7.503	4.635	155.500

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

Table 2: Total mean values for the traits of promising lentil genotypes under rain-fed and irrigated conditions

Traits	Stress (rain-fed)	Non-stress (irrigated)
Days to maturity	94.389	99.500
Electrical conductivity	40.352	29.472
RWC	51.081	72.763
Grain yield (kg ha^{-1})	612.906	864.483

Table 3: Simple correlation of traits among promising genotypes studied

Traits	Days to maturity	Electrical conductivity	RWC	Grain yield
Days to maturity	-	0.01^{ns}	-0.77**	-0.78**
Electrical conductivity	-	-	-0.32^{ns}	-0.34^{ns}
RWC	-	-	-	0.97*
Grain yield	-	-	-	-

Ns * and **: Insignificant, significant at the 5 and 1% levels of probability, respectively

CMS: There was significant difference at 1% probability level between genotypes with rain-fed and irrigated environments, but their interaction effect was not significant. Regarding to the genotypes mean comparisons related to this trait (Table 1) it was cleared that ILL 1878 genotype with 43.62 μμos cm^{-1} electrical conductivity was located in group (a) having the most electrical conductivity rate and was considered as the most cell membrane injury. ILL 9832 genotype with 27.67 μμos cm^{-1} electrical conductivity was allocated to the group (d) and ILL 8095 and ILL 9893 genotypes with 30.60 and 30.66 μμos cm^{-1} electrical conductivity, were placed in (cd) group with the lowest electrical conductivity among studied genotypes and less cell membrane injury, respectively. Average total of this trait (Table 3) under irrigated conditions (29.47 μμos cm^{-1}) and under rain-fed conditions (40.352 μμosol cm^{-1}) show that there is significant difference in two environments as cell membrane sensitivity among genotypes. The correlation between EC and grain yield was negative and non-significant ($r = -.32^{ns}$) (Table 3).

DISCUSSION

Significant differences between genotypes and environments (rain-fed and irrigated) show that there is phonotypical variability to select the superior genotypes in lieu of grain yield. Anjam *et al*. (2005) reported significant statistical differences among the different growth traits in lentil.

The late-mature, ILL 9832 genotype had the less value of grain yield and the early-mature ILL 8095 and ILL 9832 genotypes were the best genotypes in terms of grain yield (Table 1). So, we can say that the early-mature genotypes complete their growth period length before drought and heat stresses more than the end of growth season under rain-fed conditions.

By this, they keep safety from the drought stress effect at the end of growth season, approximately. Significant and negative correlation among traits such as number of day till maturity with grain yield shows that the effect of late-maturity on the grain yield has been negative, because ILL 9832 genotype was the latest- maturity genotype with the maximum growth period length. While, grain yield was located in sensitive group (f) to drought of 456.6 kg ha^{-1} minimum (Table 1). Also, Saneoka *et al.* (2004) reported that there was significant and negative relationship between grain yield and the number of day till maturity, which confirms the results. In contrast, Pagter *et al.* (2005) reported the significant and positive relationship between growth period length and grain yield. So, it is suggested to pay attention to this trait more than any other traits to breed the lentil crop under rain-fed conditions. It appears to be more suitable aspect to select the early-mature or medium-mature genotypes since, one of the most important programs of rain-fed researches institute in ICARDA to develop tolerance of lentil to drought is producing the genotypes that possess traits such as early-maturity and early-flowering (Mishra *et al.*, 2007).

Leaf high amounts of RWC means that the plant can improve its inner aquatic relations under stress conditions. In terms of the mentioned trait, ILL 8095 genotype was located in better genotypes group and ILL 8173, ILL 9832, ILL 1878 and ILL 8146 genotypes were located in low-yielding and sensitive ones against drought (Table 1). So, we can say between grain yield and leaf RWC there is positive relationship. ILL 8695 genotype, compared with the other genotypes was part of the best genotypes as RWC and grain yield. So, this trait can be effective to evaluate the tolerance to drought and recognition of the best genotypes. Correlation between the RWC and number of day till maturity was significant and negative and it means that the late-mature genotypes compared with the early-mature ones have less RWC. The genotypes which remain high leaf RWC under severe and water stress conditions show the high yield. Tissues keeping high amounts of RWC are more tolerant to drought conditions and plasmolysis (Irigoyen *et al.*, 1992). Saneoka *et al.* (2004) reported that RWC in lentil genotypes under drought stress is less than normal conditions. Costa-Fraca *et al.* (2000) stated that yield is decreased under drought stress conditions. Khan *et al.* (2007) evaluated the physiological traits depending on the tolerance against drought stress in broad bean and reported that the RWC significantly was decreased during the stress and the tolerant varieties possessed the higher RWC than non tolerant ones.

Under rain-fed conditions, the cell wall is destroyed because of the water deficiency and in such a condition, different inner ions of the vacuole are percolated into the intercellular solution as a liquid and caused to increase in the electrical conductivity rate and eventually, decrease in the CMS compared with the irrigated conditions. ILL 9832 genotype was located in the tolerant group to drought in terms of CMS, but was not part of the better genotypes as grain yield. This genotype was located in the late-mature genotypes group, but ILL8095 and ILL 9893 genotypes were part of tolerant genotypes to drought as CMS (Table 2). Existing of the mentioned contradiction to know the better genotypes means that introduction of tolerant genotypes to drought stress only by this way can not be suitable. Measuring by this way needs to high accuracy. Costa-Fraca *et al.* (2000) reported that there is significant difference among lentil genotypes as electrical conductivity.

The relationship between these traits to know the tolerant varieties to drought stress with high yield can be positive. Saneoka *et al.* (2004) demonstrated that CMS has not near relationship with yield and has not ability to determine the varieties with high yield and tolerant to drought stress lonely. Chandrasekar *et al.* (2000), reported the positive relationship between CMS and yield to introduce the high productive and tolerant varieties to drought stress. It should be said that to know the superior genotypes, we must avoid relying only on CMS, since among the genotypes tolerant to the drought (ILL 9832, ILL 6031 and ILL 8095) the ILL 9832 genotype had the less grain yield under two environments. Saneoka *et al.* (2004), who evaluated this trait in lentil reported the similar results.

CONCLUSION

Among the evaluated genotypes, there were significant variations in terms of grain yield, RWC and number of day till maturity in two environments (rain-fed and irrigated). Evaluated traits showed that ILL 8095, ILL 9893 and 6031 genotypes had the most grain yield and the highest RWC and were among early-mature genotypes. ILL 9832 genotype compared with the other evaluated genotypes was part of tolerant genotypes to drought stress and was the latest-mature genotype as CMS, but was part of less productive and sensitive ones to drought stress as grain yield. Simple correlation among the studied traits with grain yield shown that there was significant and positive correlation between RWC and grain yield. Existing of the positive and significant correlation between RWC and grain yield shows that the use of these traits can be beneficial in breeding programs. Correlation between grain yield and number of day till maturity was significant and negative, but correlation between grain yield and electrical conductivity was insignificant and negative. Also, grain yield was decreased in rain-fed conditions than irrigation of 29%.

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