Chapter

Influence of Water Stress on Growth, Chlorophyll Contents and Solute Accumulation in Three Accessions of *Vicia faba* L. from Tunisian Arid Region

Hanen Enneb, Leila Ben Yahya, Mohamed Ilyas, Datta Asaram Dhale, Mohamed Bagues and Kamel Nagaz

Abstract

In this study, we aim to investigate the physiological and biochemical adaptations of *Vicia faba* plants to moderate irrigation regime (T1) and describe the effects of water stress on their growth performance and chlorophyll contents. For this reason, three Tunisia accessions (ElHamma, Mareth and Medenine) were studied. An experiment was conducted for one month. Faba bean plants were first grown in a greenhouse and then, exposed to water stress, whereby they were irrigated up to the field capacity (FC) of 0% (control, T0) and 50% of the control (moderate stress, T1). The effect of water stress on physiological parameters showed differences in relation to the accessions studied and the water regime. Relative water content (RWC) of ElHamma accession does not seem to be affected by stress as compared with the control regime. Total chlorophyll content decreases, whereas soluble sugar contents increases for all accessions studied. ElHamma has the highest content. About morphological parameters, bean growth varies according to the ascension and treatment. Hydric stress impedes the growth of the root part and caused a significant reduction in the shoot and root Dry Weight (DW) of the T1-stressed beans, compared to the optimal irrigation (T0).

Keywords: water stress, *Vicia faba* L., RWC, growth, chlorophyll, soluble sugars

1. Introduction

Environmental stresses, especially water stress, seriously limit plant growth as well as plant productivity [1–3]. Thus, we speak of water stress when the demand for water exceeds the available resources. This category includes countries where the availability of water per year and per inhabitant is less than 1700 m^3, particularly in semi-arid regions. Arid zones are characterized by a negative hydric balance, mean annual rainfall of less than 800 mm, with a mean insolation of 2800 h/year and means annual temperatures of 23–27°C. These conditions lead to high evapotranspiration rates, carrying a water deficit in nearly the entire region [4].

Abiotic stresses and inefficient production techniques are the obvious factors limiting crop productivity, especially in the southern parts characterized by severe aridity [5–7].

Faba bean (*Vicia faba* L.) is a crucial grain crop through the word; ordering as the fourth crop legume after dry beans, dry peas and chickpeas. The bean is of great importance for nutrition, it enters in human consumption and also for animal feed [8]. North Africa appears to be one of the best faba bean output global regions and especially, Tunisia.

In Tunisia, faba bean covers more than 68% (70,000 ha) of the total area annually devoted to grain legumes crops and the national average yield is around 50,000 tons [9, 10]. Although the agro-economic importance of the bean is known, its cultivation is in decline, both in some countries of the world and in Tunisia [9]. The areas reserved for legumes in Tunisia in gradual fall [11]. This mainly affected the peas and broad beans. The national average bean yield is low and fluctuates widely from year to year. This is mainly due to climatic variations [6].

The seeds of the bean are very rich in proteins (25% of the dry matter of the seeds) and especially in lysine but poor in amino acid [12]. It is also a source of carbohydrates, mineral salts, fiber, vitamins and little lipids. The seeds are the most consumed parts and like all vegetables; the bean is self-sufficient for nitrogen nutrition. The bean is used as a green manure which serves as well for the improvement of the structure of the soil as for its enrichment in mineral elements. In addition to its nutritional and fertilizing (green manure) utility, the bean also has medicinal interests [13].

The success of agriculture in arid and semi-arid zones worldwide will be tremendously dependent on the ability of agricultural systems and farmers to adjust to climate change [14]. A vast majority of these areas, despite the huge production, suffer from limiting resource of water [15]. As that resource becomes more scarce and availability more difficult to predict, water managers and farmers will be forced to implement new creative solutions to water supply challenges. Expected exposure indicates improvement and better understanding of culture adaptation to water stress in such areas is high-priority to successful development in these regions under a changing climate. This work focuses on understanding the effect of water stress on morphological, physiological and biochemical aspects of *Vicia faba* L. plant grown in Tunisian Arid Region.

