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Physiological and growth response traits to water deficit as indicators of tolerance criteria between quinoa genotypes

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Abstract

The growth of crops is dependent on water availability, therefore, plant act by different mechanisms to overcome drought stress. Here, physiological and growth responses of six genotypes of Chenopodium quina to water stress were investigated in field condition under four irrigation treatments (100%ETc; 50%ETc; 33%ETc and rainfed). Results show that the six genotypes display different levels of tolerance to water stress.. Both tolerant genotypes L143 and L119, responded to the increase of water stress by decreasing leaf water potential, stomatal conductance and leaf area index. Furthermore, the chlorophyll a and b were increased in both genotypes. However, physiological traits indicate that under the half irrigated treatment (50%ETc) quinoa plant present an interesting tolerance to water stress, so using just half water requirement we can get comparative results to the control.

1. Introduction

Water scarcity is one of the major constraints of plant growth, productivity, and adaption worldwide. The lack of water resource and irregularity of precipitation had significantly impacted the sustainability of the crops production[1, 2]. Most plant species respond to abiotic stress through, molecular, biochemical and physiological modifications, and ultimately morphological adaptations [3, 4]. Plants have evolved many different mechanisms to deal with the occurrence of water limited conditions [5]. One of the most common mechanism is the stomatal closure, which reduces water loss and regulates plant water potential [6]. Leaf water potential was used as a sensitive indicator of plant water stress [7]. Withholding water was shown to reduce leaf water potential in quinoa [3] and tomatoes plants [7] as well as many other plant species. It was widely reported in agricultural crops that moisture stress affects stomatal conductance, resulting in a decline in the availability of internal CO_2 and hence in photosynthesis [8]. Quinoa (*Chenopodium quinoa* Willd.) is cultivated in the Bolivian Altiplano region as a staple crop [9]. It is well adapted to grow under unfavorable soil and climatic conditions [10, 11] and the crop is also rapidly gaining interest throughout the world [12] because of its robust character and its high nutritional value [13]. Its robust character is due to a high tolerance level of frost [14], drought [15] and soil salinity [16].

A deeper understanding of the mechanisms of quinoa tolerance for water deficit is crucial to identify genotypes having aptitudes to grow in arid areas. Moreover, it is important to use physiological traits to assess and screen the quinoa genotypes for their tolerance to water deficit tolerance. Therefore, the main objectives of this study were to (1) determine physiological traits that contribute to tolerance to water stress in quinoa cultivars, (2) characterize quinoa cultivars growing under different water stress treatments, and (3) to select the most adapted and tolerant genotypes to water stress.

2. Material and methods

2.1 *Plant material and experimental location*

The study was carried out on six quinoa cultivars (*Chenopodium quinoa* Willd) provided under the EU 7th Framework Program through the project "Sustainable water use securing food production in dry areas of the Mediterranean region (SWUP-MED)". Seeds were grown in a sandy soil in farmer's fields at "TninBouchan" Experimental Station of Cadi Ayyad University is located in 70km south West Marrakech (32°14.6267'N, 8°19.8181'W, 280 m.a.s.l.). Field trials were conducted in February and harvested in June 2012 during two successive years 2011 and 2012 as previously reported by Fghire, et al. [17] and Fghire, et al. [3].

2.2 *Experimentation and measurements*

2.2.1 *Experimental treatments*

The trial consisted of a rain feed treatment (0% of crop evapotranspiration (ETc)), full irrigated treatment (100% ETc), and two others treatments with deficit irrigation DI (50% of ETc and 33% of ETc). The experimental trial was arranged in a randomized complete block design with four plots replicate (15m²/plot). Quinoa seeds were sown directly on a sandy loam soil (62% of sand, 36% of silt and 12% of clay) with spacing of 0.2m between sowing pits of the same row and 0.8 m between rows. Buffer areas of 1 m between experimental units were sown to avoid border and interaction effects.

2.2.1 Irrigation strategies

Meteorological data (minimum and maximum temperature, minimum and maximum relative humidity, wind speed, solar radiation) are continuously monitoredwith iMETOS® agweather stations installed in the field and automatically sent to internet climate data base. Irrigation planning was based on a daily follow up of Reference evapotranspiration (ET_0), calculated with the FAO-Penman-Monteith equation [18].

