Physiological and biochemical changes in Moroccan barley (Hordeum vulgare L.) varieties submitted to drought stress

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Abstract

Barley (*Hordeum vulgare* L.) is the second cereal consumed and cultivated by the Moroccan population. However, with climate change, it is predicted that frequent drought periods can cause serious plants growth problems. Thus, the selection of drought tolerant barley varieties becomes highly required to ensure the security of barley's need. Here, we tested drought tolerance of nine barley Moroccan varieties (ADRAR, AMALOU, AMIRA, FIRDAWS, LAANACEUR, MASSINE, OUSSAMA, TAFFA, and TAMELLALT) based on physiological and biochemical parameters. Drought stress was applied by maintaining filed capacity of pots at 40% (90% for the control), and plants were randomly arranged in greenhouse, under 25°C and natural light conditions. Drought stress reduced relative water content (RWC) and chlorophyll content (SPAD index). It also induced a significant increase of electrolytes leakage, hydrogen peroxide, malondialdehyde (MDA), water soluble carbohydrates, and soluble proteins contents. Higher RWC and proline content levels were recorded in FIRDAWS, LAANACEUR, MASSINE, OUSSAMA and TAFFA**,** which can be interpreted by high drought tolerance. On the other hand, ADRAR, AMALOU, AMIRA, and TAMELLALT showed higher values of MDA and H_2O_2 contents, which can be linked with drought sensitivity**.** Physiological tolerance could be a good criterion for selecting barley drought tolerant varieties.

Introduction

Cereals in their growth period face various biotic and abiotic stresses apart or combined, which impact morphological and physiological parameters, biomass and grain yield [1], [2],[3]. Climate change-induced global warming may cause increasingly severe and frequent droughts and intensify abiotic impacts on agricultural productivity in various parts of the world [4].It's expected that these environmental conditions could cause serious plant growth problems for more than 50% of arable areas by 2050 [5].

In Morocco, barley is the second cereal cultured and consumed after wheat [6], making the cultivation of barley in Morocco very important. It's well established that drought stress has different impacts in barley plants. Morphologically, drought induces decreases in root and shoots dry weight [7], [8], [9]. Physiologically, water deficit decreases the relative water content in leaves' [10], [11], [12], [13] and induces cell membrane deterioration [14], [9]. Previous works clearly showed that water withdrawal causes serious damages in photosynthesis apparatus [15], [16], [17], and nutrients uptake [18]. It is also widespread that physiological damage resulting from drought stress induces the oxidative stress resulting from reactive oxygen species generation (ROS) [19], [20] and increases synthesis of stress hormones like abscisic acid (ABA) [[18]].and affects grain yield [[3]]. Indeed, a reduction of grain yield indexes was noted in different studies [21], [1], [22]. Thus, the study of physiological and biochemical parameters in barley under drought stress is very important regarding the comprehension of the reaction of different barley varieties against this abiotic stress.

Intraspecific variability between barley varieties and lines regarding the adaptation to drought conditions is widely spread in the literature [16], [23], [8], [24], [25]. However, to the best of our knowledge, such studies describing physiological and biochemical behavior of Moroccan barley varieties are very limited. To ensure food quantity, quality, and security, the selection and description of tolerant varieties becomes urgent. Thus, this work aims to screen nine Moroccan varieties of barley (*Hordeum vulgare* L.) for their tolerance to drought stress through the study of their physiological and biochemical responses to drought conditions.

Material And Methods

1. Soil and growth conditions

Homogenous barley seeds (*Hordeum vulgare* L.) of nine Moroccan varieties (Table 1) (OUSSAMA, LAANCEUR, ADRAR, MASSINE, AMIRA, AMALOU, TAFFA, FIRDAWS, TAMELLALT) were obtained of seeds stock of RNE-lab at polydisciplinary faculty of Taza and the Moroccan National Institute of Agricultural Research (INRA).

Table 1
Names and characteristics of the barley collection varieties used in this study

Official name	Country of origin	Reference describing the genotype	Row type	Spring/winter type	Hulled/hulless	Earliness of maturity [69], [70]	Disease resistance [69], [70], [71]	Year of release
ADRAR	Morocco	(Hellal et al., 2019) [68]	2 rows	Spring Type	Hulled	Medium type	Resistant to oïdium; Sensitive Rhynchosporium. Moderately resistant to Rust	1998
FIRDAWS	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Medium type	Resistant to oïdium	1998
AMALOU	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Early type	Moderately resistant to oïdium, Sensitive to Rhynchosporium. Moderately resistant to Yellow and Brown Rust	1997
AMIRA	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Medium type	Resistant to oïdium. Sensitive to Rhynchosporium and Rust	1996
OUSSAMA	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Medium type	Sensitive to oïdium and Rhynchosporium. Sensitive to Yellow and Brown Rust	1995
TAFFA	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Medium type	Moderately resistant to oïdium and Rust; Sensitive to Rhynchosporium.	1994
MASSINE	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Medium type	Moderately resistant to oïdium and Yellow Rust; Sensitive to Rhynchosporium and Moderately sensitive to Brown Rust	1994
LAANNACEUR	Morocco	(Hellal et al., 2019) [68]	6 rows	Winter type	Hulled	Medium type	Moderately sensitive to oïdium and Rhynchosporium. Sensitive to Rust	1991
TAMELLALT	Morocco	(Hellal et al., 2019) [68]	2 rows	Spring Type	Hulled	Medium type	Moderately sensitive to oïdium, Sensitive to Rhynchosporium. Moderately resistant to Yellow and Brown Rust	1984

The seeds surface was disinfected in sodium hypochlorite (10%) bath and rinsed with distilled water. Seeds were sown in Petri dishes containing wet Whatman paper and incubated at 25°C up to the coleoptiles reaches 1 cm length. Then, seeds were placed in pots containing a mixture of soil, sable, and peat (1:1:1). Field capacity (FC) of each pot was estimated by measuring the volume of water retained 24 h after drawing. Three weeks after sowing (third leaf stage), two plants were maintained in each pot, and drought stress was applied by maintaining water content in stressed pots at 40% of FC. For control pots, FC was maintained at 90%. Pots were arranged randomly in greenhouse under natural light, temperature was maintained at 25°C and humidity at 75%.

