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Research Article

CHARACTERIZATION OF SOME MORPHO-PHYSIOLOGICAL RESPONSES, SEED CHEMICAL COMPOSITION AND GRAIN YIELD IN LENTIL VARIETIES (Lens *culinaris Medik culinaris*) UNDER IRRIGATED AND RAINFED CONDITIONS

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ABSTRACT

In Algeria, pulses are rain crops and are drought-prone during their breeding period, resulting in a loss of yield. To this end, four varieties of lentil are tested for their drought tolerance through bio-stress indicators in a field trial during the 2016/2017 crop year. Plants are grown under rained and irrigated conditions (irrigation + rainfall), from the "flowering" stage to the "filling of grains" stage. The results indicated that drought stress significantly decreased the relative water content (13.4%), leaf surfaces (32.21%), and grain yield of all genotypes tested (58.37%). The chemical composition of lentil seeds showed a reduction in starch content (4.41%) and an increase in protein content (32.21%). Potassium ions and Sodium ions were present at the highest and lowest concentrations (747.91ppm- 4.5ppm) in rainfall conditions, respectively, while Ca++, PO4-, and Mg++ concentrations were not affected by drought.

In conclusion, the emphasis on bio-indicators of water stress tolerance at the reproductive period is of great importance in minimizing grain yield losses and the quality of seeds.

Keywords: drought tolerance, breeding period, lentil seeds, rainfall conditions, yield.

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1. INTRODUCTION

Lentil (*Lens Culinaris Medik*, *culinaris*) is the most important legume crop in Algeria after Faba bean and chickpeas, the grain production remains very low and cover only 30% of consumption needs evaluated at 1.01 million quintals and an individual consumption of 2.5 kg/habitant/year. During the last six years (2010-2016), Lentil production has reached an average of 100,713 quintals [1]. As for yields, they remain quite low and have slightly improved in 2015-2016 as a result of farmers' incentives and support measures introduced by the state to improve farm incomes and create employment and reduce imports.

In various parts of Algeria, Lentil constitutes the main ingredient for low income people meals due to its affordability and its consistence of high quality proteins (18-30%), minerals (242-290g.kg^{-1}of K, 5.4-14.4 g.kg^{-1} of P, 54-504mg.kg^{-1}of Fe,), vitamins (βcarotene), fibers and carbohydrates [2,3], it is widely consumed as a soup or joined with vegetables and boiled to a stew (ragout).

In the Mediterranean regions of Algeria such as Mila, Constantine, Guelma, Tiaret: sersou plain, Medea and Setif, leguminous plants (lentils, chickpeas...) are exclusively rain fed but because of the climate change and the rarity of rain, they are subjected to dryness. This latter has a harmful effect on the productivity of these products and their nutritional quality. Thus, the tolerances of these crops to the terminal drought stress remains the best solution [4,5] since irrigation is not possible due to the scarcity of water resources, its high cost equipment and the risk of soil salinity.

Crop resistance to drought has been attributed to different mechanisms leading to different types of responses. Plant responses to this stress can be phenological (shortening of stages), morphological (reduction in height, and in leaf area...), physiological (maintaining high potential, decrease in transpiration...), biochemical (osmotic adjustment by accumulation of osmoregulators) and agronomic (ensuring maximum yield in limited conditions).

Compared to other cultivated food products, lentil is relatively resistant to drought while being very sensitive to water deficit at the reproductive period where it risks important losses in yield and quality [5,6]. The reproductive period (flowering and seed filling stages) is a crucial stage of growth in lentil crops. When drought occurs at flowering, it causes a reduction in the production of flowers leading to pods and the falling of young flowers and pods before seed formation and filling [5]. Seed filling is the final stage of growth. It involves the supply of various components and precursors from the leaves into developing seeds and many biochemical process take place for production and translocation of photo assimilation, importing precursors for biosynthesis of seed reserves, minerals and other functional constituents (carbohydrates, proteins and lipids) [7,8]. According to [8, 9], when occurring at this stage, drought slows down the filling process and reduces its duration, as a result, scalded grains and low grain yields are got.