The crop coefficient of quinoa presented by Garcia, et al. [11] was corrected with validated procedures [18] for incomplete cover, incomplete wetting by irrigation and high convection due to the arid climate. Irrigation was efficiently applied by drip irrigation. Drip emitters were spaced 0.10 m along the lateral with a discharge of 1 LPH under an operation pressure of 1.5 kg cm^{-2}. The rate of water flow in all drip laterals was equal and constant under all the treatments. The net irrigation requirement is derived from the field balance equation [19]:

$$IRn = (ET_0*Kc)-(Pe + Ge + Wb)*LRmm$$

Where:

IRn = Net irrigation requirement [20]

ET_0 = Reference evapotranspiration [20]

Kc = Crop coefficient

Pe = Effective dependable rainfall [20]

Ge = Groundwater contribution from water table [20]

Wb = Water stored in the soil at the beginning of each period [20]

LRmm = Leaching requirement [20]

The gross irrigation requirements account for losses of water incurred during conveyance and application to the field. This is expressed in terms of efficiencies when calculating project gross irrigation requirements from net irrigation requirements, as shown below [19]:

$$IRg=IRn/E$$

Where:

IR_g = Gross irrigation requirements [20]

IR_n = Net irrigation requirements [20]

E = Overall project efficiency

2.3 *Stomatal conductance*

Stomatal conductance (gs) was measured at midday during the whole growing season with a portable porometer (Leaf Porometer, Decagon Device, Inc., Washington, USA). The device was calibrated before use using the supplied calibration plate. The terminal part of the main leaf lobe was placed into the cup on the head unit, which was positioned normal to the sun. Measurements were conducted during cloudless periods on exposed leaves around noon.

2.4 *Plant water status*

Pre-dawn stem water potential (Ψpd) was measured using a Scholander pressure chamber (SKPD 1400, Skye Instruments, Powys, UK). A branch with four newly expanded leaves per plant (four plants per treatment) was detached, enclosed in a plastic bag, immediately severed at the petiole, and scaled into the humidified chamber for determination of balancing pressure. The stem was covered with black plastic to avoid light assimilation.

2.5 *Leaf area index*

Leaf area index was measured with a 0.8-m long ceptometer (Decagon Devices Inc., Pullman, Washington) between 11:30 and 14:00h on clear days. Four measurements were taken in each replicate. The measurements were taken at soil surface level placing the sensor below the canopy and moving it parallel to rows at regular intervals.

2.6 *Photosynthetic pigments*

For each sample 50 mg of fresh leaves were cut and ground in 3 ml of cold 90% acetone. The extracts were centrifuged at 1000g for 10 minutes. The supernatants were then collected in test tubes and incubated in the dark for two hours before the assay. The optical density (O.D.) of the extract was measured at wave lengths 663 and 645 [21] to estimate chlorophyll 'a' and 'b' respectively. Three replicates were used for each treatment, and die amount of pigment present in each sample was calculated according to the following equations:

$$\mu g \text{ (chlorophyll a)/g (FW)} = .2.7 \text{ (O.D)}_{663} - 2.69 \text{ (O.D)}_{645} \times \frac{v}{w \times 1000}$$

$$\mu g \text{ (chlorophyll b)/g (FW)} = 22.9 \text{ (O.D)}_{645} - 4.68 \text{ (O.D)}_{663} \times \frac{v}{w \times 1000}$$

whereas W, the fresh weight by grams for extracted tissue; V, the final size of the extract in 90% acetone; O.D., optical density at specific wave length.

2.6. Statistical analysis

The experiments were carried out with a randomized complete block design. Values are means of four replicates, the means were separated with least significant difference test using CoStat version 6.3.

3. Results

3.1 Stomatal conductance

The variation of the stomatal conductance (gs) during the cycle of culture (Figure 1) showed high values at the beginning of the cycle. The genotypes studied showed a maximum of stomatal opening in the beginning of plant cycle; with a dependant variation to treatments. Indeed, the variation between the rainfed treatment and 100% ETc was between 138 and 266, 115 and 214, 118 and 216, 150 and 344, 147 and 376, and 138 and 266 mmol $(H_2O).m^{-2}s^{-1}$ in respectively Titicaca; L11; L119; L123; L142 and L143.

