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Impact of limited irrigation on the growth characteristics *of Lotus ornithopodioides* L.

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Abstract:
Background:
An increase in the severity of drought events on Mediterranean climates highlights the need of using native species adapted to drought. Thus, we investigated morpho-physiological differences in Lotus ornithopodioides L., a forage legume species endemic from Mediterranean, for traits that could effectively discriminate species performance in response to water restriction testing.
Material and Methods:
Results:
Greenhouse trials were set to study the effect of water deficit on growth parameters of Lotus ornithopodioides L.. A completely randomized design with two water regimes (100% and 40% of field capacity) were applied to plants of these Lotus specie grown in pots for seventy days of their vegetative development cycle. Roots and shoot dry weight, Root and shoot elongation, Water content (RWC) Root to shoot ratio (R/S of DM) and Chlorophyll content (a, b and total) were studied. The limited irrigation significantly reduced the growth of stem and root elongation, shoots dry weight and water content. Also, limited irrigation decreased chlorophyll (a), chlorophyll (b) and total chlorophyll content. However, limited irrigation increased the root to shoot ratio of DM.
Conclusion:
This study showed a L. ornithopodioides drought tolerance and its adaptation to extreme water stress condition (70 days), manifested by persistence and continuous aerial and root growth.
Key Word: *Drought, stem elongation, Dry matter production, relative water content, Shoot ratio of DM*

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I. Introduction

Drylands over 40% of the earth's land surface where semi-arid areas are most extensive followed by arid areas and then dry sub-humid lands (Huang *et al*., 2016). Nearly 20% of the degrading land is cropland, and 20-25%, rangeland. In these areas, agriculture is the major source of survive of local populations, whether through rain-fed and irrigated farming and pastoralism, where desertification and soil salinization, and increasing water stress are widespread (Peterson, 2018).

As climate change alters temperature and rainfall patterns, yields of some crops are decreased in rain-fed agricultural systems and leads to grassland productivity loses between 49-90% (Gaballah *et al.*, 2021). Preventing land desertification and degradation and supporting sustainable development in arid and semi-arid lands has major implications for food security, climate change and human settlement (UNEP, 2011). To overcome this constraint, it is imperative to find out the genotypes that can grow under water-scarce conditions. The use of naturally tolerant species for revegetation may be an interesting practice in arid and semi arid environments (Hessini *et al*., 2020).

Bowles *et al*. (2021) acknowledged that spontaneous plants growing naturally have evolved many adaptations to withstand drought. Acclimation of plants to drought is the result of various events, which affect some morphological, physiological, and metabolic changes lead to (Kapoor *et al*., 2020). Native Mediterranean species are usually considered tolerant and adapted to dry conditions and to soil salinity of semi-arid regions (Rejili *et al.*, 2010). *Lotus* species thrive in several regions of Tunisia which frequently undergo water and salt stress, and soils are vulnerable to desertification to erosion and desertification. These species endemic to these regions produce high biomass and are potentially high quality animal fodder. Further, their extensive root systems can contribute to the mitigation of soil erosion (Jaballah *et al*., 2010, Rejili *et al*., 2009 and Talbi *et al*., 2009).

Germplasm collections in Mediterranean basin have highlighted that there are species of *Lotus* that may have potential to be domesticated and benefic Mediterranean farming systems (Howieson and Loi 1994). In fact, only a few *Lotus* species have been domesticated and improved by selection and plant breeding, to be used as forage for livestock (Escaray *et al*., 2012). One of these is *Lotus ornithopodioides* L., an annual species of *Lotus* which was found in many collecting sites of Mediterranean areas growing on different soil types, from sand to clay loam, and different parent rocks which included granite, limestone, schist and basalt (Loi *et al.*, 1995; Nutt *et al*., 1996). Several authors showed, L. ornithopodioides has a number of desirable characteristic such as a deep root system, excellent pod retention on stems and important pod and seed production (Loi *et al.*, 2016 and Hajri *et al*., 2018a). Hajri *et al.*, (2018b) report the presence of *L. ornithopodioides* L. in arid and semi arid regions. This is an indicator of their adaptability to drought and soil salinity stress that affect their growth and yield. Therefore, they are considered promising forage legumes for arid and semi-arid areas.