2. Determination of plant physiological parameters

Eight weeks after sowing, leaf samples were harvested and stored at -20°C up to analysis.

2.1. Relative water content (RWC)

Leaves Relative Water Content (RWC) was estimated following the Weatherlay's method [26] using formula:

$$\mathrm{RWC}(\%) = \frac{\mathrm{Freshweight} - \mathrm{Dryweight}}{\mathrm{Turgorweight} - \mathrm{Dryweight}} \times 100$$

2.2. Proline content

Proline content was evaluated following the method described by Bates et al, [27]. Briefly, 200 mg of leaf sample was homogenized with 4 mL of sulfoscalicylic acid (3%) and centrifuged at 10000 rpm for 10 min. 2 mL of supernatant was mixed with 2 mL of glacial acetic acid and 2 mL of Ninhydrin reagent was added to the mixture. The set was incubated 1 h at 100°C. Proline was extracted in toluene and the absorbance was measured at 520 nm. Proline content was measured using a standard curve of L-proline.

2.3. Electrolyte leakage (EL)

Electrolyte leakage (EL) was evaluated using the technique of Szalai et al, [28]. Briefly, leaf samples were emerged in distilled water overnight at room temperature, then, electrical conductivity was noted (EC1). The samples were incubated at 95°C for 10 min and the electrical conductivity was measured (EC2). EL was calculated using the formula:

EL% = (EC1/EC2)*100

2.4. SPAD index (Chlorophyll content)

Chlorophyll content was measured undestructively using SPAD meter (SPAD KONICA MINOLTA, made in Japan). In barley, a significant correlation between SPAD value and chlorophyll content was noted [29].

2.5. Hydrogen peroxide (H_2O_2) content and lipid peroxidation

250 mg of leaf sample was homogenized with 5 mL of Trichloroacetic acid (TCA) 0.1%. The supernatant was taken after centrifugation (10000 rpm/5 min) and considered as the extract of H_2O_2 and malondialdehyde (MDA).

To quantify H_2O_2 content, 0.2 mL of extract was added to 0.8 mL of phosphate potassium buffer (10 mM, pH7), then, 1 mL of KI (1M) was added to the mixture. The set was incubated 10 min at room temperature, and the absorbance was measured at 390 nm (Spectrophotometer JASCO V-730, made in Japan). H_2O_2 content was measured using a standard curve of H_2O_2 [30].

Lipids peroxidation was evaluated by quantifying leaf MDA content. It was estimated using the method of Heath & Peaker [31]. Briefly, 1 mL of the extract was added to 4 mL 20% TCA containing Thiobarbituric acid 0.5%. The set was heated at 95°C for 10 min. After centrifugation (10000 rpm /5 min), the absorbance was noted at 532 nm and 600 nm (Spectrophotometer JASCO V-730, made in Japan). MDA content was estimated using using the extinction coefficient of MDA at 532 nm which is 155 $mM^{-1}.cm^{-1}$.

2.6. Total soluble sugars content (TSS)

The extract of total soluble sugars (TSS) was prepared following the method of Erice et al, [32]. Briefly, 100 mg fresh leaf was mixed with 5 mL of phosphate potassium buffer (50 mM, pH 7.5) and centrifuged 15 min at 10000 rpm. The supernatant was used as the extract of TSS.

TSS content was measured using the method of Yemm & Willis [33]. Briefly, 0.1 mL of supernatant was added to the anthrone reagent, and the mixture was heated at 90°C for 10 min, and the absorbance at 625 nm (Spectrophotometer JASCO V-730, made in Japan). TSS was measured using a standard curve of D-glucose.

2.7. Soluble proteins content

Leaf sample (200 mg) was homogenated on ice in 2 mL of refrigerated phosphate sodium buffer (100 mM, pH 7.5). The supernatant taken after centrifugation (8000 rpm/15 min) was considered as proteins extract. Soluble proteins content was determined photometrically (Spectrophotometer JASCO V-730, made in Japan) following Lowry's method [34] using a standard curve of Bovine Serum Albumine (BSA).

2.8. Data analysis

All measurements were made in triplicates. Analysis of variances (ANOVA) was performed over drought stress and varieties. Means were compared at 5% as a level of probability adopting the Fisher's Least Significant Difference's test (LSD). Correlation matrix was performed based means values of parameters studied. These statistical analyses were established using Minitab 18 statistical software. Principal component analyses (PCA) and Agglomerative hierarchical cluster analysis were carried out based on means values using XLSTAT software (XLSTAT Version 2016.02.28451).

Results

1. Physiological characteristics of Moroccan barley varieties under drought stress

Based on the Figs. 1, 2, and 3, it's clear that drought stress influenced most of biochemical and physiological parameters studied for most of varieties. Other than TAFFA, there was a significant reduction ($P < 0.05$) of RWC (Fig. 1A) in leaves of the varieties studied. When compared to controls, the varieties of ADRAR, AMALOU, AMIRA, and TAMELLALT had the most important decrease of RWC in their leaves, with reductions of 13.52%, 9.12%, 10.79%, and 10.24% respectively. The lowest reductions were noted in FIRDAWS (5.13%), LAANACEUR (5.27%), MASSINE (3.04%), and OUSSAMA (7.01%).