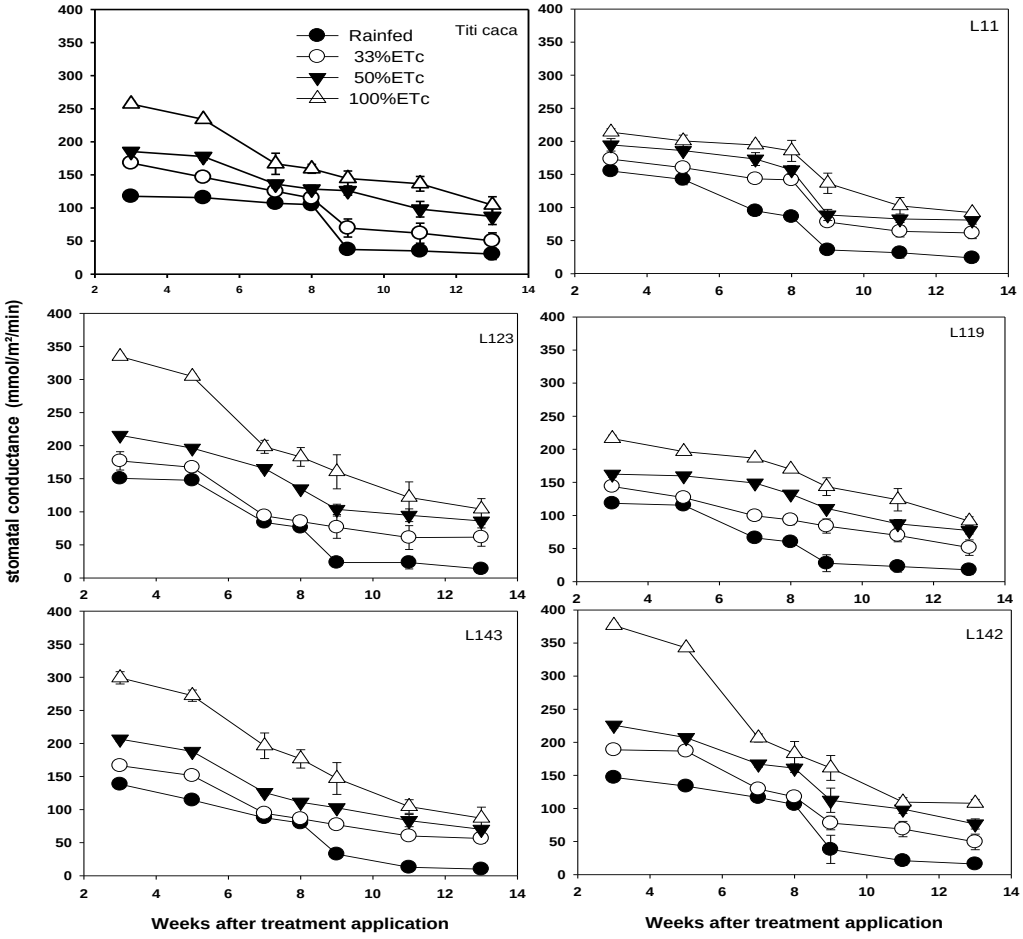

Figure 1: Evolution of stomatal conductance measurement along the experience at the six genotypes of quinoa: Titicaca; L11; L119; L123; L142 and L143, subjected to four water treatments of irrigation: 100% ETc (Δ), 50% ETc (▼); 33% ETc (○); and Rainfed (●). (The values represent the average of 4 replicates ± standard error).

Based on these values, we find that the behavior of the genotypes varies from one treatment to another. The L142 line showed a 61% reduction compared to control after three weeks of rainfed treatment. Towards the end of the cycle, reductions were more pronounced and above 70% for all genotypes. However, in the semi-irrigated treatment (50% ETc), stomatal closure was less intense 40% and 32% respectively for L142 and L143 and 30% for the remaining genotypes (L11, L119, L123 and Titicaca). Towards the end of the cycle, the leaves gs of all genotypes and in all treatments were less than 100 mmol $(H_2O).m^{-2}s^{-1}$.
Statistical analysis of data showed that stomatal conductance was significantly ($p < 0.001$) affected by genotype, water treatment and stage of growth (Table 1). All interactions between different treatments were shown to be highly significant.

3.2 *Leaf water potential*

Figure 2 shows the evolution of leaf water potential along the crop cycle of six quinoa genotypes. The pattern of water potential was similar for the six genotypes. However, Ψ began to decline from the 3rd week in rainfed treatment and from the 5th week for the other treatments. The Ψ reached under rainfed conditions 3.6 MPa for L119 and L143 lines. These values highlight the adjustment capabilities of quinoa water status. Water potential has fallen by 169-288% for L119 respectively. However, under 100% ETc, the variation of Ψ fluctuated between -1.7MPa in the L11, L119 and the Titicaca and -2MPa among the other genotypes. Fewer than 50% ETc treatment water potential discriminates between genotypes; on one side, the variety Titicaca presented a more than 45% reduction from the 3rd week, and on the other, all other genotypes maintained their Ψ around 20% up to the 9th week.