2. Concept of water deficit on plants

Water is a vital element for the growth and development of plants. It generally constitutes 85–90% of the total fresh weight of plants. Water is the natural vehicle for all the substances circulating in the plant, the elements absorbed in the soil or the organic substances produced in the leaves circulating in the saps of the xylem and phloem [16]. The need for water for the plant is proven by multiple observations such as wilting and death of plants left without water, hence the importance of irrigation and the relationship between vegetation and rainfall [17]. Plants can get the water they need through their roots. These extract water from the soil to pass it through the plant; part of this water is then released into the atmosphere (a phenomenon called transpiration) [1]. Omprakash et al. [18] defines water deficit or water stress as a period of insufficient water activity in the plant. According to this same author the notion of stress implies on the one hand a more or less abrupt deviation from the normal conditions of the plant and on the other hand a sensitive reaction of the plant in the different aspects of its physiology which changes appreciably with either adaptation to the new situation, ie to the limit degradation leading to a fatal outcome. On the contrary, according to Dodd and Ryan. [19] any water restriction resulting in a drop in the plant's production potential, following a disturbance of its physiological activity caused by a deficit in water consumption is called water stress.

3. Analyses of faba bean responses to water deficit stress

The experiment was carried out during 2019/2020, in a plastic greenhouse at the IRA (Institute of Arid Regions), located 22.5 km north-east of the city of Medenine. This region is located in the lower arid bioclimatic stage characterized by a low annual rainfall of around 150 mm/year; high temperature and frequent winds. *Vicia faba* seeds used in this test is the *Vicia faba* L. originating from Medenine and Gabès (ElHamma and Mareth). The latter ascensions tested were sown in pots filled with soil in February 2019 Three seeds were planted per pot; Three repetitions for each ascension were established. Water used for irrigation is tap water, titrating ±2 g/l and a pH of 7.6. Two water treatments were tested: T0 = 100% CC (capacity in the fields 100%) and T1 = 50% CC. The quantities of water (in ml/kg of soil) added each week (220 ml/kg for T0 and 110 ml/kg for T1). The dry weight of leaves and roots was carried out separately. The above-ground and underground dry matter is obtained after passing in an oven for 48 hours at 75°C. Relative water content of faba bean for each treatment were determined by the following method: Cut the leaf blade into small squares, weigh them to determine the fresh weights (FW) and then place them in a box of petri dish at 5°C. After 24 hours, the pieces of leaves are removed and then weighed at full turgor (TW). They are then placed in an oven at 75°C and then weighed again to determine their dry weight (DW). Chlorophyll contents determined by Gross method [20] and to quantify soluble sugars, the phenol method of Dubois et al. [21] was used.

4. Morphological changes in faba bean under water stress.

4.1 Plant biomass

Three accessions (El Hamma, Mareth and Medenine) of faba bean have been analyzed for dry matter accumulation. Significant effect of treatment ($P < 0.005$) was observed and no difference has been established between ascensions (**Figure 1**). Under moderate water stress treatment (T1), faba bean (i.e., El Hamma, Mareth and Medenine) accessions were significantly decreased their shoot and root DW as compared to control plants. Total leaf and root dry masses were all two to three times greater in the well-watered treatment (T0) than those in the water-limited one (T1). The watering treatment showed significant effects among localities on apparent shoot biomass and apparent root biomass (**Figure 1**). Risk-taking by minimal reduction of shoot DW under water stress was revealed as a physiological indicator for drought resilient at the flowering stage for faba bean [22]. The capacity of populations to maintain shoot biomass in water shortage was displayed to be an important trait for drought tolerance in soybean plants [23]. Under drought faba bean decreased their root DW (**Figure 2**). Root growth reduction was also reported for pea plant [24]. According to Chavoshi et al. [25] severe drought stress reduces biomass and seed yield (by 20–90%).