For electrolytes leakage (Fig. 1B), ADRAR, AMIRA, FIRDAWS, TAMELLALT, and TAFFA showed a significant increase ($P < 0.05$) of EL percentage under drought stress, with the highest score (61.65%) observed in TAMELLALT leaves. For AMALOU, LAANACEUR, MASSINE, and OUSSAMA there was no significant difference in EL% between ($P \geq 0.05$) controls and stressed plant leaves.

All varieties showed a significant ($P < 0.05$) decrease of SPAD values (Fig. 1C), and a significant accumulation of proline (Fig. 2A) in their leaves under stress, but with different degrees. The varieties that accumulated the most of proline in leaves are: FIRDAWS (81.43%), LAANACEUR (70.23%), MASSINE (89.12%), and OUSSAMA (70.45%). When compared to controls, the reduction of water content in the medium to 40% of FC did not affect the Total Soluble Sugars (TSS) in some varieties of barley. In the conditions of our experiment, no significant impact ($P \geq 0.05$) on leaves TSS content (Fig. 2B) was recorded in AMALOU, MASSINE and TAFFA. However, FIRDAWS and TAMELLALT showed the highest TSS contents with increases of 26.98% and 23.75%, respectively, compared to controls. According the results obtained for soluble proteins (Fig. 2C), it was noted that drought stress increased soluble proteins content in the leaves of all varieties, except in AMIRA and MASSINE. In these later, no significant difference between control and treatment was recorded (Fig. 2C).

Except OUSSAMA and TAMELLALT, all varieties considered in this study had a significant increase ($P < 0.05$) of H_2O_2 content (Fig. 3A) under drought stress (ADRAR (76.65%), AMALOU (79.95%), AMIRA (86.95%), FIRDAWS (78.79%), LAANACEUR (40.53%) MASSINE (39.33%), and TAFFA (45.6%). For MDA content (Fig. 3B), no significant difference was noted in FIRDAWS and LAANACEUR between control and stressed plants. However, there was a significant increase ($P < 0.05$) of MDA content in the leaves of the other varieties. The highest increases were 80.79% and 67.17% for ADRAR and TAMELLALT respectively, compared to controls. For OUSSAMA, a significant decrease ($P < 0.05$) of MDA content was recorded under drought stress.

2. Analysis of variances (ANOVA)

Table 2 shows means squares of combined analysis of variances for RWC, SPAD, Electrolytes leakage (EL%), proline content, H_2O_2 content, MDA content, TSS content and total soluble proteins content in leaves of the nine Moroccan barley varieties exposed to drought stress. It's shown that varieties, treatment and their interaction influenced the biochemical and physiological parameters studied significantly. However, treatment was the most important source of variability for all parameters studied except TSS and soluble proteins contents (36 and 22% respectively), the effect of this factor is more than 80% for RWC and SPAD index, about 69% for proline and MDA contents, and little more than 50% for H_2O_2 content and EL%. For TSS content the major source of variability was variety factor with 60% of total variability, on the other hand, Treatment by variety interaction is the important source of variability concerning soluble proteins content parameter with 42% of total variability.

Table 2
Mean squares of physiological parameters of leaves of nine barley Moroccan varieties

Source	Df	RWC	EL	SPAD	Proline	H_2O_2	MDA	TSS	Proteins
Variety	8	28,39***	211,47***	34,73***	36,04***	4,645***	80,335***	82,097***	1,653***
Treatment (trt)	1	201,28***	380,17***	532,04***	148,12***	7,898***	359,625***	49,035***	1,050***
Replicates	2	1,81	7,86	6,64	4,03	0,33	0,784	0,662	0,027
Variety*trt	8	13,41***	143,07***	15,92***	22,90***	1,759***	77,139***	3,435***	2,021***
Error	34	3,61	8,31	3,22	3,45	0,313	0,237	0,959	0,109
Total	53								
Df : Degree of freedom. *: significant at 0.05, **: significant at 0.01. ***: significant at 0.005.									

Correlation Among The Biochemical And Physiological Parameters Studied

Table 3 shows the correlation matrix among the physiological parameters studied under drought stress. Positive and negative correlations were noted. Our results showed that the RWC was correlated negatively and significantly ($P < 0.05$) with EL% and MDA content. Regarding the results recorded for EL, this parameter was positively correlated with MDA and TSS contents. SPAD value was negatively correlated with proline and proteins contents, while H_2O_2 content was positively correlated with TSS content. Also, significant positive ($P < 0.05$) correlation was observed between soluble proteins and TSS contents.

Table 3
Correlation matrix (Pearson) among parameters studied under drought stress.

Variables	EL%	SPAD	Proline	H_2O_2	MDA	TSS	Proteins
RWC	**-0,549***	0,366	-0,305	**-0,510***	**-0,745*****	-0,245	-0,412
EL%		-0,229	0,234	-0,029	**0,505***	**0,530***	0,386
SPAD			**-0,781*****	-0,439	-0,230	-0,186	**-0,566***
Proline				**0,510***	0,140	0,366	**0,730*****
H_2O_2					0,451	-0,141	**0,471***
MDA						0,049	0,257
TSS							**0,519**
*: siginificant at 0.05, **: significant at 0.01. ***: significant at 0.005							

3. Principal component analysis (PCA)

PCA was used as a multivariate statistical tool to represent the variability of physiological parameters of different varieties studied under drought stress. This analysis showed three top components (Eigen value ≥ 1), accounting 88.13% of the total variation of the physiological parameters under drought stress as delivered in Fig. 4. PC1, PC2, and PC3 explain respectively 42.36%, 28.69%, and 17.08% of the total variation. PC1 was positively and significantly ($P < 0.05$) correlated with RWC, proline content, and it was also negatively correlated with SPAD value and MDA content. PC2 was positively correlated with EL%, TSS content, and proteins content. On the other hand, H_2O_2 content was positively correlated with PC3.

