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RESEARCH ARTICLE

Study of Morpho-Physiological and Biochemical Behavior of Cultivated Legume (*Lens culinaris Medik* Ssp *culinaris*) in Dry Area of Algeria

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The victory over the protein deficiency afflicting millions of human beings is a crucial and urgent task for world agriculture. The legume Lentil (*Lens culinaris Medik*) is one of the largest sources of protein with an average of 25% of all seeds produced worldwide. When used in crop rotation, it fertilizes the soil, as is the case in North Africa and this plant holds an important place throughout the world. In Algeria, lentil is largely growth in the semi-arid zones of the interior plains characterized by various abiotic constraints such as terminal drought which causes significant losses in lentil yield every year. For this purpose, the cases in this study aims to evaluate the effect of end of cycle water stress on the behavior of four varieties of lentil (Syrie 229, Metropole, Balkan 75 and Ibela) and to elucidate the plant's morpho-physiological and biochemical parameters involved in tolerance and to assess varieties which could be grown under water stress conditions. The experiment was conducted in a laboratory and greenhouse with a well-controlled condition. The experiment was arranged in a completely randomized design of two factors (water stress and varieties) with four replications and two treatments. Not treated plants (without water stress application) were utilized as control. The four tested genotypes were subjected to two water regimes, one irrigated throughout the cycle (No stressed) used as a control, the other one stressful from the beginning of flowering till seed's filling stages (stressed). The study covered the variation of the relative water content in leaves as well as the leaf surface, the rate of proline and soluble sugars of the leaves and the total seed protein content. The obtained results showed that the studied genotypes behaved differently to the water stress. Thus, a significant decrease was observed in the relative water content from 75.29% to 70.71% and in the leaf area from 16.76 cm^2 to 13.63 cm^2. An opposite behavior was observed on the osmoticum accumulated as a response to water deficit. A significant increase in proline (150.14 µg/g DM to 203.69 µg/g DM) and in soluble sugars stress (60.42 µg/g DM to 110.21 µg/g DM) was detected in leaves under stressed conditions. The terminal water stress resulted also in an increase in protein content in lentil seeds from a mean value of 22.08% to 24.84%. Regarding the obtained results, Metropole genotype seems to be the most tolerant cultivar to water stress followed by Balkan 755.

Key words: Water deficit; RWC; Varieties; Lentil; Morpho-physiological; Biochemical; Protein; Tolerance

Introduction

The victory over the protein deficiency afflicting millions of human beings is a crucial and urgent task for world agriculture. The legume Lentil (*Lens culinaris Medik*) is one of the largest sources of protein with an average of 25% of all seeds produced worldwide (Van Hameren et al., 2013). The food legume crops, particularly lentil, occupy a prominent place in world agriculture and the main producers are: Canada, the USA, Australia, Turkey, India and Syria. Unfortunately, Algeria is one of the main importing ones. In Algeria, Lentil production was so old, it experienced a significant expansion during the colonial period and since the 2000s, some importance has been attached to this crop thanks to the policy of agricultural renewal following to its wide use in human nutrition as an important source of high protein content, vitamins and minerals. However, the low yields recorded each year are the result not only of farming practices but also of environmental conditions where water stress remains the main factor limiting yields (Adda et al., 2013). Moreover, the lentil production areas in Algeria are located in the interior plains and highlands of Constantine, Tiaret: sersou plain, Medea and Setif (Mefti et al., 2001). These areas belong to the semi-arid bioclimatic zones where End–cycle drought is very frequent, seriously altering yield development and consequently lens productivity (Hamadache., 2014).