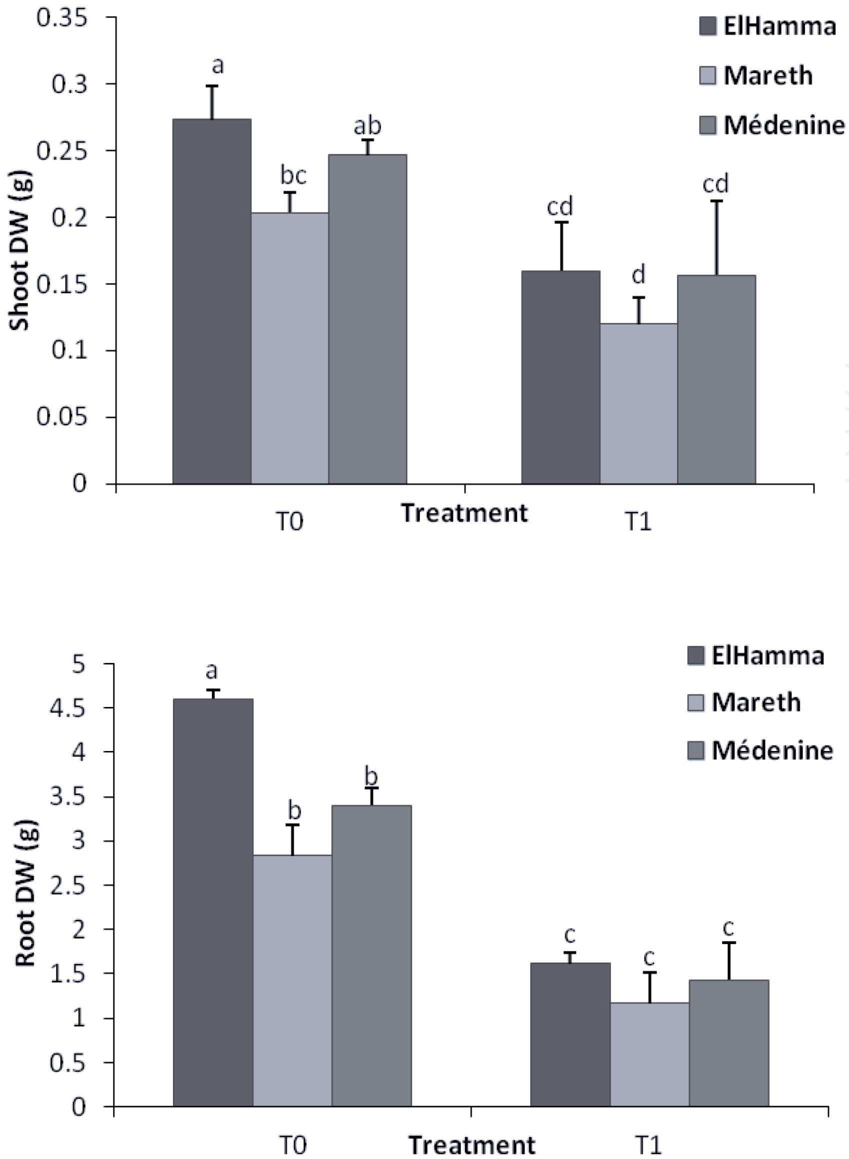

Figure 1.
Changes in (a) Shoot DW, (b) Root DW for Vicia faba *L. accessions in To (control) and T1 (moderate deficit irrigation (50%FC)). Means ± S.E. (n = 3). Means with different letters (a, b, c) are significantly different at P < 0.05 and values sharing a common letter are not significantly different at p < 0.05.*

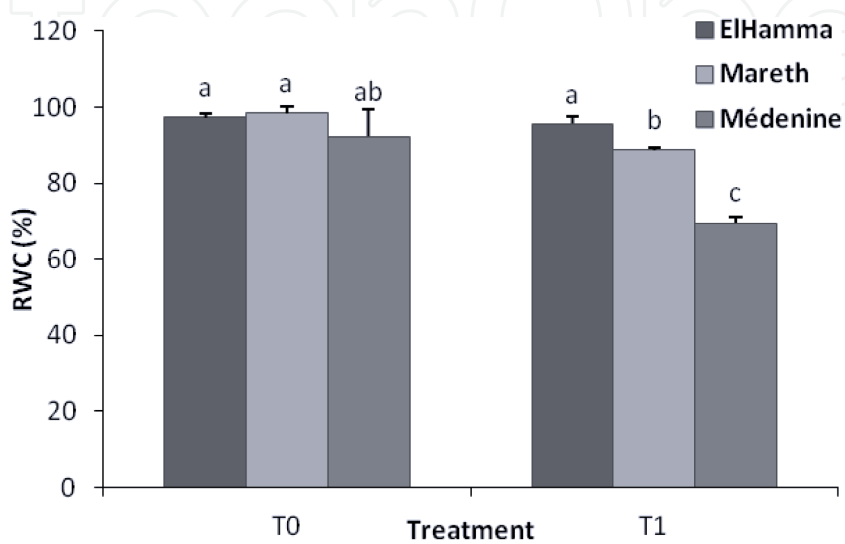

Figure 2.
Changes in relative water content (RWC) for V. faba *leaves in To (control) and T1 (moderate deficit irrigation (50% FC)).*