In this context, exploration of the variability in water deficit stress responses would permit not only to identify some tolerant genotypes, but also to determine useful criteria for genetic improvement of water deficit tolerance. As part of this approach, the present study aimed to investigate the impact of limited irrigation on the growth and allometric parameters of *Lotus ornithopodioides* L. in vegetative stage.

II .Material and Methods

Fully ripened seeds of *Lotus ornithopodioides* L. from a natural population of a semi-arid area of Northern Tunisia were collected during July from different sites in northern Tunisia. Seeds of trefoil specie were sown in plastic pots filled with 5 kg soil. Pots were arranged in complete randomized design with three replications were established. After a period of plant establishment, irrigation was applied at two levels: full irrigation up to field capacity (100% Field capacity (FC) and limited irrigation (40% of FC). Water deficit treatment was applied up during the vegetative phase. The soil field capacity was estimated according to the technique of Bouyoucos.

Vegetative growth analysis

Growth parameters were evaluated at the end of the experiment (70 days after sowing). Plants were harvested and divided into roots and shoots. Length were immediately determined for roots (RE) and stem (SE). Dry weights (DW) were obtained by weighing the plant material after drying at 80°C until a constant mass was reached. Leaf root to shoot ratio of DW (R/S of DW) was calculated as DW of root /shoot dry weight.
Relative Water content (RWC) was calculated as (FW-DW) / DW.
Where:

DW: dry weight
FW: fresh weight

Chlorophyll analysis

Chlorophyll content (a, b and total) measurement was performed according to Wintermans and Mots (1965), and total chlorophyll concentration was calculated as in Horchani et *al* (2010).

Statistical analysis

The analysis of variance (ANOVA) for each character was performed followed by Levene's test to reveal the significance difference between means. The data were statistically analyzed by SPSS software, version 20.0. The confidence interval was calculated at the threshold of 95%.

III. Results and Discussions

The study of morphological traits showed that water regime was significantly affected on almost variables analyzed (Table 1). Water treatment affected stem and root elongation, shoot dry weight and Root: Shoot ratio of DM of the *Lotus ornithopodioides* specie (P<0.05). However, root dry weight of *L. ornithopodioides* L. plants was no significant variation under water treatment. Under deficit irrigation (40% FC), plants of *L. ornithopodioides* L. showed a significant decrease in stem and root length. This reduction was about 42.5% and 17% ~~42%~~, respectively. The reduction in stem length resulted in a reduction in shoot dry weight growth of 75%. Moreover, no significant differences ($P > 0.05$) in the values of mean root dry weight between the two treatments were found. These results corroborate those obtained by Acuňa *et al.* (2010) and Clua *et al.* (2009) in *Lotus tenuis* populations. Moreover, the aerial biomass reduction of *L. ornithopodioides* is coincident to others forage legumes responses, as *Medicago laciniata*, where severe water stress caused a significant growth reduction (Yousfi *et al.,* 2016).The root shoot ratio of DM was significantly affected by availability of water in the soil. When water was 40% FC this ration increased by more than 73% for *L. ornithopodioides* plants in comparison with the treatment without water stress (100% FC). The water deficit is reflected in resource allocation to roots. In fact, these ratios are 0.13 and 0.48 respectively for full irrigated treatment and severe stress. These results agree well with those conducted in certain arid regions. Thus, Ferchichi et *al* (2010) found for *Lotus creticus a* ratio between 0.293 and 0.663 respectively under ideal conditions and severe stress. Under ideal growing conditions, plants with a high root: shoot ratio of DM have a high energy cost of respiration (Slama *et al.*, 2006). Gargallo-Garriga *et al,* (2014) reported that shoots, considered as heterotrophic tissue, are metabolically deactivated during drought to reduce the consumption of water and nutrients, whereas roots are metabolically activated to enhance the uptake of water and nutrients, together buffering the effects of drought. Therefore, they considered the root: shoot ratio of DW is one of the most important determining facts in plant growth. It is considered as a criterion of adaptation to drought, which allows for more exploration of the soil to capture resources (Slama *et al*., 2006).