In view of this situation, it will be necessary to develop a strategy to escape and reduce the effect of water stress. According to (Hassani, 2009), the selection of drought tolerant plants appears to be one of the most effective solutions to mitigate the effect of these constraints. This strategy is based on the knowledge of mechanisms developed by plants to adapt to environmental conditions. The Lens crop (Lens *culinaris*, Medik) is particularly sensitive to drought during the flowering and grain formation stages which negatively effects yield component and thus causes significant losses in grain yields (Hamadache., 2014). In addition, according to many authors, Plants have involved many mechanisms for drought stress tolerance including a number of physiological, morphological and biochemical processes such as maintaining of water-use efficiency, reduction in plant height and leaf areas and an accumulation of osmoregulators. For this purpose, this work aims to study the effects of end–of cycle water stress on the behavior of four varieties of lentil using morpho- physiological and biochemical characteristics of water deficit tolerance.

Materials and Methods

The plant material

The plant material used consist of four varieties of lentil from different origins selected at the ITGC stations of the technical institute for field crop of Tiaret and which are in seed production in Algerian semi-arid areas (Table 1).

Table 1. Origin of tested plant material.

Genotypes	Origins	Agronomic characters	Sensitivity to drought
Syrie229	Icarda (syria)	Early, round seed (microsperma), green yellow color, high yield, HWS <4 g	Mid tolerant
Metropole	French	Semi-late, upright habit, high-yield, large seed (macrosperma), HWS> 6g	Tolerant
Balkan 755	Icarda (syria)	Semi late to late, upright habit, large brown seed , medium yield, HWS> 6g	Sensitive
Ibela	Spain	Early, semi-upright, , broad pale green seed ,medium yield, HWS>6g	Mid tolerant

HWS: Hundred weight seed (Source: Hamadache, 2014)

The experimental device

The experiment was conducted under controlled conditions in a greenhouse in the University of Tiaret (Algeria) during the 2016/2017 crop season in order to evaluate the behavior of four varieties of lentil under dry conditions applied from flowering stage and to identify mechanisms involved in end-of-cycle water stress tolerance. The seeds were germinated in Petri dishes in incubator at 25°C, after appearance of the radical; the germinated seeds were immediately transferred into PVC cylinder of 50 cm in length and 8 cm in diameter at the rate of 04 seedlings by cylinder. The substrate used was a mixture of sand, soil and compost in proportion of 2:1:1. The cylinders were arranged as a randomized blocks design with four replications and two water regimes were applied, one irrigated throughout the cycle (NO Stressed: NSTR) and the other one Stressful from the beginning of flowering (stressed: STR) to seeds filling stages. As soon as the first flower appears, the No stressed blocks continued to be watered throughout the cycle once/48 hours at 250 ml/pot, while the stressed ones received a water supply only once a week at the rate of 210 ml. The studied parameters were essentially related to some morpho-physiological and biochemical characteristics of the leaves. The total seeds protein content was also determined.

Measurement and analysis

The relative water content RWC in %.

It was determined by Barrs and Weatheley, (1962). The excised leaves were immediately weighed to give initial weight Wi, then, they were stored in distilled water in the dark at 4°C for 24 hours, after full turgidity, the leaves were wiped with blotting paper and weighed again to give the full turgidity weight Wft. Then, they were placed in an oven for 48 hours at 80°C and the dry weight Wd was determined. The RWC was calculated by formula:

$$RWC\% = (W_i - W_d)/ (W_{ft} - W_d) \times 10$$

The leaf area LA in cm^2:

The leaf area was directly measured by an Automatic area meter (model AAM-8) with a resolution of 1 cm^2.

Proline determination

Proline content was determined According to TROLL and LINDSLEY (1958) method improved by Lahrer and Magne cited by Leport (1992); a plant sample composed of 100 mg of dry leaves matter was treated with 40 % of ethanol then heated in water bath at 80 °C for 10 minutes then 1 ml of the extract was added to a mixture of distilled water, acetic acid (60%) and ninhydrine (500 mg/100 ml) and heated again in water bath at 92°C for 25 minutes. After cooling, 3 ml of Toluene were added, then stirred and left in the dark. Two hours later, the absorbance was read at 528 nm with a 1200 UV Spectrophotometer. Proline concentrations were determined using calibration curves.