The chemical composition of lens crop can vary according to cultivators, soil and the climatic conditions of the area. The grain protein content is a major determinant of grain nutritional value of legume crops in general and lentil in particular: its synthesis is mostly related to Nitrogen remobilization from the vegetative organs [7]. Some authors indicated that protein content in seeds was not associated with drought parameters or yields of lentil but with a genetic character and gene effect. Starch is also emerging as a key molecule in mediating plant responses to abiotic stresses, such as water deficit, under drought conditions. Indeed, plants generally remobilize starch to provide energy and carbon; while photosynthesis may be potentially limited, starch degradation in response to stress has often been correlated with improved tolerance [10]. The amount of mineral contents is also influenced by environmental conditions [11]. Thereby, this study was undertaken with a focus on some characteristics of tolerance throught bio-stress indicators, on grain protein content, starch content and some mineral concentrations including grain yield of four varieties of lentil under irrigated and rained conditions.

2. MATERIALS AND METHODS

2.1 Location of the experiment

The field experiment was carried out during 2016/2017 cropping season in Sebaine , station of the technical institute for arable crops (ITGC), located at 35km from Tiaret province west of Algeria at 35°21'N latitude, 1°28'E longitude and 1023 m altitude. This semi-arid area received a mean annual rainfall less than 400mm.

2.2 Weather information at the experimental site

The climate of the experimental site was characterized in 2016-2017 agricultural season by a wet and cold winter; the month of January was very rainy with a cumulative rainfall of 201mm and an average temperature of 3.98°C. Spring was marked by a water deficit from February to July. March and April were almost dry (4mm and 6.8mm respectively), sunny with dominance of dry winds that dried out the surface soils. Monthly, minimum and maximum temperatures as well as precipitation are monitored by an IMETOS weather station located at the experimental site. (Figure 1a, b).

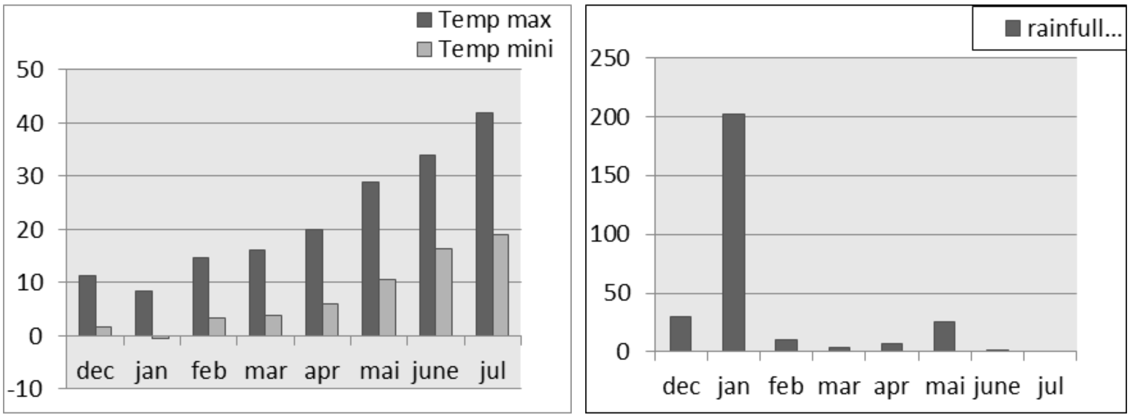


Fig.1. Temperatures and Rainfall data during 2016-2017crop season

2.3 Soil analysis

Prior to sowing, soil sampling was carried out using a soil auger at a depth of 0-20 cm. Soil samples were bulked, dried and sieved with a 2 mm mesh sieve for the determination of the physical and chemical properties. Results were given in Table 1.