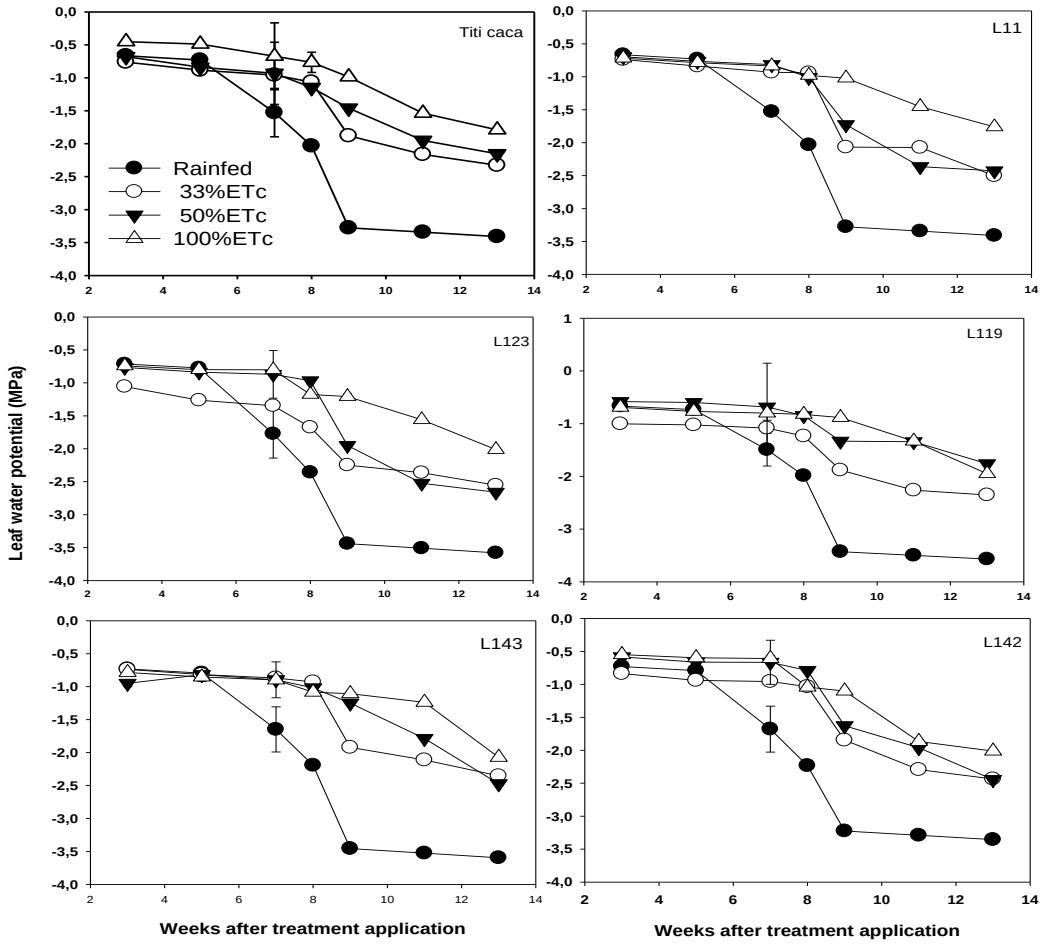

Figure 2: Evolution of Leaf water potential measurement along the experience at the six genotypes of quinoa: Titicaca; L11; L119; L123; L142 and L143, subjected to four water treatments of irrigation: 100% ETc (Δ), 50% ETc (▼); 33% ETc (◌); and Rainfed (●). (The values represent the average of 4 replicates ± standard error).

Statistical analysis showed that the water treatment, genotype and growth stage, and the various interactions between these factors have a significant effect on leaf water potential (Ψ) ($p < 0.001$) (Table 1). The values of (Ψ) under 100% ETc were all significantly higher compared to other diets ($p < 0.001$).

Table 1: Analysis of variance of the effect of water treatment, genotype and stage of growth on physiological parameters studied

	Genotypes	Water treatment	Genotypes × Water treatment	stage of growth	Genotypes ×stage of growth	Water treatment × stage of growth	Genotypes × Water treatment × stage of growth
Ddl	6	3	18	6	36	18	108
Leaf water potential (Ψ)	466,14***	27283,43***	249,91***	34590,16***	40,95***	2307,92***	55,45***
Stomatal Conductance (gs)	139,14***	585,15***	45,04***	1522,35***	40,68***	220,28***	26,67***
Leaf area index (LAI)	112,03***	2478,21***	67,86***	976,62***	22,30***	54,88***	16,48***
Chlorophyll a	1660.44***	3834.49***	213.42***	9236.09***	258.69***	1859.93***	154.18***
Chlorophyll b	137.63***	729.56***	60.18***	2193.32***	60.10***	195.98***	54.99***

* : difference Significant at p <0.05
** : difference Significant at p<0.01
*** : difference Significant at p<0.001
ns : difference not Significant

3.3 *Correlation*

The graphical representation of the relationship between stomatal conductance and water potential data shows a close dependence between the two physiological parameters (Figure 3). Indeed, the water potential drop as soon as the present stomatal conductance values of less than 130 mmol. m-2 .s-1. Water potential reaches very low values when the conductance becomes of the order of 50 mmol. m^{-2} s^{-1}.