Soluble sugars determination

Soluble sugars determination was carried out according to Shields and Burnett (1960) method used by Rekika (1997) based on the extraction of soluble sugars (glucose, fructose, and saccharose) by the maceration of a plant sample composed of 100 mg of dried leaves in 2.5 ml of 80% Ethanol for 12 hours (reagent A). In the Other hand, a reagent B was prepared four hours before testing from 01 g of Anthrone in 1l of sulfuric acid. The reagent A was diluted 10 times with Ethanol then, 4 ml of reagent B were added, then, stirred and heated in water bath at 92°C for 10 minutes. After cooling, the optical density was read at 580 nm with 1200 UV Spectrophotometer. The obtained values were plotted on a standard curve.

Determination of total seed protein content

The total seed protein content was determined using total seed N value multiplied by 6.25. The nitrogen content of the seeds was analyzed according to The Kjeldahl method (Kjeltek 1002, Manuel part N°1000 1535, Técator AB).

Statistical analysis

The analysis of variance and the correlation matrix were carried out with the Software statbox version 6.10. Means and homogenous groups were compared to the student Newman Keuls (SNK) test at 5% level of probability. The histograms were constructed with Excel 2007.

Results

The relative water content (RWC)

Leaves' relative water content was influenced by the water stress (P=0.003). Genotype variations within the variability conducted do not cause any significant variation (P>0.05) as well as the interaction between the two factors, which is not significant on the expression of this parameter (P>0.05) (Table 2). Our statistical results were highly significant for the water treatment effect on the relative water content at 5% of probability and no significant for the genetic variability and the interaction (water treatment × genotypes), the four genotypes studied behaved similarly for this parameter (Table 2). According to the SNK (Student Newman Keuls) comparison test at P<5%, the NSTR treatment (control) exhibited the highest value of RWC with 75.29% and the STR one had the lowest RWC with a mean value of 70.71%. Thus, the Metropole variety retained a high RWC (74,3%) under water stress conditions following by Syrie 229 and Ibela (71.22% and 71.19 % respectively) while the cultivar Balkan 755 registered the low value of RWC with a rate of 66.12% of RWC. The reduction in the value of RWC of all the studied varieties fluctuates between 2.8 % to 9.7 % compared to the control treatment (Figure 1a).

Table 2. Statistical analysis results of the morpho- physiological parameters.

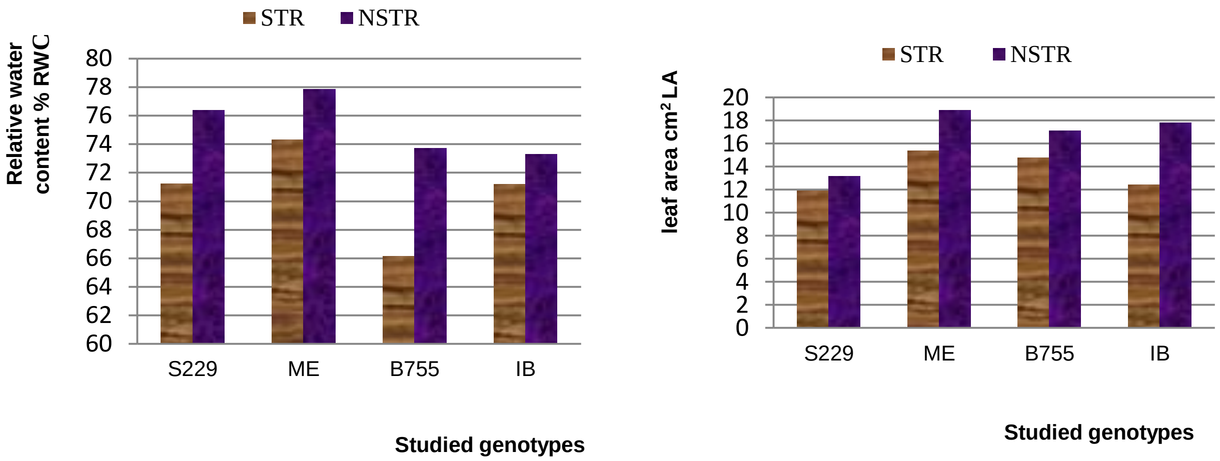
Variables	Water stress effect (F1)	Genotype effect (F2)	Interaction effect (F1*F2)
RWC%	.003**	.210 ns	.803ns
L a cm^2	.000***	.000***	.012**