Table 1. Soil characteristics in the experimental site

Properties	Soil texture	pH	ECe (ms/cm)	Organic matter %	N %	P_2O_5 Ppm	K_2O ppm
Rates	Silty –clay	8.1	0.25	1.56	0.035	4.36	272.14

2.4 Plant material used

The plant material used consist of four varieties of lentil from different origins selected at the ITGC stations of the technical institute for arable crops (Algeria) and which are in seed production programms and consumed in Algerian semi- arid areas. They are differing in origins and sensitivity to drought stress including a local variety (Metropole) introduced from France during the colonial period, two cultivars from Icarda in Syria (Syrie 229 and Balkan 755) and the last one (Ibela) from Spain.

2.5 Land preparation and planting

The soil was prepared by deep ploughing with a disc plough followed by a pass of cover crop to break up clods, levelled with a power harrow, a phosphate and sulfate fertilizers were applied at 100-50 Kg.ha^{-1} using triple super phosphate (TSP) and Potassium sulfate (K2O).

Urea (40kg.ha^{-1}) was added at branching stage.

The field experiment was arranged in factorial experiment based on a randomized complete block design with four replications. The factors include the water regime with two levels (Irrigated from the flowering to seed filling stages: IRR, Rained: RE) and varieties with four levels (syrie229, Metropole, Balkan 755 and Ibela). Seeds of lentil varieties were previously treated with a fungicide (Apron Star 250 g/100 kg) to prevent attack of soil pest and diseases then sown on 12 December 2016 at a density of 200grains.m-^{2} and a depth of 4 cm using an experimental in-line seeder. Each elementary plot (6m^{2}) was of 5 m long and 1.2 m wide with four sowing lines separated by 30 cm. Each variety was repeated four times in each water regime. Plots and blocks were separated by 1.5m unplanted spaces. Rolling was carried out after sowing to ensure good soil and seed adherence. A post sowing weeding with Gesagard at a rate of 3l.ha $^{-1}$ was also carried out to prevent weed infestation. In the irrigated plot, irrigation was triggered at the beginning of March, coinciding with the starting of flowering, with three water supplies per week, maintaining soil moisture at 100% field capacity. The irrigation was stopped after seed formation and the beginning of leaf senescence. A hand weeding was applied when necessary.

2.6 Crop harvesting and analysis

At maturity, plants of each plot were harvested in 8 of June 2017 manually (the harvest was carried out manually by tearing, which led to losses by ginning), then threshed using a sheave thresher. The resulted seeds were weighed to give real grain yield. Seeds samples were dried at 65°C for 72 hours then stored at 4°C. Before analysis, the grains are reduced to a fine powder which was used for protein, starch and mineral analysis.

The relative water content (RWC) was determined according to [12] method and the leaf area by a direct measure using an automatic area meter (model AAM-8).

The total protein content was calculated by multiplying % nitrogen with the factor6.25. Total Nitrogen content in the grain sample was determined following Kjeldahl's method as described in the device manual of Kjeltek 1002, Manuel part N° 1000 1535, Técator AB.

Starch was determined according to [13] methods**.** A 100mg of fine powdered seeds were homogenized in test tubes with 5ml of 1N KOH then neutralized with 5ml of 1N chloridric Acid. The mixture was boiled in a water bath for 10mn and readjusted to 10ml, after centrifugation, the supernatant was collected to starch determination by spectrometry UV at 580 nm.

Mineral ions were analyzed according to the method used by [14]. A 0.5g of fine powdered seeds were incubated in Teflon tubes with nitric Acid overnight at room temperature then digested under increased temperature for 1 hour. After digestions, the tubes were cooled and 30% hydrogen peroxide were added then incubated for 1 hour at 125°C two times. The samples were dried at 200°C.The cooled digester was resuspended in 2%HNO_3 then filtered through an ash-free filter. The filtrate was recovered for mineral dosing. . The ions: Ca, K, Mg, Na and P were analyzed by inductively coupled plasma optical emission spectroscopy (ICP-OES, ICAP 6300 marque Thermo Fisher).