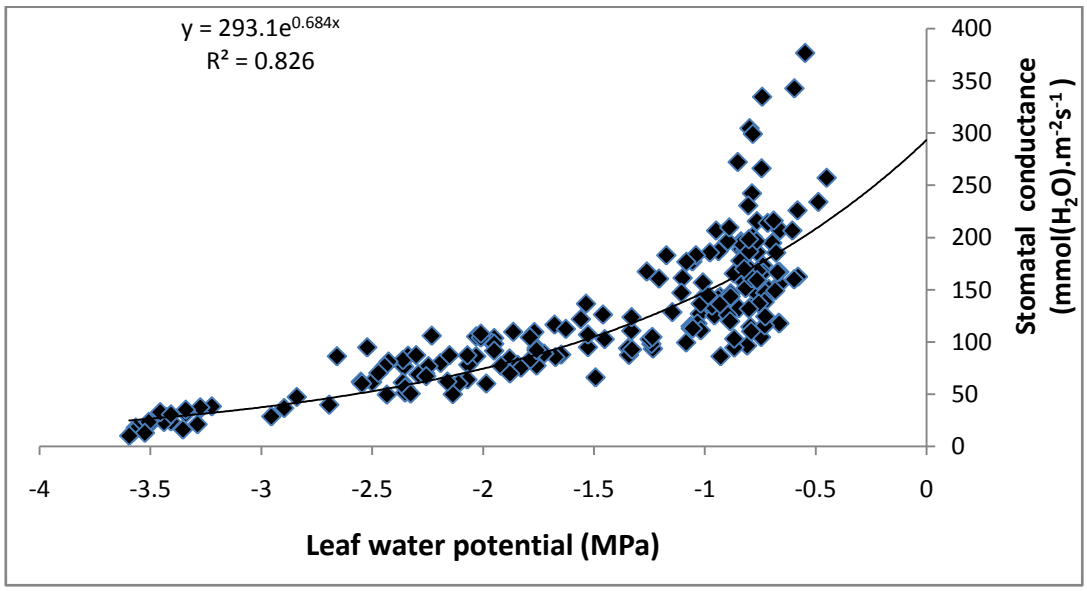

Figure 3: leaf water potential and stomatal conductance correlation

3.4 *leaf area index (LAI)*

Figure 4 shows the effect of water stress on leaf area index which increases significantly (P <0.001) and a continuously and peaked at filling stage seed for all water treatment and from that stage the (LAI) starts fell. The leaf area index exhibits an increase until the 9th week (seed filling stage) to reach higher values in all genotypes studied under 100% ETc treatment with a maximum of 5.26 noted in the L119 line. However, the LAI showed significant decreases in response to increased water stress, reaching minimum values (in the 9th week) due to the rainfed treatment ranging from 1.9 noted in L119 to 3.13 in L11.

Deferent studied genotypes show a similar evolution of LAI them, where irrigation effect and genotype are highlighted (Figure 4). In addition, the LAI decreases with increasing water stress; which manifests itself by a

minimum level in plants subjected to rainfed treatment for all genotypes (1.9 to 3.13 in L119 at L11 at the seventh week), and a maximum for the well irrigates treatment (100% ETc) with 3.72 (Titicaca variety) to 5.26 (the L119 line).The statistical analysis (Table 1) shows that the LAI is significantly affected (P <0.001) by irrigation, genotypes, the growth of the plant and the interaction between these factors.