To select tolerant varieties, a biplot was created using two first principal components (Fig. 4). The negative side of PC1 shows ADRAR with high scores of SPAD value and MDA content. The positive side shows MASSINE and OUSSAMA with high values of RWC and proline content. The positive side of PC2 discriminates TAMELLALT with high value of EL%, and FIRDAWS and TAFFA with high levels of TSS and proteins contents.

4. Agglomerative hierarchical cluster analysis

Values of leaves biochemical and physiological parameters under drought stress can be used to classify barley varieties by tolerance degree to drought stress. This analysis is based on regrouping the varieties with similar biochemical and physiological characters under drought stress on the same cluster. Cluster analysis for the nine Moroccan barley varieties divided them into three classes (Fig. 5). ADRAR, AMALOU, and TAMELLALT were grouped in first class. AMIRA was classified alone in the second class. However, FIRDAWS, LAANACEUR, MASSINE, and OUSSAMA were classified in the third class.

The dendrogram (Fig. 5) showed that the percentage of similarity between the varieties classified in first cluster reaches about 98.5%, where AMALOU and ADRAR are most similar (99%). Even if the second cluster is containing one variety (AMIRA), it’s similar to the varieties of the third cluster (about 97.2%). On cluster 3 varieties, the percentage of similarity reaches about 98%, where OUSSAMA and MASSINE are very similar under drought stress (99.9%). LAANACEUR and FIRDAWS also showed an important similarity between each other estimated to 99.8%.

Discussion

Drought stress is the most prevalent and prominent abiotic stress influencing plants growth and development [2]. In cereals, water deficit affects many morphological, biochemical and physiological parameters. Leaf and root growth are inhibited under moderate and severe stress [35]. Drought stress affects photosynthesis, water relations, nutrients uptake, oxidative status, osmotic balance and hormonal balance which impact the yield [3],[18]. Therefore, to ensure food security, the selection of tolerant varieties becomes indispensable. In this study, Moroccan varieties of barley (*Hordeum vulgare* L.) were screened for physiological and biochemical characteristics to select varieties able to cope with drought stress.

The results showed a significant intraspecific variability regarding the adaptation to drought conditions, as reported in many papers studying barley growth under drought stress [36],[12],[20],[37]. RWC is a critical physiological criterion for determining the degree of tissue and cell hydration required for optimal physiological growth and functioning in plants [2],[38]. Preservation of high value of RWC under drought stress indicates an important drought tolerance [39],[40]. In our results, a significant reduction of RWC was noticed under drought stress in leaves of barley varieties. Similar results were reported in other studies when barley plants were exposed to water deficit [10], [11], [12], [13]. As shown in Fig. 1A, RWC of ADRAR, AMALOU, AMIRA, FIRDAWS and TAMELLALT are the most influenced by drought stress. This means that these varieties are less able to keep cell turgor in their leaves under drought stress. However, LAANACEUR, MASSINE, OUSSAMA, and TAFFA are less influenced, meaning high capability of these varieties to keep cells turgor in their leaves under drought conditions.

Drought stress induced a significant decrease of chlorophyll content (SPAD value) as shown in Fig. 1C. Such results were also reported by other studies dealing with cereals [2], [41].Chlorophyll reduction may be due to electrolytes leakage from thylakoïds membrane and lipids peroxidation [2], protoplasm dehydration, and less photo-assimilation level [9]. The increase of electrolytes leakage in leaf tissues is considered as an index of membrane damage and deterioration [42] and as the parameter indicating membrane steadiness [43]. The increase of EL percentage under stress conditions is always associated with sensitivity of plant to oxidative stress [44]. In our findings, different levels of increase of EL% were noted, which is in agreement with the literature [9], [14]. As presented in Fig. 1B, TAMELLALT variety was the most influenced by water deficit regarding electrolytes leakage, followed by

FIDRAWS, TAFFA, AMIRA and ADRAR. This indicates membrane cell damage in the tissues of those varieties under drought stress. However, for the other varieties, there was no significant impact of drought stress on their EL% indicating an important cell membrane steadiness under drought stress and high tolerance.

Under drought conditions as other abiotic stresses, the accumulation of proline is common in most of cereals [9] [18], [45], [46]. This molecule is an important osmoregulator for membrane stability, buffering cellular redox potential, and scavenging free radicals [18]. Proline can also play an important role in activation of detoxification pathway [47], which makes varieties with ability to produce more proline under stress, to be more tolerant. This was the case in FIRDAWS, LAANACEUR, MASSINE, and OUSSAMA. These varieties accumulated the highest levels of proline under drought stress as shown in Fig. 2A, elucidating that these varieties are more resistant to drought stress. The accumulation of proline is widely reported as a known response in stress tolerant plants. Many roles have been attributed to this molecule regarding its involvement in plant tolerance to abiotic stress, but the accurate mode of its action is still unclear. It is also important to keep in mind that high levels of proline could have negative impact on plant cells. The ability to maintain the intracellular proline content in balance seems to be essential for plant tolerance and survival [48]. Actually, Proline levels in the cell are determined by the balance between biosynthesis and catabolism. The proline is synthesized from ornithine and glutamate, the glutamate pathway being more predominant in the plant cell [48]. The glutamate is converted to pyrroline 5-carboxylate (P5C) by the action of the pyrroline-5-carboxylate synthase (P5CS). The intermediate P5C is then reduced to proline by the P5C reductase (PC5R). Under stress conditions, transcriptional activation of P5CS and PCR genes was reported to increase while less impact was recorded in the ornithine route [49]. P5CS accumulates in the chloroplasts, leading to enhanced proline biosynthesis [50]. Two forms of P5CS exist in plant cell. P5CS1 which is chloroplastic and involved in the stress induced proline biosynthesis and P5CS2 is responsible for development processes [51]. In the other hand, the level of proline content in the cell is regulated by the action of proline dehydrogenase (proDH) and pyrroline 5-carboxylate dehydrogenase (P5CDH). These enzymes are transcriptionally regulated by developmental and environmental signals and proline catabolism is enhanced during recovery period from stress [49]. The level of accumulation of proline is reported to vary markedly between species and between genotypes within species [52], [53]. This could be linked to the complexity of the signaling networks and the multigenic processes involved in the regulation of the proline biosnynthesis pathway and its accumulation in plant cells under environmental constraints. In *Medicago trancatula*, *Helianthus annuus*, and *Sesamum indicum* plants, it has clearly been reported that the extent of oxidative stress varies among genotypes within a species, when submitted to stress conditions [54].