2.6 Statistical analysis

Statistical analysis of the data was performed with STAT-BOX software version 8.40. Newman Keuls test was applied to compare means of each trait at 5% probability. Principle component analysis (PCA) based on the correlation matrix was used to give relationship between studied parameters.

3. RESULTS

3.1 The relative water content

The results obtained from the relative water content parameter vary significantly under the effect of the water treatments adopted than that of the genetic variability tested and the interaction (water regimes* varieties). The water deficit (Rained conditions) was accompanied by a significant decrease in the values of RWC. The relative water content decreases differently among the tested genotypes, thus, Balkan 755 and Ibela were most affected by the water stress; they registered a significant rate of decrease estimated at 17.32% and 14% with 67.93% and 65.87% of RWC respectively. The reduction in Syrie229 was of 11.61% (71.07%) while, Metropole registered the lowest decrease with a rate of 10.23% (69.76%). Under well watered conditions (irrigation +rainfall), the values of RWC were ranging between 76.91% recorded by Ibela and 82.17% registered by Balkan755 (Table 2).

3.2 The leaf area

The analysis of the value of the leaf area parameter illustrated in Table 2, showed a highly significant effect of both the water situation adopted, the variety factor and their interaction. In the absence of irrigation, under rainy conditions, the leaf area was reduced in all genotypes, Thus, the Balkan755 variety registered the highest reduction with a rate of 24.6% and the lowest decrease was marked by Syrie229(12.83%). Under Well watered conditions (irrigated + rainfall), Balkan 755 and Metropole recorded the highest value of leaf area (18cm^2 and 17.75cm^2 respectively), Ibela registered 15.80cm^2 while the lowest value of leaf area

(13.56cm^2) was achieved by Syrie229.

Table 2. Analysis of variance and mean values of morpho-physiological parameters and grain yield of lentil cultivars under irrigated and Rain fed conditions

Water treatments	varieties	RWC %	L.A Cm	Grain Yield Kg.h^{-1}
Irrigated	Syrie229	80.34	13.56	655.28
	Metropole	77.71	17.75	937.80
	Balkan755	82.17	18.00	689.05
	Ibela	76.91	15.80	672.00
Rainfed	Syrie229	71.01	11.82	288.83
	Metropole	69.76	14.95	370.80
	Balkan755	69.93	13.57	299.70
	Ibela	65.87	13.20	342.50
LSD values		3.02	1.07	0.94
P $_{water\ regimes}$		0.0000***	0.0000***	0.0000***
P $_{varieties}$		0.2036ns	0.0000***	0.0022**
P $_{(water\ regimes*varieties)}$		0.4830ns	0.0125**	0.0168*
C.V%		5.62	4.98	16.32
Type Ecart		4.15	0.73	0.90

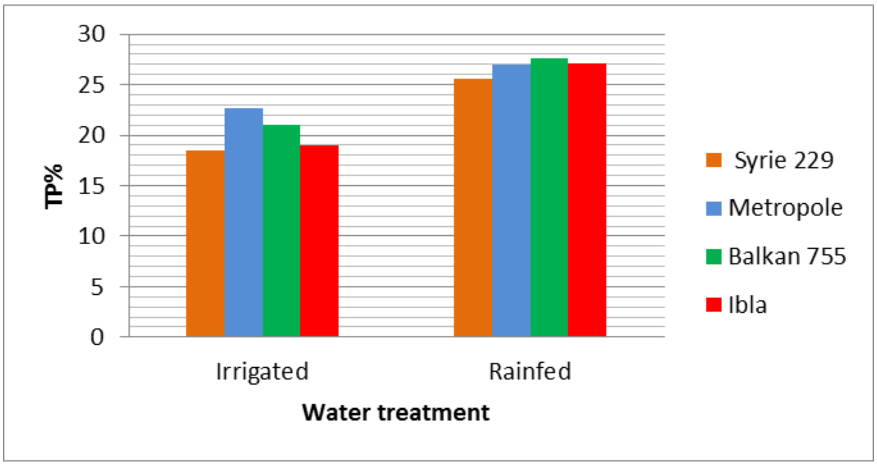
***: P Significant at 0.001, **: P significant at 0.01, *: significant at 0.05, ns: not significant
RWC: relative water content; L.A: leaves areas.