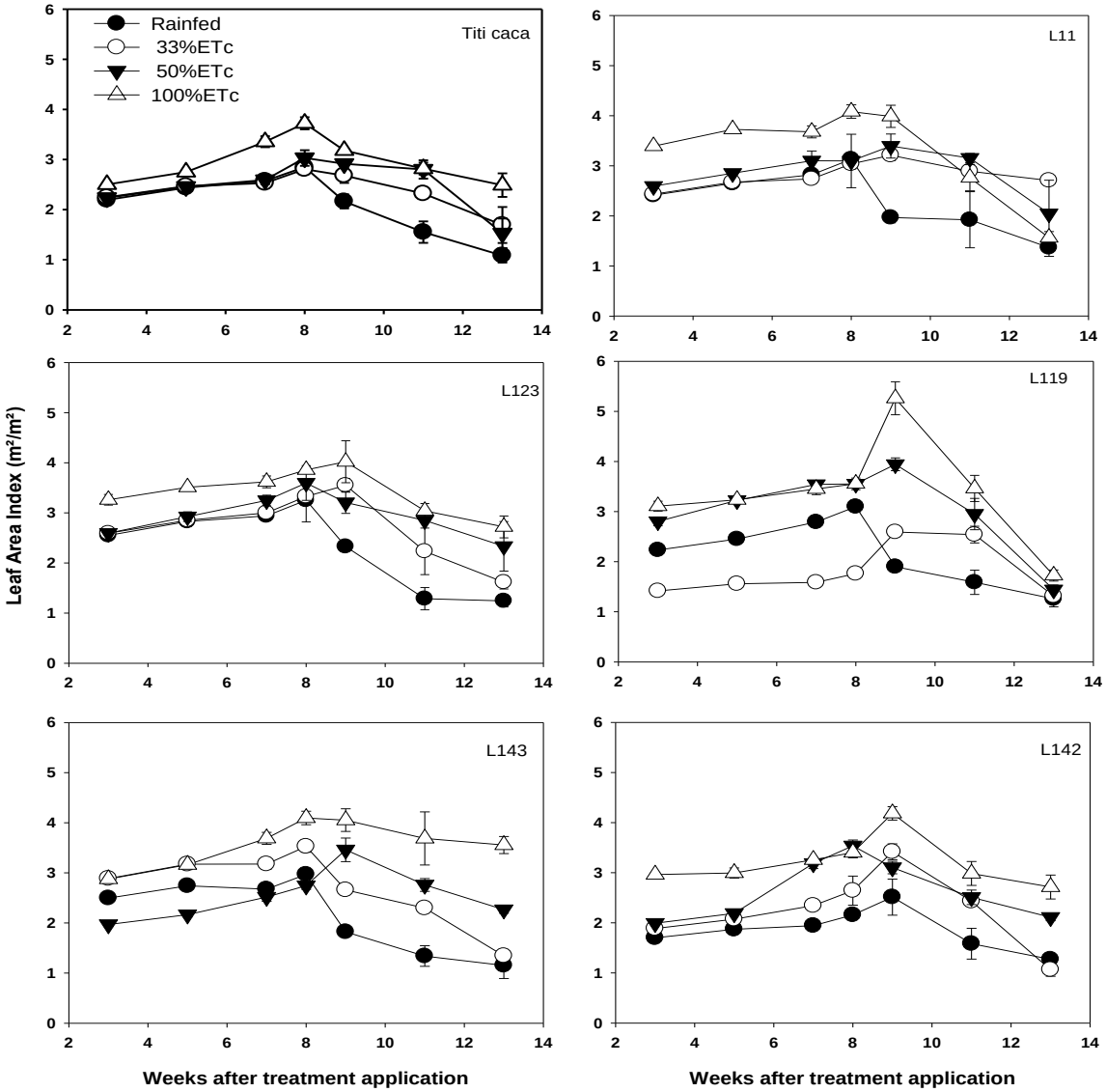

Figure 4: Evolution of Leaf Area Index measurement along the experience at the six genotypes of quinoa: Titicaca; L11; L119; L123; L142 and L143, subjected to four water treatments of irrigation: 100% ETc (Δ), 50% ETc (▼); 33% ETc (◌); and Rainfed (●). (The values represent the average of 4 replicates ± standard error).

3.5 Content of chlorophyll a and b

Figures 5 and 6 present the evolution of chlorophyll a and b under the effect of water stress applied through four water stress (100% Etc, Etc 50%; 33% ETc and non-irrigated). Among the seven genotypes studied, we found that the control plants keep a relatively stable trend of around 300µg /g FW for Chl a and about 200 µg /g FW for Chl b, along the cycle plant. the stressed treatments (50% ETc, 33% ETc and rainfed) generated an increase in the content of Chl a and b in the first weeks after the application of water stress levels to reach 2 to 3 times more larger than that of the control. These chlorophyll concentrations have been reduced from the third week for the Chl a and from seventh week for the Chl b. At the last week, the concentrations of chlorophyll a and b reched a values close to those observed in controls.

Nevertheless, the ANOVA showed, for both types of chlorophyll, strictly dependent (p <0.001) genotypes, water treatment, growth stage, and that various combinations of these factors.

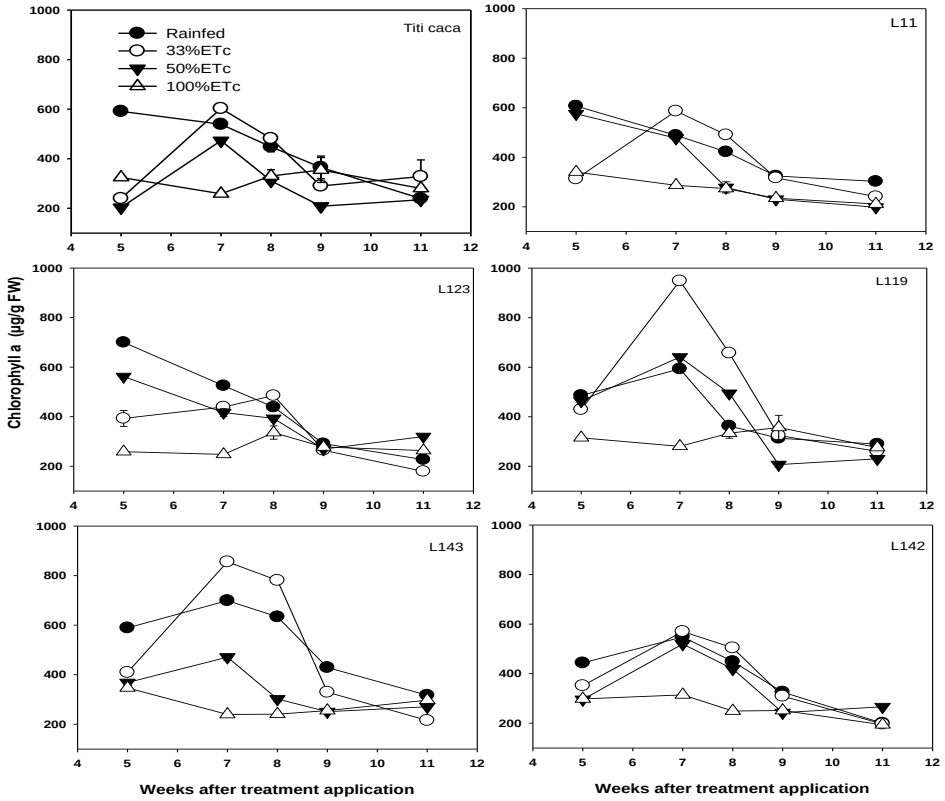

Figure 5: Evolution of chlorophyll a content along the experience at the six genotypes of quinoa: Titicaca; L11; L119; L123; L142 and L143, subjected to four water treatments of irrigation: 100% ETc (Δ), 50% ETc (▼); 33% ETc (◌); and Rainfed (●). (The values represent the average of 4 replicates ± standard error).