5. Physiological and biochemical responses of faba bean to water stress

5.1 Water status (RWC)

As shown in **Figure 2**, RWC varied significantly among accessions and RWC was significantly higher as expected in non-stress conditions (T0) than in

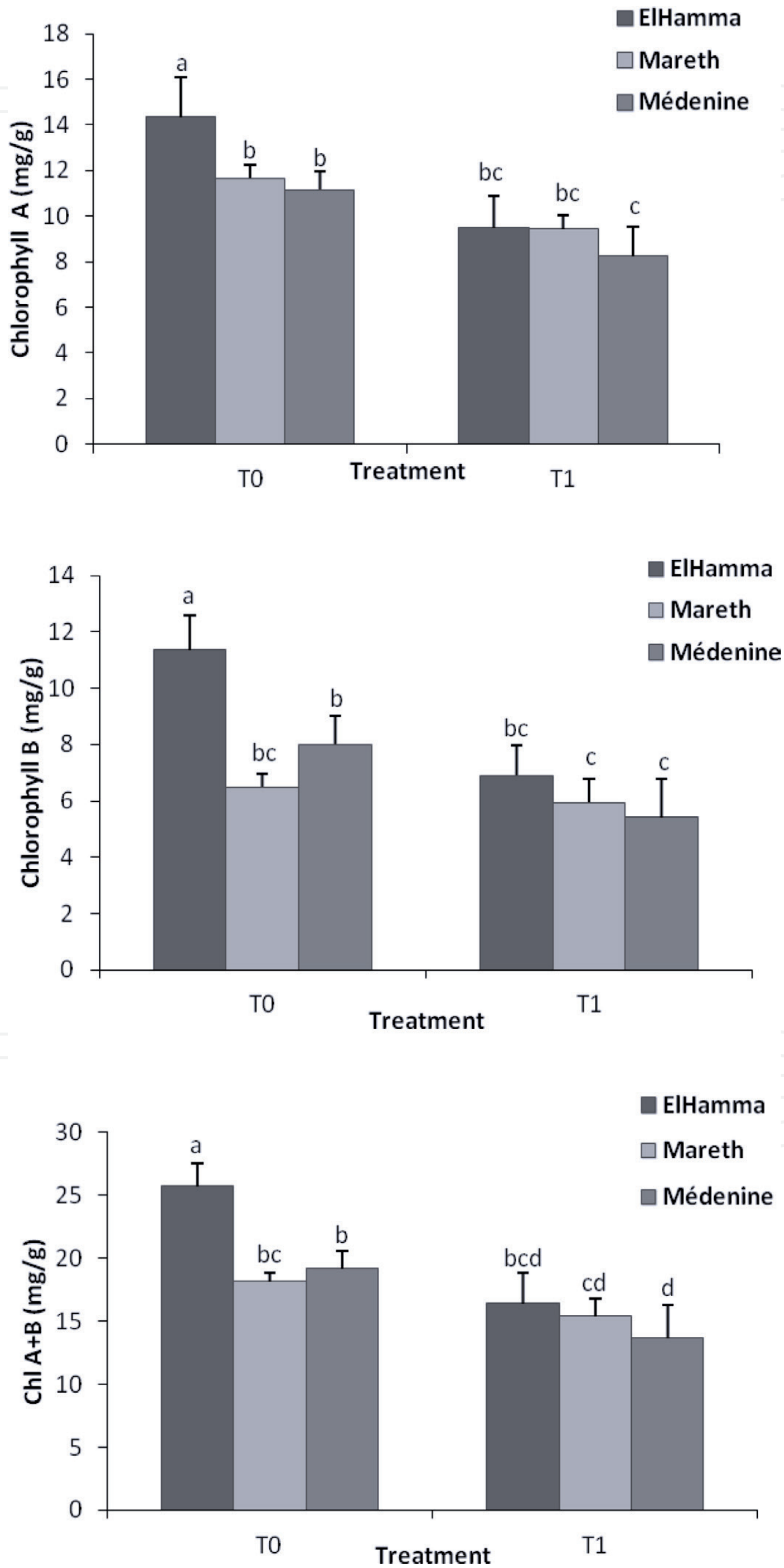

Figure 3.
Changes in Chlorophyll (a), (b) and (a+b) for V. faba *leaves in T0 (control) and T1 (moderate deficit irrigation (50% FC)).*