3.3 Total protein content

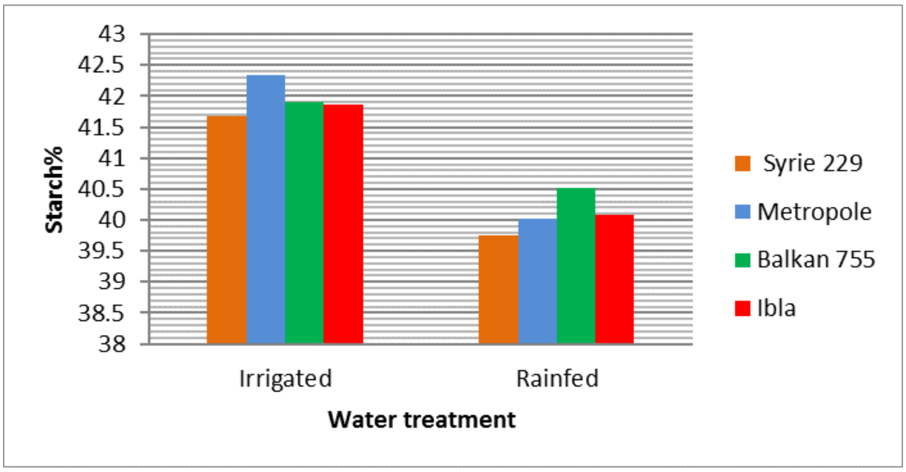
Based on the seed protein data (Figure.2a), all evaluated varieties showed similar responses in grain protein content under the two water treatments. The effect of terminal drought stress on the variation of this parameter was highly significant at the level of 5% probability as well as the nature of genotypes and the interaction (water regime*varieties). As compared to irrigated plants, total seed protein content in stressed plants (under Rained conditions) increased significantly ($P<0.0001$) in both varieties. The mean value of total protein content increased from 20.30% to 26.84 %. The maximum increase of 43.12% was observed in Ibela variety and the minimum increase of 18.89% was reported in Metropole variety.

3.4 Starch content

The results (Fig. 2b) showed that cultivars exhibited neighboring starch concentrations; the varietal effect on the variation of this parameter seems insignificant. However, all cultivars showed a reduction in their starch content under terminal drought stress conditions (Rainfed). The mean value of starch content decreased from 41.94% to 40.09%. The highest decrease was of 5.43% reported in starch content of Metropole variety and the lowest reduction was of 3.29% marked by Balkan 755variety.



(a)



(b)

Fig.2. Variation of Seed Protein content (a) and Starch content (b) of lentil seed varieties under irrigated and Rain fed conditions.

3.5 Mineral composition

The chemical analysis of mineral composition of lentil *culinaris Medik* varieties showed an increase in the accumulation of K as a result to terminal drought. The increase in K+ content varied from 6.40 % (Balkan 755) to 17.40 % (Syrie229). The maximum increase was of 102.75 ppm in Syrie 229 cultivar and the lowest increase of 46.02 ppm was reported in Metropole variety subjected to Rained conditions.

Regarding the content in P and Mg ions, they were not affected by both factors studied (water regimes, varieties and their interaction). Under irrigation treatment, the highest P content was reported by Syrie 229 with a concentration of 33.50ppm and the lowest value of 30.21ppm was exhibited by Metropole variety. Whereas, under drought conditions, phosphor content ranged from 29.36ppm (Ibela) to 32.82ppm (Metropole). Indeed, the grain samples collected from irrigated plants showed a content in Mg ranging from 5.58ppm (Balkan755) to 6.24ppm (Syrie) while those from stressed plants (carried out under Rained conditions), reported a Mg content ranging from 5.78ppm (Syrie229) to 6.15ppm in Metropole variety (Table 3).