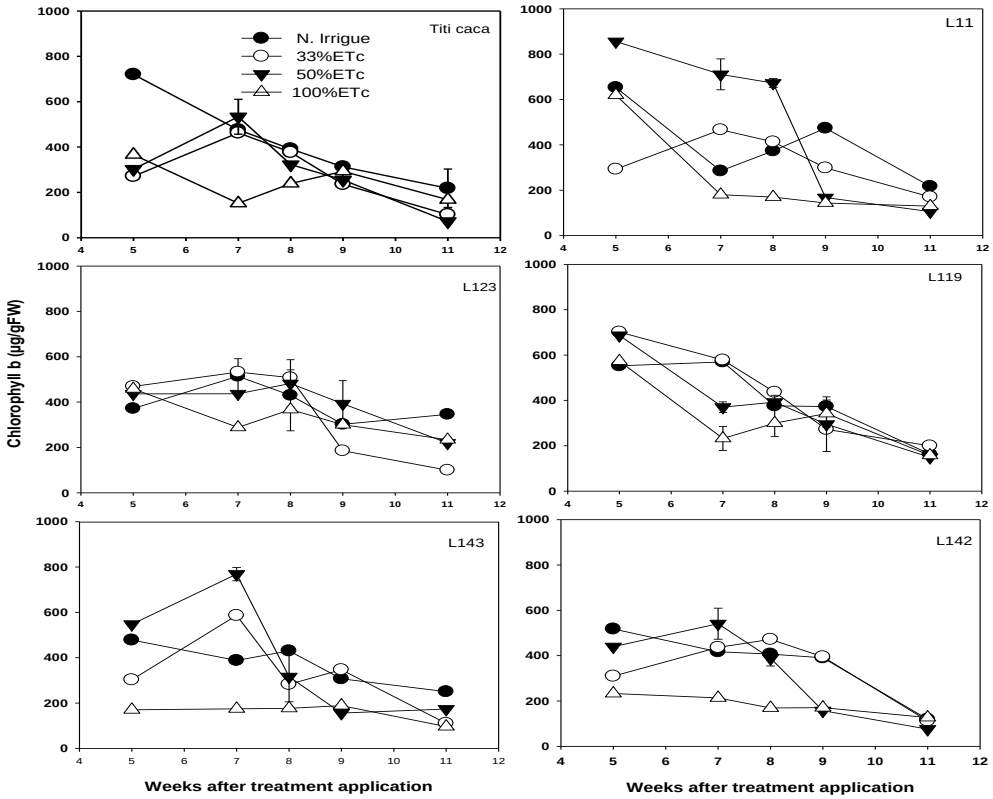

Figure 6: Evolution of chlorophyll b content along the experience at the six genotypes of quinoa: Titicaca; L11; L119; L123; L142 and L143, subjected to four water treatments of irrigation: 100% ETc (Δ), 50% ETc (▼); 33% ETc (◌); and Rainfed (●). (The values represent the average of 4 replicates ± standard error).

4. Discussion

Water deficit is one of the most important environmental stress, affecting agricultural productivity in worldwide [22]. It occupies and will continue to occupy an important place in the agri-business news. This is a serious problem in arid and semi-arid environments, where precipitation change from year to year and which plants are subjected to more or less long periods of water shortage.

Increased soil moisture deficit is normally accompanied by changes in roots and leaves water potential, the concentration of nitrate and the pH of xylem [23]. Soil moisture is the available water resource, which controls plant growth and water use, including the reduction and expansion of leaf area and stomatal conductance during drought [24]. Previous studies have shown a consistent relationship between the physiological processes of the plant (eg, leaf expansion, the stomatal conductance, gas exchange) and the fraction of water in the soil breathable in drought conditions [25-27]

The water status of the plant, characterized by the water potential reflecting the status of the binding of water within plant tissues, and allows to make the connection with the flow of water existing in the plant, resulting from the evaporative demand in the leaves, is governed by the law of the tension -cohesion in the soil-plant-atmosphere [28].