stressed plants (T1). This parameter is sufficiently influenced by water restriction ($P < 0.001$). Thus, leaves of the three ascensions studied reacted with a considerable drop in their relative water content (RWC). In El Hamma, the RWC is maintained at high values for irrigated plants and stressed plants, while in Mareth and Medenine, plants cultivated under T1, exhibited a significant decrease compared to the control of about 9.74% and 24.73% respectively. Repeated measures ANOVA, indicated significant interaction between accession and stress deficit ($P < 0.01$) for RWC. The analysis of the relative water content makes it possible to give a clue and describe in a global way the water status of beans in response to water stress. Decrease in RWC in plants under drought stress have been perceived in many plants and may depend on plant vigor. Likely, in this constraint, the ability to achieve good osmoregulation and to preserve cell turgor is reduced [26]. It seems that concentration of organic solutes to maintain membrane is not sufficient in this case [27, 28].

5.2 Chlorophyll contents

Chlorophyll 'A', 'B' and total chlorophyll contents, showed no significant difference among the accessions in the stress conditions, but significant difference under the non-stress conditions (**Figure 3**). The decrease in the chlorophyll contents in the three ascensions studied during the period of stress would be most likely due to the increased in catalytic activity of chlorophylls and degradation of photosynthetic pigments and this process is also the result of not providing the necessary factors for the synthesis of chlorophyll and the destruction of its structure under stress conditions [24]. Zhu [29] has been reported that a decrease in the chlorophyll content will be due to a decrease in the stomata aperture and aimed at limiting water losses by evaporation and by increased resistance to the entry of atmospheric CO_2 necessary for photosynthesis [30]. These results are in agreement with Tairo et al. [31] who reported a significant increase in chlorophyll 'A', 'B' and total chlorophyll contents was seen in varieties 1(KAT B9), 4(F8 Drought line) and 5(JESCA) of common bean *Phaseolus vulgaris* (L.).

5.3 Osmoregulation (accumulation of soluble sugars)

Induced drought stress influenced ($P < 0.001$) soluble sugar contents (**Figure 4**). Untreated plants showed the lowest concentration (0.54 mg/g for Mareth accenssion).

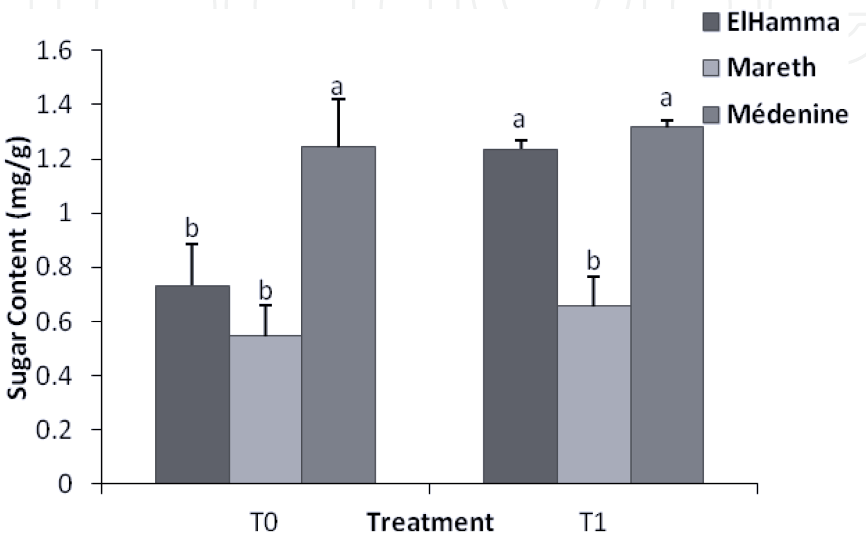

Figure 4.
Effect of water stress on soluble sugar contents of V. faba *L. leaves.*

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However, the highest soluble sugar content (1.31 mg/g) was noticed under water deficit condition for ElHamma cultivar. These metabolic adaptations that improve plant tolerance to osmotic or water stress involve an increased synthesis of compatible osmolytes as soluble sugars and proline. This increase in sugar content under stress might contribute for osmotic adjustment and plays an important role in scavenging free radicals and protect DNA from damaging effects of increased reactive oxygen species (ROS) levels due to water stress [32, 33]. Moreover, soluble sugar accumulation was considered as an indicator of plant tolerance to drought conditions. It improves water absorption and reduces water loss when stress leads to slower growth [34, 35].