The accumulation of Calcium ion in lentil seeds was not influenced by the practice of irrigation (water regime factor no significant), whereas, the effect of varieties factor was highly significant ($P<0.001$). The values oscillate between a maximum achieved by the Balkan755 cultivar of 8.68ppm and a minimum marked by the Ibela variety of 7.12ppm.

As regards the sodium content, the grains collected from Rained plants were poorer in this element. The Na ion was influenced by both water regimes factor, varieties factor and their interaction which was confirmed by the analysis of variance illustrated in Table 3. In the Absence of irrigation, under drought conditions, Na concentration decreased, it showed a mean decrease from 5.91ppm to 4.50ppm with a maximum reduction of 3.35ppm (Ibela) and a minimum decrease of 0.69ppm registered in Metropole variety. The sodium content ranged from 3.76ppm (Syrie229) to 8.15ppm in Ibela variety (Table 3).

3.6 Grain yield

The analysis of variance of grain yield parameter illustrated in Table 2 indicated that irrigation and varieties treatments as well as their interaction significantly affected lentil grain yield. Thus, the grain yield was reduced under Rainfed conditions (only 10.8 mm of rainfall received during reproductive period), the rate of reduction was of 59% in comparison with those obtained under irrigation. Drought at reproductive period of lentil cultivar decreased grain yield in all varieties tested, the lowest reduction was observed in earliest varieties and it was of 49% and 59% in Ibela and Syrie229 respectively. Metropole registered a rate of reduction of 60.5% while the highest reduction of 66.36 % was exhibited by the latest variety Balkan755.Under irrigated conditions, Metropole obtained the highest grain yield with 937kg.ha^{-1}; the lowest one was recorded in Syrie229 variety with a value of 655kg.ha^{-1} .

Table 3. Means results of mineral elements under irrigated and Rained conditions

Characters	Varieties	Water regimes		Pvalue(<0.05)water regime)
		Irrigated	Rained	
K+ ppm	Syrie229	590.32	69307	0.0000***
	Metropole	678.62	751.85	
	Balkan755	718.15	764.17	
	Ibela	715.12	782.57	
Ca++ ppm	Syrie 229	7.80	7.01	ns
	Metropole	7.70	8.19	
	Balkan755	8.52	8.84	
	Ibela	6.98	7.26	
PO4- ppm	Syrie229	33.50	29.76	ns
	Metropole	30.21	32.82	
	Blkan755	31.30	30.25	
	Ibela	32.84	29.36	
Mg++ ppm	Syrie229	6.24	5.78	ns
	Metropole	5.65	6.15	
	Blkan755	5.58	5.90	
	Ibela	5.77	5.82	
Na+ ppm	Syrie229	4.26	3.25	0.0003***
	Metropole	3.73	3.04	
	Blkan755	6.06	5.24	
	Ibela	9.82	6.47	

***: P Significant at 0.001, ns: not significant

4. DISCUSSIONS

In the arid and semi-arid Mediterranean areas such as Algeria, plants encounter various abiotic and biotic stresses during their growth cycle. A significant negative effect occurs to the development, metabolism and yield of plants that undergo stress [15,6]. Among the abiotic stresses, terminal drought has been considered as one of the main factors responsible for crop failure in global agriculture leading to drastic reductions in yield and the quality of seeds and grains [16,17]. Lentil is grown in Algeria as a Rain fed crop; it is facing to terminal drought which occurs at the reproductive period. It is more sensitive to drought at flowering and filling stages [5,18].

Drought tolerance in crops has been attributed to different mechanisms leading to different physiological, morphological, agronomical and biochemical responses. According to our results, the terminal water stress at reproductive period of Lentil crop was associated with a significant decrease in RWC values. The rate of reduction was between 10.23% and 17.32% and tolerant cultivars had the highest RWC. Similar results were observed by [5,6, 18,19] under controlled conditions on lentil cultivars. The relative water content is widely used to asses' plant water status and also as an index for dehydration tolerance. It is recommended by previous studies as an effective physiological indicator of water status [21,22].