Relative water content (leaf RWC) varied significantly among water treatments (Table 1). *Lotus ornithopodioides* L. plants grown under limited water treatment showed lower shoot RWC, compared to well-irrigated plants. Result indicated that RWC decreased by more than 54% under severe conditions of water stress. Acuña *et al.* (2010) found that with decreasing irrigation RWC in *L. tenuis* decreased about 17%. Piltz *et al*., (2007) reported that the use of leaf RWC as an indicator of plant water status and plant drought tolerance is the best indices revealing the stress intensity. The rate of RWC in plants with high resistance against drought is higher than others. In other words, plant having higher yields under drought stress should have high RWC. So, based on the results, L. *ornithopodioides* can classified as medium tolerant genotype.

Table 1. Average values of growth parameters of *Lotus ornithopodioides* under two irrigation treatments

Treatment	SE (cm)	RE (cm)	SDW (g plant $^{-1}$)	RDW (g plant $^{-1}$)	R/S of DW	Leaf RWC
Full irrigation	43,76 ± 4,08	15,7 ± 1,15	1,33 ± 0,16	0,17 ± 0,03	0,13 ± 0,03	5,55 ± 0,98
Limited irrigation	25,14 ± 5,64	13,02 ± 1,45	0,33 ± 0,2	0,16 ± 0,03	0,48 ± 0,07	2,54 ± 1,01
Sig. P<0.05	***	*	***	NS	***	**

RE: Roots length, SE: Stem length, SDW: Shoot Dry Weight, RDW: Root Dry Weight, R/S of DW: Leaf root to shoot ratio of DW, RWC: Relative Water content

Chlorophyll concentration parameters plants under limited irrigation presented significantly (P<0.05) lower concentration than under full irrigation. As shown in Figure 1, water treatment reduced total leaf chlorophyll concentration by 21.6% for limited irrigation plants compared to full irrigated plants (Fig. 1). Chlorophyll a and chlorophyll b was decreased by 19.5% and 23% in water deficit treatment relative to control plants, respectively. This result suggesting chlorophyll degradation, leaf senescence, and reduced photosynthetic capacity.

Sosnowski *et al.* (2021) observed that increasing water stress caused a significant decrease in the content of chlorophyll pigments in leaves of *Medicago* × *varia* T. Martyn. However, Nunes *et al,* (2008) indicate, no significance change in the leaf pigments content (chlorophyll *a*, total chlorophylls and carotenoids) of *M. truncatula* leaves between water treatments.

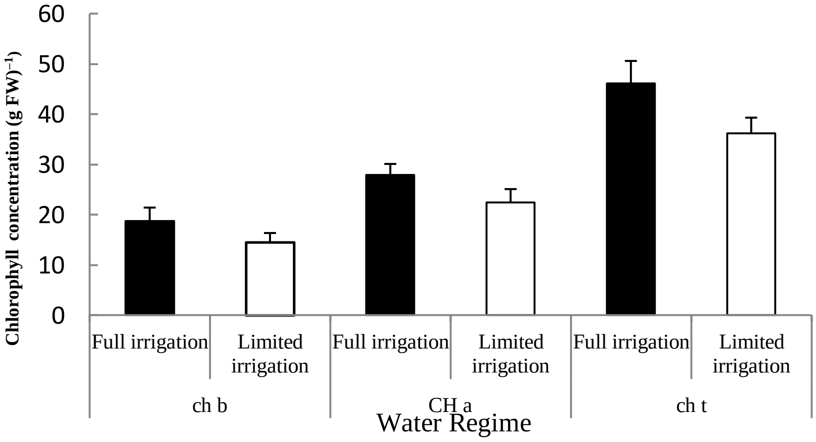

Figure 1. Total leaf chlorophyll concentration, chlorophyll a and chlorophyll b in *Lotus ornithopodioides* L. leaf in response to water treatment.

IV. Conclusion

The present pot study indicated that limited irrigation significantly reduced the growth parameters stem and root elongation, shoots dry weight, Root: Shoot ratio of DM of *L. ornithopodioides* L. and water content. Water deficit also reduced chlorophyll contents of *L. ornithopodioides* L. plants compared to non limited water treatment at 100% of field capacity. However, further work is needed to test this population, including additional morphological and physiological traits and more severe drought conditions.

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