***: very highly significant at 5% probability, **: highly significant *: significant, ns: not significant

The leaf area (La)

The results provided in Table 2 showed a very highly and highly significant effect of the imposed water regimes, the genetic variability tested and their interaction (F1 × F2) on the expression of the leaf area parameter at 5% of probability. the water stress reduced the leaf area (P=0.000). The terminal water stress induced at flowering to seed filling stages affected the leaf area slightly, thus, the No stressed (control) plants registered the highest L.A of 16.76 cm^2 and declined to 13.63 cm^2 for the leaves of stressed plants. For the genotype factor, four homogenous groups were obtained by the Student Newman-Keuls comparison test, it showed that the highest leaf area was recorded by the Metropole cultivar with a value of 17.15 cm^2 and the lowest one was given by the

Syrie 229 genotype with a leaf area of 12.54 cm^2. Thus, the reduction of foliar areas of all tested genotypes under water stress conditions fluctuated between 9% to 30.2 % compared to the control (Figure 1b).



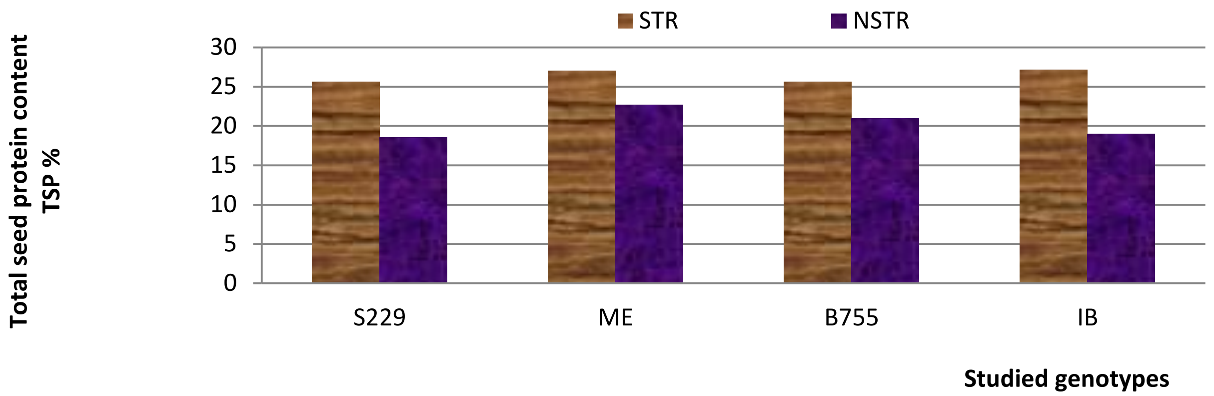
(b)

Test drugs: Significant from normal control, * P < 0.0 5; Mean=Mean values of four replications.

Figure 1. Effect of water stress on the relative water content (RWC) (a) and the leaf area (L a) (b) of the four lentil genotypes studied.