As shown in Fig. 2B, except AMALOU, AMIRA, and MASSINE, other varieties recorded an increase of TSS content. For proteins content, a great increase was marked under drought stress in all varieties (Fig. 2C), which is in agreement with literature results where tolerant barley varieties accumulates sugars and proteins in their leaves under drought stress [44]. The accumulation of soluble sugars and proteins is associated with osmotic regulation of cells. TSS and proteins accumulation decreases osmotic potential inside the cell, making cells able to keep a high turgor potential [44],[55] and stabilize cell membrane by reacting with lipid bilayer [20].

Based on biochemical, molecular, and genetic findings, it has been determined that soluble sugars play a critical role in web regulation of plants adaptation against biotic and abiotic stresses [56]. In last years, sugars are more studied for their hormone-like functions, as a primary messenger in signal transduction [57]. Glucose is widely known with his role as a modulator in repression of genes implicated in ABA catabolism and activation of genes implicated in ABA biosynthesis [58]. Moreover, It's proved that high level of sugars lead to the repression of the genes implicated in photosynthesis [59],indeed, it's well documented that the repression of Rubisco small subunit (RBCS) gene is associated with high sugars concentration in potato and maize [56], [60]. Furthermore, sugars accumulation is linked with down regulation of many genes implicated in photosynthesis such as atp-δ thylakoid ATPase (ATP- δ) gene, chlorophyll a/b binding protein (CAB) gene, pyruvate phospho dikinase (PPDK) gene, C4 malic enzyme gene (ME1) and C4 PEP carboxylase (PEPC1) gene [56]. In 2012, Hu et al, [61]showed that proline accumulation varies increasingly with glucose concentration applied on wheat plants under salt stress. This can help plant to be more tolerant to abiotic stress by increasing proline content, with proline functions cited above.

Under drought conditions increases of MDA and hydrogen peroxide contents in leaves were observed (Fig. 3). This was also the case of other studies [19], [20]. Various environmental stresses including drought constraint induce the generation of reactive oxygen species (ROS), which can lead to the oxidation of DNA and proteins, the peroxidation of membrane lipids, hydrogen peroxide accumulation and oxidative brust cell [62], [63]. In the other hand, the lipid peroxidation could be triggered by an increased lipoxygenase activity [18]. Both enzymatic and non-enzymatic processes are reported to be involved in the formation of lipid peroxidation products in plants such as MDA and jasmonates under oxidative stress conditions [64]. In cereals, low H_2O_2 and MDA contents are associated with high stress tolerance ability [18]. Actually, the role of aldehyde compounds (MDA) produced under stress environmental or developmental signals depends upon their accumulation levels which are controlled by the balance between the lipid peroxidation intensity and activity of aldehyde dehydrogenases (ALDHs). It is well established that the expression and activity of ALDHs are induced by H_2O_2, abscissic acid and MDA [36]. These molecules could serve as signals of protection processes where ALDHs contribute to maintain the cellular redox homeostasis and reducing potential NADPH required for antioxidant activity of the ascorbate-glutathione cycle and photosynthesis process [65], [66]. In our study, MASSINE, OUSSAMA and TAMELLALT varieties are characterized both by lower values of H_2O_2 and MDA contents compared with other varieties, which makes them less affected by ROS and/or lipoxygenase under drought conditions. The lower content of MDA in plant cells could be interpreted as a defense mechanism signaling rather than an indicator of membrane damage and protein carbonylation [64]. When MDA accumulated in the cells at high level, proteins are carbonylated, such as PSII core proteins and Rubisco, leading to disturbances in all plant cell metabolism which may trigger cell death. All previous published results converge towards the synthesis and involvement of MDA in plant metabolism under environmental stress. However, the role of this molecule remains unclear and needs more investigations.

It is widespread in the literature that drought tolerance is associated physiologically with high values of RWC and proline content [2], [18], [38], [67]. In our results, the biplot created (Fig. 5) discriminates MASSINE and OUSSAMA as the two varieties with higher percentages of RWC, and FIRDAWS is discriminated as the variety that accumulated most of proline in their leaves under drought stress. Also, the agglomerative hierarchical cluster analysis (Fig. 5) classified these varieties with LAANACEUR in the third class considered including varieties showing high physiological tolerance against drought stress. Furthermore, high scores of H_2O_2, MDA contents [18], [19], [20], and EL% [9], [42], [43] were always considered as signs of plants sensitivity against abiotic stresses. The biplot of our results (Fig. 5) discriminates TAMELLALT with high percentage of electrolytes leakage, ADRAR with high value of MDA content, AMALOU and AMIRA with high scores of H_2O_2 content. Except AMIRA, using agglomerative hierarchical cluster analysis, these varieties were classified in the first class of sensitive varieties under drought stress. AMIRA is classified alone in the second class showing more similarity with the first class (Fig. 5).