6. Other environmental stresses effects on *Vicia faba* L.

The effect of sodium chloride (NaCl) concentrations (0.0, 60, 120, 240 mM) on growth and chlorophyll content of (*Vicia faba* L.) seedlings caused an increase for both fresh and dry weights of the shoot. Salinity significantly reduced chlorophyll 'A' content. It also significantly reduced chlorophyll 'B', total chl., and carotenoïds contents after ten days of treatment [36]. High-temperature stress gravely reduced the photosynthesis, stomatal conductanceand water use efficiency of *V. faba* [37–39]. The effects of magnesium (Mg) supplementation on the growth performance as well as, on photosynthetic pigment synthesis, were studied in *Vicia faba* L. plants exposed to heat stress and non-heat-stress conditions. Results revealed that growth attributes and total chlorophyll decreased in plants subjected to heat stress, whereas accumulation of organic solutes had seen. These results suggest that adequate supply of Mg is not only essential for plant growth and development, but also improves plant tolerance to heat stress by suppressing cellular damage induced by ROS through the enhancement of the accumulation of Proline and glycine betaine [40].

7. Improvement of crop production under environmental stresses

Crop production is influenced by multiple environmental abiotic factors including drought, soil salinity, waterlogging and temperature interferes with the whole metabolic activities in plants [41, 42]. Plant adopted different mechanisms and modifications to cope with stresses such as plant hormones that facilitating growth, nutrient allocation and development [43, 44]. Salicylic acid (SA) significantly increased the Mn^{+2}, Co^{+3}, Fe^{+2}, Cu^{+3}, K^{+} and Mg^{+2} while decreased the Na^{+}, Ni^{+3}, Pb^{+4}, Zn^{+2}, and Na^{+}/K^{+} content of roots and soil content under salinity stress. SA (10-5 M) can be implicated to mitigate the adverse effects of salinity on maize plants [45, 46]. Additionally, exogenous application of gibberellic acid (GA3) reduced oxidative stress in *C. capsularis* seedlings. GA3 not only ameliorate photosynthesis, biomass and plant growth, foliar application of GA3 but also, increases metal (Cu) concentration in different organs of the plants when compared to 0 mg/L of GA3. GA3 plays a promising role in reducing ROS generation in the plant tissues [47, 48]. Furthermore, melatonin application (100 mM and 500 mM) maintaining plant growth and relative water content. More than that, improving the photosynthetic characteristics, total carbohydrate, and total phenolic content in leaf tissues of faba bean plants irrigated with saline water. Melatonin might directly eliminate ROS when produced under stressed conditions [49].

Mineral compounds play too a key role in the structural and functional integrity of the plant to better respond to abiotic stresses. Silicon application enhances drought tolerance of *Kentucky bluegrass* by improving plant water relations and morpho-physiological functions [50, 51]. Moreover, the applications of N fertilizer linearly increased kernel weight during both growing seasons [52, 53]. N deficiency reduces photosynthates production in plants while an optimum N fertilization may increase weight and kernel corn quantity resulting in higher crop growth rates [54]. In addition, a combined application of phosphorus (P) and biochar mitigates heat-induced adversities on physiological, agronomical and quality attributes of rice. The application of biochar+P recorded a grain yield of 7% (plant-1) of rice compared with control throughout different temperature treatments and cultivars. The highest grain production and better grain quality in biochar+P treatments might be due to enhanced water use efficiency, photosynthesis and grain size, which offsetting the adversities of high temperature stress [55–57]. So, fertilization enhances heat injury in rice (*Oryza sativa* L.) [58].

8. Conclusions

In conclusion, water stress seems to be the main limitation factor to growth process in *V faba* L. Under moderate stress (50%CC), the tested ascensions responded similarly on how to cope with water stress. But, El Hamma had the highest performance as compared with Medenine and Mareth accessions. *V. faba* maintains their relative water content at high values and accumulates a high concentration of soluble sugars in their tissues. Accordingly, the dehydration avoidance is considered to be an adaptive mechanism for minimizing water loss. The evaluated *V. faba* accessions responded to water stress also, by reducing shoot and root biomass. Besides, morpho-physiological attributes relative to shoots and roots play an important role in the response to water stress and contributed to drought tolerance of plants. Therefore, it is imperative to identify the specific physiological process of drought tolerance in faba bean, and to define as valuable selection criteria under stressful conditions. Thus, nutritional and economic values of *V. faba* L. can be enhanced in Tunisia.

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Conflict of interest

The authors declare no conflict of interest.

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