Regarding the morphological parameter expressed as a leaf area, was very sensitive to the water treatments (r=-0.67**). In fact, the reduction in leaf area values as a result to terminal drought was between 1.74cm^2 and 4.43cm^2 with a significant genetic variability (P=0.000). The decrease in leaf area parameter is considered as a form of adaptation to water stress, it is the immediate response of plants to water deficit and it contributes to the conservation of water resources by reducing transpiration [23,25]. The leaf area is an important determinant of transpiration, the reaction of plants to drought is to reduce the area of their foliar system. These results go in agreement with those obtained by [6,24,25]. This is confirmed by the significant relationship established between the relative water content and the leaf area parameter(r=0.51**). A higher leaf area induces a higher transpiration.

Drought at seed filling is crucial for determining seed weight, seed composition and therefore quantitative and qualitative yield. Drought can hinder the accumulation of various seed constituents primly protein and starch. The results illustrated in the present study show an increase in seed protein content under Rain fed conditions. All evaluated varieties provided similar responses. The protein content in grains was higher under water deficit and lower under irrigation conditions. The maximum increase of 42.12% in lentil seed protein content was observed in Ibela genotype and the lowest increase of 18.89% was obtained with Metropole variety. The effect of water stress on lentil seed protein content was evaluated in several studies and different responses have been observed. An increase in protein content under drought was reported by [17,26-28] on many legume species. According to [30], the water shortage can reduce production quantity without reducing the amount of protein in seeds. The synthesis of grain protein content is mostly related to Nitrogen remobilization from the vegetative organs, drought during grain development may reduce the carbon fixation by partial closure of stomata and the rate of its accumulation declined which accentuates the increases of grain protein concentration [31,32].

As opposed to the protein content which has increased in lentil grains of the studied varieties, the terminal drought induced a significant reduction in starch content. This result goes in agreement with the finding of [33-35]. According to [8,11], plants generally remobilize starch to provide energy and carbon at times when photosynthesis may be potentially limited, its degradation in response to stress has been correlated with improved tolerance in many crops.

The findings of the present investigation on lentil mineral composition indicated that the concentration in Ca++, total P and Mg++ in lentil seeds remained unchanged throughout the water treatments (Rain fed and irrigated), the content in Calcium was highly dependent of the genetic variability tested. In contrast, K+ and Na+ accumulations were mostly affected. In fact, under Rained conditions, K+ had increased (11% of increase) and Na+ had decreased (25%). The results are similar to the finding of [20,29,35,36]. Potassium ion play a key role in formation of the osmotic adjustment ability, maintains higher turgor pressure, relative water content and lower osmotic potential, thus improving the ability of plants to tolerate drought stress [37]. Under stressed conditions, tolerant plants try to keep higher K^+ and lower Na^+ concentration in the cytosol. This is done by the regulation of the expression and activity of K^+ and Na^+ transporters [37] which is in agreement with [38,39]. [38] For his part detected an increase in Ca, K and total P in field and green house experiment on common bean in response to drought stress.

The water deficit induced at flowering and seed filling stages had a more negative impact on grain yield. A significant reduction in grain yield was observed in all lentil varieties under Rain fed condition where Metropole and Syrie229 varieties had the highest and the lowest grain yield (370.8kg.h^{-1} and 282.8kg.h^{-1}) respectively. The decrease in grain yield under limitant water supply was reported by [5,8,39] on lentil cultivars. According to [6] drought at reproductive period severely influenced the biochemical process associated with seed filling causing a drastic reduction in seed yield as a result of physiological factors and metabolic alteration of Photosynthetic components (starch metabolism,...) limiting the supply of grains with sugar thus reducing their size and number.