Conclusions

In this study, we screened physiological and biochemical parameters in barley leaves of nine Moroccan varieties grown under drought stress. A remarkable variability was recorded between varieties. A significant impact ($P < 0.05$) of drought stress with different levels was described in our results. Drought treatment induced a significant reduction of RWC and chlorophyll content (SPAD index), accumulation of proline, increase of hydrogen peroxide, MDA, soluble sugars and proteins contents in leaves of barley varieties. FIRDAWS, LAANACEUR, MASSINE, OUSSAMA and TAFFA maintained high values of RWC and proline content under drought conditions, which could be linked to their drought tolerance. ADRAR, AMALOU, AMIRA, and TAMELLALT showed high values of MDA and H_2O_2 content which indicates drought sensitivity of these barley varieties. Further assessments are needed to evaluate drought effect on field grain stage, phenology, grain quantity and quality in these Moroccan varieties.

Declarations

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Authors' contributions: MF: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. NS & SB: Methodology, Formal analysis, NE & SL: Conceptualization, Supervision, review & editing, Validation, Resources, Funding

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Figures

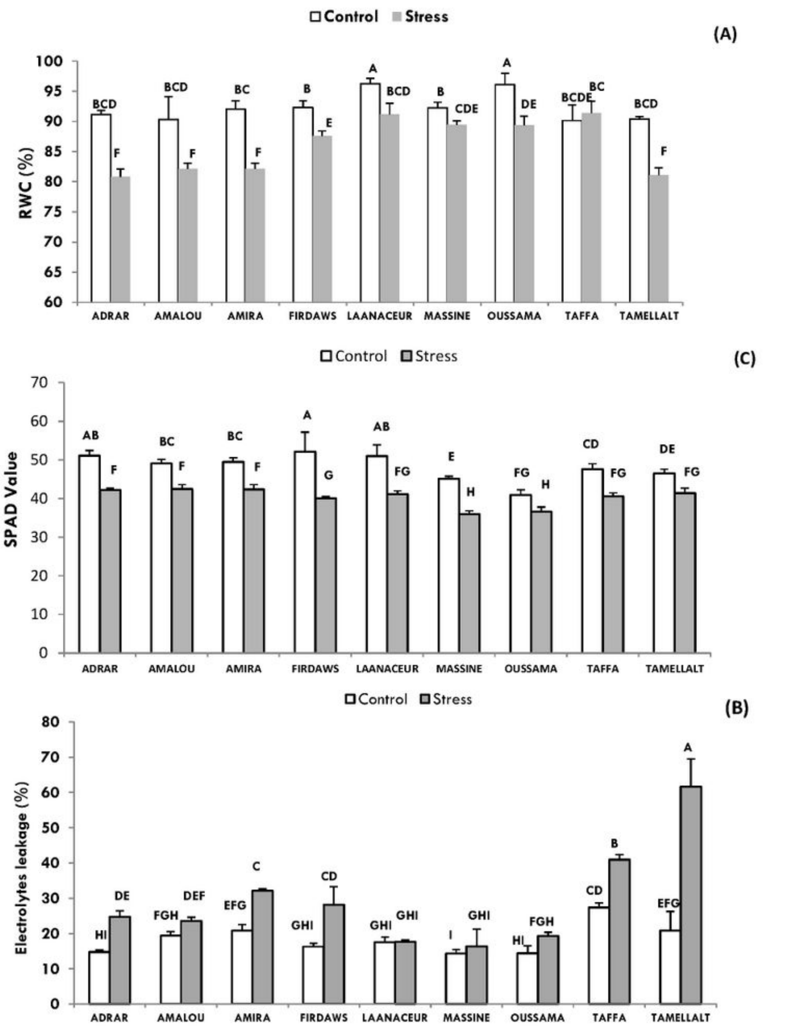

Figure 1: Means values of RWC (A), EL% (B), and SPAD value (C) of barley varieties under optimal irrigated conditions (Control) and drought stress. Values indicate the mean (±SE) of n=3. Scores with the same letter are not significantly different at P = 0.05.

Figure 1

See image above for figure legend.