Our results show that the water status of the quinoa plant evaluated by the leaf water potential [29] was significantly affected by irrigation; the leaf water potential was closely affected by water stress treatments. In fact, we found in the six genotypes a variable reduction depending to water treatment. This reduction has increased significantly with decreasing amounts of irrigation water. However, levels of Ψ obtained are in agreement with the results of Fghire, et al. [3]. They showed that under irrigation at 100% Ψ was -0.5 to -1.0 MPa, and under stress conditions, it was reduced to -1,5MPa. Similarly, Jacobsen [30] showed that the quinoa Ψ in control plants was maintained above -1MPa. Jensen et al. [31] demonstrated that as a result of drought, stressed plants reached values of -2MPa. Other authors [7,32,33] reported in many cultures link between Ψ and soil water potential explored by the roots. At the quinoa plant Garcia, et al. [9] showed a linear relationship between Ψ and the water content of the soil. Relations between Ψ potential and irrigation dose found in this study also highlight the close relationship between changes in Ψ and soil water content. However, in all genotypes the Ψ exchange in a similar manner. The data were clearly different between treatments, especially at the end of the experiment, with a difference of 2.5MPa between the full irrigated and rainfed treatments. These differences between treatments could be partly the result of differences in levels of soil water. Although many studies of vines cultivated in the field [34,35], Bean [36], tomato [7] showed that Ψ of the sheet was significantly decreased in case of water stress as the case of this study.

This high capacity of quinoa to reduce Ψ of leaves contributes to its tolerance to water deficit. Vacher [37] showed the quinoa's ability to change its water status during the period of drought, with a daily amplitude of leaf water potential (big Ψ at night and very low Ψ during the day), which promotes the extraction of ground water and survival in drought conditions.

However, the leaves Ψ may not be the best indicator of water stress. Indeed dehydrated roots can produce chemical signals inducing stomatal closure, before the change of Ψ of the sheet is detectable [7, 38].

It is now well established that a signal is emitted from the roots to the leaves, favored by desiccation of the soil, it reaches the leaves by water transpiration appeal, inducing stomatal closure. That chemical signal is identified to abscisic acid (ABA), which is synthesized in the roots in response to the soil drying [39]. The stomatal reacts differently depending on the duration of the drought and also depending on the soil type [31]. Our results, demonstrated the significance ($p < 0.05$) reduction of gs under the water stress. Thus, indicate that the decrease in stomatal conductance could be a good indicator to the water stress and explain the reduction in leaf water potential. During a water shortage, the stomatal close to reduce water losses [7, 36]. The Stomatal closure and the limition of the photosynthesis depend to the severity of water deficit [40]. Previous results indicated that quinoa gas exchange parameters are within the normal range of other C3 plants such as lupine [31] and barley [41]. Others indicate that stomatal closure of quinoa grown in the field or greenhouse did not take place before the Ψ was below -1.2 to -1.6 MPa, why quinoa is characterized as tolerant crop dehydration [3, 31]. In this study, stomatal closure had already started a Ψ of -1MPa. Our results are similar to those found by Jacobsen, et al. [30] indicating that stomatal closure began in stressed plants when Ψ reached -0.8 MPa. While much of the water deficit induced reduction in CO_2 uptake can be attributed to stomatal closure, another part was attributed to direct effects of dehydration on the biochemical reactions of photosynthesis [40].

It is now known that the water status of the sheet interacts with stomatal conductance and transpiration and a good correlation is often observed between leaf water potential and stomatal conductance under water deficit [42]. However, several authors have reported a large heterogeneity at the correlation between stomatal conductance and water potential [43]. Stomatal response to other environmental factors may be responsible for

this dispersion [44]. Indeed, the precise relationship appears to be particularly dependent on the species studied, the stage of the water deficit, growing conditions and timing of the measures [45, 46].
Crop development in plants grown under limited moisture conditions is greatly disturbed [47]. A significant reduction of size and leaf area is generally observed [48]. The reduced leaf surface can come from a reduction in leaf expansion and/or an accelerated senescence of the leaf. Leaf growth is stopped quickly by water deficit, since it occurs at water potentials of -0.4 MPa [29]. Thus, plants subjected to water deficit generally exhibit a significant loss and leaf senescence accelerated [48]. The ABA is generated during water stress in the roots to be transported to the aerial part of the plant. This induce a decrease in the rate of elongation and leaf stomatal conductance in a number of species such as the tomato [7], corn [23], soybeans [49]. In quinoa, LAI of well-irrigated plants were significantly higher than that of plants subjected to different stress levels. This index, similar to the gs, has a degree of sensitivity to drought. Similar results have been demonstrated by Jacobsen, et al. [30] stating that under water stress, the rate of expansion of the leaves of the quinoa plants was significantly lower than the control from the beginning of water stress and that this index is more sensitive to drought than the gs.

Conclusions

Studies conducted as part of this work have broadened our knowledge of physiological responses of quinoa under different deficit irrigation schemes it has also allowed us to evaluate its potential for adaptation to climate semi-arid likes Marrakech region.
The results obtained showed that deficit irrigation mainly causes stomatal closure in quinoa. The plants under rainfed and 33% ETc close early stomata compared to the control. Furthermore, the plants under plan 50% ETc exhibit stomatal conductance similar to that of controls. Stomatal closures help plant to maintain the water status of the plant to reach the water potential of very low values, which are positively correlated with the intensity of stress.
The deficit treatments caused a significant reduction in leaf surface. Since the leaf area of plants is reduced under stress, the water used for transpiration is reduced; efficiency of water use is remarkably higher in these plants compared to controls.

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