4.1 Relationship between studied parameters

Terminal drought affects many morphological, physiological, productivity and grain chemical composition parameters which were interlinked with each other and induce different responses and modifications to mitigate the effect induced by drought. Using the principal component analysis (PCA) based on the correlation matrix (Figure. 3), It was observed a positive relationship between RWC and leaf area parameters(r=0.51**) which suggest that under drought stress a higher leaf area caused more transpiration by this reason the relative water content decreased. The results of this study indicated that a lower leaf area in lentil cultivars is desirable under drought stress. As relationship were established between RWC and seed protein content (r=-0.75**), between RWC and starch content (r=0.63**), between RWC and Potassium content (r=-0.53**) between RWC and grain yield (r=0.65**) and between grain yield and Seed protein content (r=-0.67**), we noted that a reduction in RWC value had a drastic effect on grain yield due to metabolic alterations of the photosynthetic components (starch metabolism., protein synthesis,...), a higher seed protein content and a lower starch content were observed under Rainfed conditions with an increase in K and a reduction in Na contents. The other minerals analyzed (P, Ca and Mg) were not as affected by drought during the reproductive period of lentil growth.

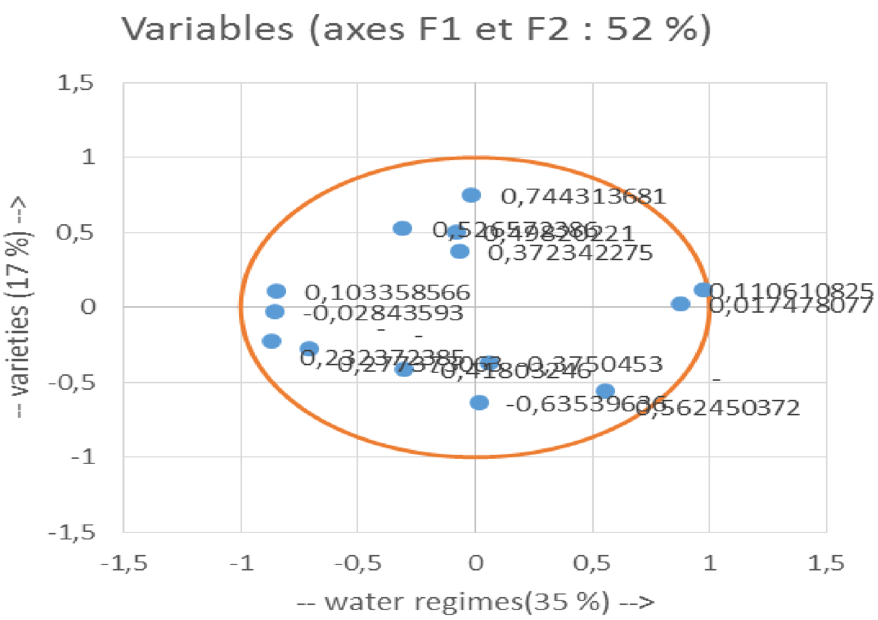


Fig. 3. Principle component analysis (PCA) based on the correlation matrix between studied parameters

5. CONCLUSIONS

The effect of drought during the reproductive period of lentil cultivation offers a wide choice of morphological, physiological, biochemical and nutritional criteria involved in tolerance to terminal water stress and can serve as a basis for rapid selection tests of efficient and resistant genotypes. The finding of this study indicated that drought at the time of flowering and seed filling stages impacted the morpho physiological parameters studied, the seed composition and the seed yield in lentil varieties. The first response of lentil plants to water stress was a reduction in leaf area parameter and a decrease in RWC values. A high relative water content was maintained by reducing transpiration which affect the photosynthetic capacity by partial closing of stomata, thus the remobilization and the accumulation of protein, starch and minerals were affected by terminal drought, indeed a higher RWC was associated with an increase in total protein content and a degradation of starch in grains of tolerant varieties with an important accumulation of K+ ion. The rate of total P, Ca++ and Mg++ minerals remained unaffected by drought. The effect of terminal drought was detrimental on Lentil grain yield and the highest yield was obtained under irrigation. In conclusion, the emphasis on bio-indicators of water stress tolerance at the reproductive period is of great importance in minimizing grain yield losses and the quality of seeds.

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