The seed protein content

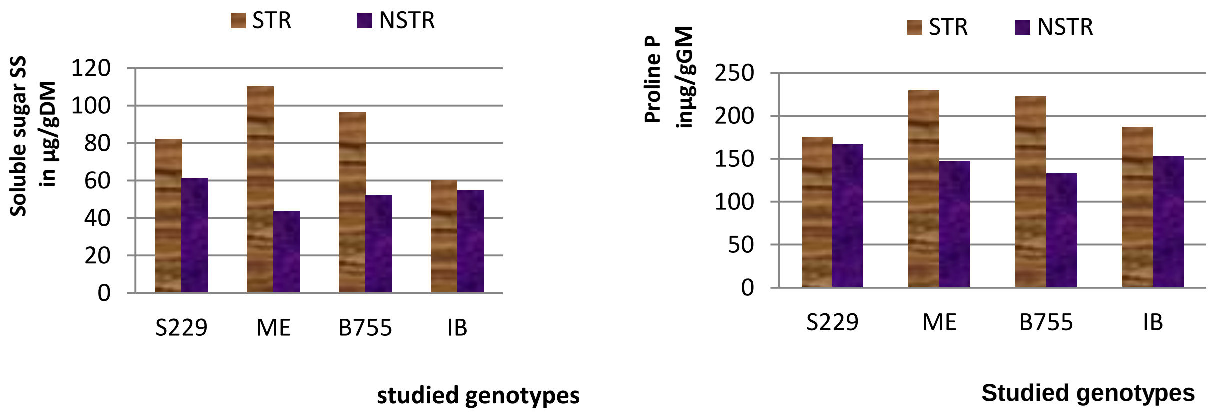
The results of analysis illustrated in Table 3, showed that the total protein content resulting from the accumulation of nitrogen in the grain was strongly influenced by the water situation adopted, thus, a very highly significant to highly significant effects of the water regimes, the genetic variability and their interaction were observed on the variation of this parameter (P=0.000). According to the Student Newman Keuls test, for the water stress factor, the highest level in protein was obtained in stressed plants with a mean value of 26.84 % while, In favorable water supply conditions, the protein content accumulated in the seeds was significantly lower (20.29%). For the genotype factor, Metropole cultivar accumulated the highest amount of protein in their seeds under water stress conditions (24.84 %), the lowest value was registered by the genotype Syrie 229 with a mean value of 22.08% of protein (Figure 2).



Test drugs: significant from normal control, P < 0.05; Mean=Mean values of four replications

Figure 2. Effect of water stress on the total seed protein content of the four lentil genotypes studied.

Soluble sugars (SS) in µg/g DM
Soluble sugar accumulation is usually used for diagnosing the biochemical state of plants during acclimation and adaptation to different stresses like drought. According to our results, the analysis of variance (Table 3) showed a very highly significant to highly significant effect of the water treatment (P=.000) as well as the interaction (water effect*genotype effect) (P=0.004) on the variation of this parameter except for the cultivars which had the same tendency for the expression of this parameter. Indeed, the means comparison results showed that the stressed plants respond to drought by increasing the amount of sugars in their cells. So, under limited water supply, sugar accumulation increased strongly in the four genotypes. The increase is higher in Metropole (252.77%) than in Balkan-755 (185.50%) than in Syrie-229 (134.02%), However Ibela accumulated the lowest value (110.03 %) (Figure 3).



(b)
Test drugs: significant from normal control, P < 0.05; Mean = Mean values of four replications

Figure 3. Effect of water stress on the leaf soluble sugars content (a), the leaf proline content (b) of the four lentil genotypes studied.

Proline (P) in µg /g DM
Statistical analysis of Proline results revealed a very highly significant to significant effect at P<0.05 of the water regime factor and of the interaction (water effect*genotype effect) on the fluctuation of this parameter but did not vary significantly between cultivars (Table 3). According to the SNK comparison test, under water stress conditions, Proline content strongly increased, however a highest accumulation was observed in Stressed plants with a mean value of 203.69 µg/g DM of proline compared to the control plants where Proline content registered a rate of 150.14 µg/g DM.

Table 3. Statistical results of leaves biochemical parameters and total seed protein content.