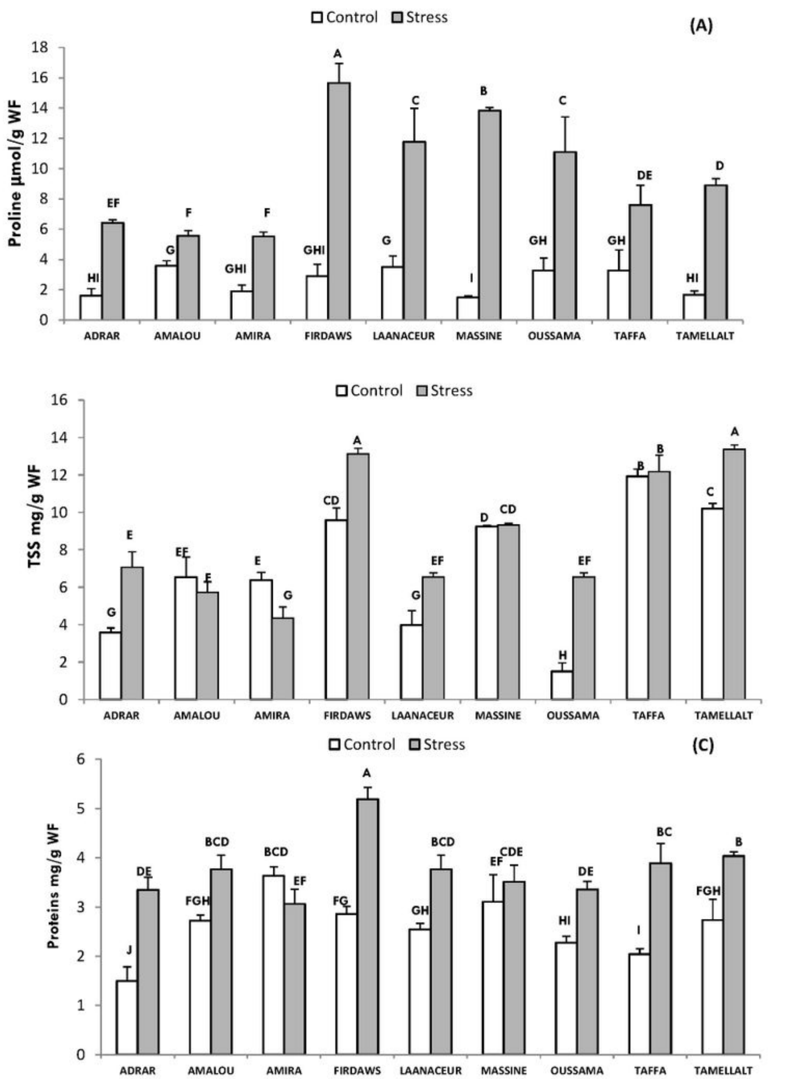

Figure 2: Means values of proline (A), TSS (B), and proteins (C) contents of barley varieties under optimal irrigated conditions (Control) and drought stress. Values indicate the mean (±SE) of n=3. Scores with the same letter are not significantly different at P = 0.05.

Figure 2

See image above for figure legend.

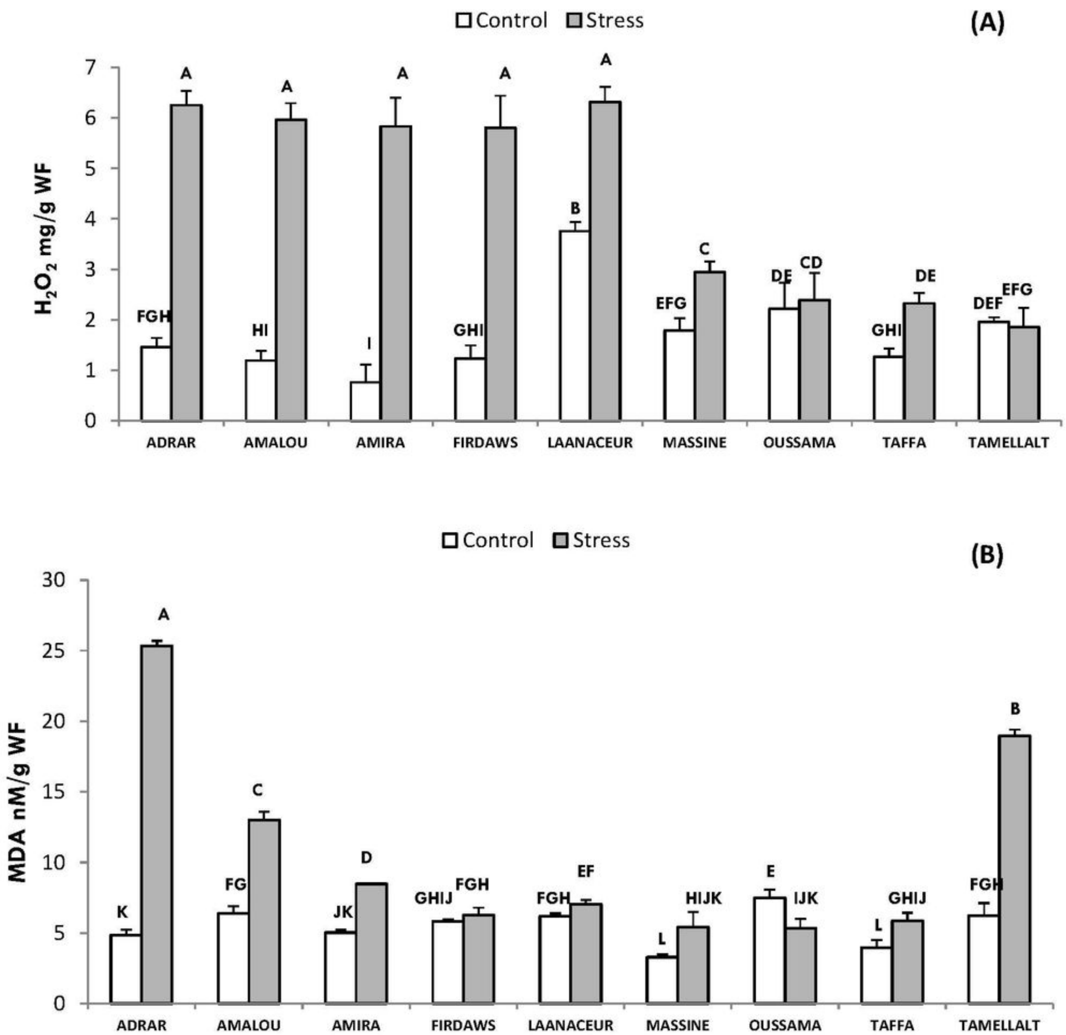

Figure 3: Means values of H_2O_2 (A) and MDA (B) contents of barley varieties under optimal irrigated conditions (Control) and drought stress. Values indicate the mean (±SE) of n=3. Scores with the same letter are not significantly different at P = 0.05.

Figure 3

See image above for figure legend.