Variables	Water stress effect (F1)	Genotype effect (F2)	Interaction effect (F1*F2)
P in µg/g DM	.000**	.622 ns	.037*
SS in µg/g DM	.000***	.089ns	.004**
TSP content %	.000***	.000***	.002**

P: Proline - DM=Dry Matter, SS: Soluble Sugars, TSP Content %: Total Seed Protein Content In Percent. ***:Very Highly Significant At P<0.05, **: Highly Significant,*Significant, Ns: Not Significant.

The means comparison of Proline accumulation showed that under water stress, Proline content increased for all genotypes, the highest accumulation of Proline was observed in Balkan-755 (167.5%) and Metropole (155.88%), however the lowest value was recorded in Syrie 229 (105.03%) compared to those achieved under control conditions where the accumulation of Proline oscillated between a minimum gave by Balkan-755 (132.85 µg/g DM) and a maximum registered by Syrie-229 (166.98 µg /g DM) (Figure 2a).

Discussion

Drought is one of the most vulnerable and frequent abiotic stresses in the Mediterranean area affecting growth and metabolism of all crops; it affects all their physiological and biochemical processes, growth and productivity. Plants experience drought stress when their roots do not get enough water or the transpiration rate is too high (Morgil et al., 2017). Like many other authors, (Idrissi et al., 2013) considered that Lentil crop is strongly sensitive to end-of-cycle dryness, Thus, Drought tolerance assessment is of a great interest in lentil genetic improvement programs to develop varieties which are productive and less sensitive to water deficit. According to the results of our study focused on the effect of the terminal water stress induced from flowering to filling stages on the morpho- physiological and biochemical behavior of four varieties of lentil (*Lens culinaris* Medik), all the parameters studied were affected by this constraint, thus, the water state of plants expressed by the relative water content of its foliar system was affected by the water treatment. The water deficit caused a significant decrease in the values of the relative water content explained by the negative correlation r=-0.36* (Table 4). This result is consistent with those obtained by (Mefti et al., 2001) on Medicago trunculata (L.) populations and (Morgil et al., 2017); on lentil, who showed that any deficiency of water supply perceptible to the plant is expressed by a decrease in the relative water content of its foliar system. The relative water content is the most appropriate parameter to measure the water status of plants under limited water supply conditions, it is an effective criterion for dry environments adaptation of different species, it indicates the state of turgescence of plant tissue and their ability to maintain a level of hydration to guarantee the continuity of its metabolism. The same effect was observed for the Leaf area parameter. The foliar area was strongly influenced by the water stress imposed which is confirmed by the analysis of the correlation matrix (r=-0.59**) in tab 4; thus, the terminal water stress caused a significant decrease in the leaf area of all the studied genotypes, the rate of reduction ranged from 9% to 32 % compared to the no stressed plants. Indeed, reducing leaf area is the immediate response of plants to abiotic stresses; it contributes to the conservation of water resources which allows the survival of plants (Lebon et al., 2004). Our results are in agreement with those of (Gharabi et al., 2018) who noted a reduction in leaf area of olives leaf as a response of salt stress and (Hassani et al., 2008) indicates that decreasing leaf area under stressed conditions is considered as a form of adaptation to salt stress by reducing water losses by transpiration but it causes a decrease in yields due to the reduction of photosynthesis. Our results were also confirmed by Morgil et al., (2017) who demonstrate in a study on physiological Tolerance mechanism in Lentil cultivars under drought stress conditions that Drought stress is characterized by reduced water content, diminished leaf water potential and turgor loss, closure of stomata and decrease in cell enlargement and growth.

Table 4. Correlation matrix between water stress, the morpho-physiological and biochemical parameters of lentil cultivars.

	Water regyme	**Genotypes**	**RWC %**	**LA cm^2**	**SS (µg/g.DM)**	**P (µg/gDM)**	**TSP %**
Water regyme	1						
Genotypes	0,00	1					
RWC %	-0,36	-0,19	1				
LA cm2	-0,59	0,28	0,21	1			
SS (µg/g.DM)	0,66	-0,20	-0,30	-0,28	1		
P (µg/g.DM)	0,64	-0,04	-0,23	-0,34	0,50	1	
TSP %	0,88	0,07	-0,32	-0,36	0,48	0,60	1

RWC: Relative Water Content, L.A: Leaf Area, SS: Soluble Sugars, P: Proline, TSP%: Total Seed Protein.