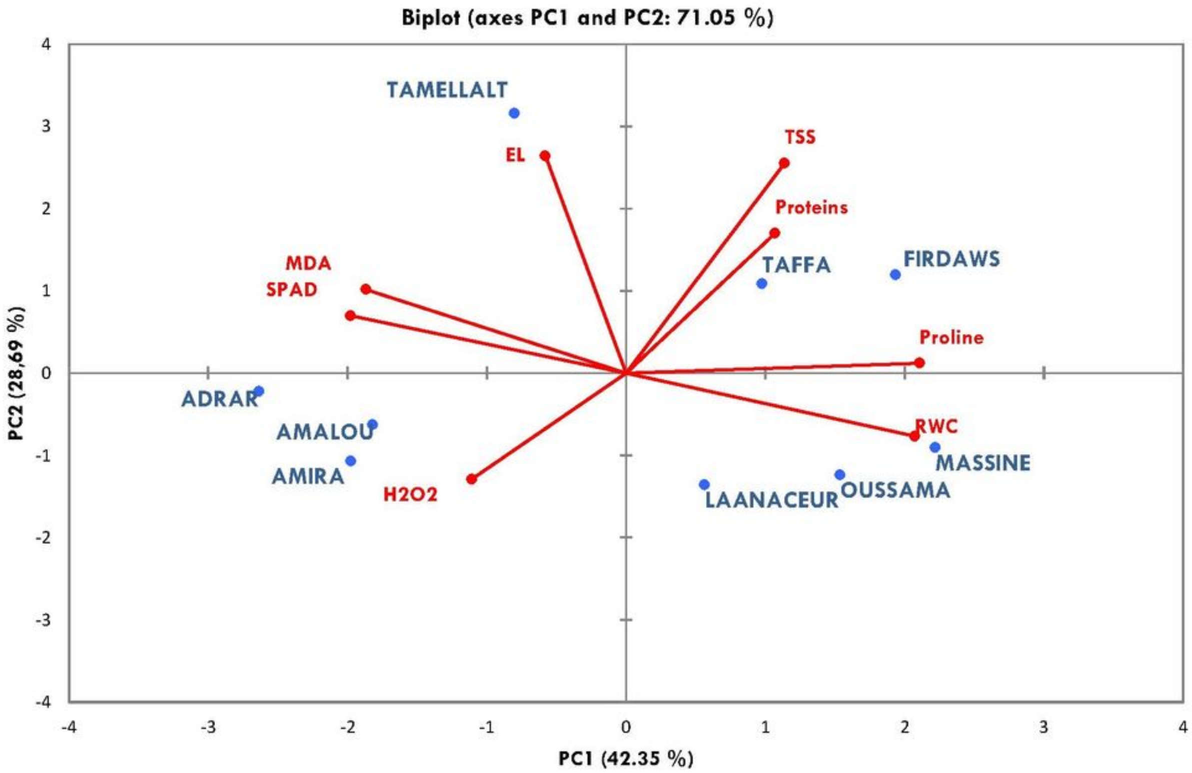

Figure 4: Biplot of principal component analysis for physiological responses in nine barley Moroccan varieties

Figure 4

See image above for figure legend.

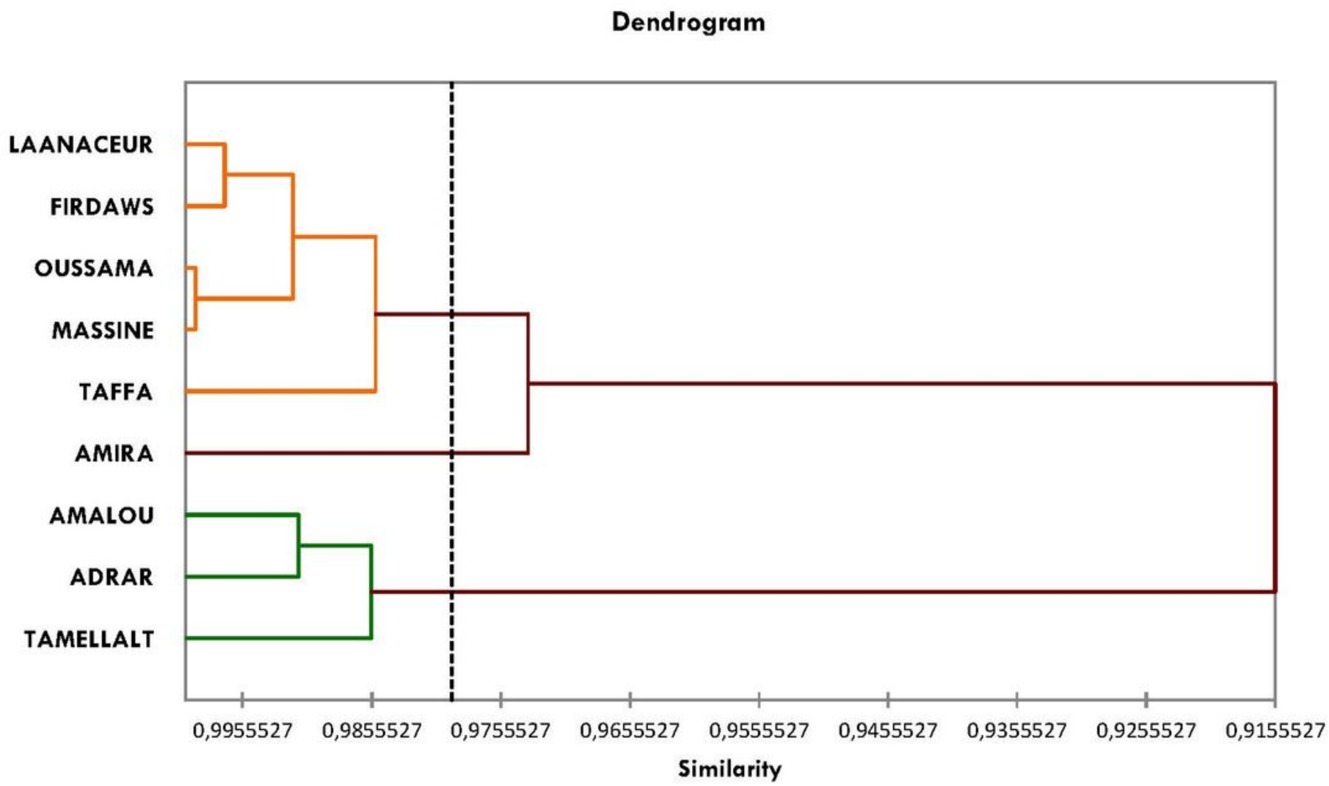

Figure 5: Dendrogram for agglomerative hierarchical cluster analysis based on physiological characteristics under drought stress of leaves of nine barley Moroccan varieties

Figure 5

See image above for figure legend.