In addition, the applied water stress from flowering to seed filling stages on four cultivated varieties of lentil caused a strong and significant accumulation of soluble sugars, whose rate had increased in stressed plants with a significant positive relationship (r=+ 0.66**), (Table 4). According to Muuns et al., (2006) and Hassani et al., (2014), the increase in soluble sugar content is the result of the degradation of starch reserves by their rapid conversion into sucrose due to an inhibition of starch synthesis. Indeed, Mishra et al., (2018) reported in a study on lentil physiological and biochemical adaptation under drought stress, that soluble sugars accumulation under water stress is an adaptation parameter, it contribute to the lowering of the osmotic potential by protecting membranes against dehydration. The same holds true for Proline in all genotypes.

This is confirmed by the analysis of the correlation matrix (r=+ 0.64**) (Tab.4). Indeed, the increase of proline level under stressed conditions was greater in Balkan 755 and Metropole with a rate of 167.5% and 155.88% respectively. The accumulation of this amino acid is a phenomenon of adaptation to water deficit allowing to plants to maintain their turgidity by reducing their hydric potential (Mefti et al., 2001), this type of tolerance allows the plant to perform its physiological functions normally. Hassani et al., (2008) reported that proline accumulation under salt stress has a cellular protection function and contributes to osmotic adjustment. Nourri et al., (2002) and Azzouz, (2009) for their part, indicate that Proline accumulation can confer tolerance to plants under stress conditions by developing an antioxidant system that can play a role as an osmotic adjustment indicator.

This is also the case for the protein content that had increased in seeds. Our results are consistent with those of Abid et al., (2015), who showed an increase in Proline, soluble sugars and protein content in *faba* beans (*Vicia faba* L) under water deficit conditions and Ardakani et al., (2013) which reported an increase in protein content under water deficit in soybeans. Pierre et al., (2014) for his part, indicate that the water stress at flowering and grain formation stages leads to a reduction in yield and weight of a thousand grains and increased protein levels in wheat grains. Similarly, Sial, et al., (2005), indicate a reduction in the weight of the thousand grains in Wheat due to the shortening of the filling period under the effect of water stress and a 4% increase in the protein content of the grains.

The protein content is the translation of nitrogen content in the grain, it is not managed at the end of the crop cycle when the stress has been declared but it is accumulated throughout the cycle of the plants. Moreover, Triboi (2014) reported that a post-flowering water deficit reduces the availability of carbon for the grain, which affects starch content. On the other hand, the levels of proteins are often increased because the metabolism linked to proteins is less affected than those related to carbon accumulation. In addition, some proteins (LEA and aquaporins) are synthesized as a response to drought; they are accumulated in dry seeds and played a role in plant adaptation (Arvalis., 2013).

Conclusion

From the work focused on the study of some morpho-physiological and biochemical parameters of the leaf and seed protein content of four varieties of lentil under terminal water stress conditions, we conclude that the studied genotypes significantly differed in their tolerance to end of cycle water stress applied from flowering to filling grain stages. All morpho-physiological and biochemical traits of lens cultivars were strongly influenced. The applied stress resulted in a reduction in relative water content RWC, in leaf area and in chlorophyll content of the foliar system of all studied genotypes accompanied by a strong accumulation in osmoticum (proline and soluble sugars) allowing to plants to perform their vital function normally. Our results showed also an increase in seed protein content under water stress, the metabolism of proteins is less affected by stress. The studied parameters are of a great importance in lentil genetic improvement programs. Finally, regarding the response of the induced plant material used to cope with this stress, Metropole cultivar showed an appreciable level of tolerance compared to the other varieties tested.

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