GSC Advanced Research and Reviews

eISSN: 2582-4597 CODEN (USA): GARRC2

Cross Ref DOI: 10.30574/gscarr

Journal homepage: https://gsconlinepress.com/journals/gscarr/

(RESEARCH ARTICLE)

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Effect of water stress imposed at vegetative stage on growth and physiological traits of subterranean clover

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GSC Advanced Research and Reviews, 2023, 14(02), 066–071

Publication history: Received on 12 December 2022; revised on 24 January 2023; accepted on 26 January 2023

Article DOI: https://doi.org/10.30574/gscarr.2023.14.2.0032

Abstract

Pasture productivity in the Mediterranean region depends mainly on the soil water deficit. Subterranean clover originates from the Mediterranean region and is adapted to areas with hot and dry summers. The response of Subclover to drought was studied in greenhouse and subjected to three soils - water levels: 100% (control), 50 and 25% of field capacity (FC). After 70 days of water deficit treatment, our results showed that increasing levels of water constraint resulted in a progressive decline in the different growth parameters (shoot and root dry matter (DM) height, leaf area, shoot and root (DM)) and those of water content and leaf chlorophyll concentration. At the limited irrigation, *Trifolium subterraneum* L. showed smaller decreases in plant height and leaf area. Despite, limited irrigation increased the root to shoot ratio of DM. Thus, it shows better adaptation and a more balanced water balance. Findings of this study suggest that subterranean clover used avoidance mechanisms to withstand drought.

Keywords: Water stress; *Trifolium subterraneum*; Growth; Water content; Leaf chlorophyll concentration

1. Introduction

The Mediterranean region has been classified as a hot spot for climate change [1] with high potential of facing important problems of water scarcity in the next decades. In this regard, a gradient of decreased rainfall and runoff has been recorded in addition to increased temperatures in the Mediterranean area [2]. Climate change could also impact soil erosion, through both higher climatic aridity leading to less vegetation cover and a decrease in precipitations [1].

To overcome climate change and increasing water consumption in agriculture, the selection adapted species tolerant to drought and the identification of physiological mechanisms used by plants to cope with drought are of paramount importance [3]. Due to their ability to fix atmospheric nitrogen and solubilize phosphorus, legumes improve productivity, help efforts to adapt to climate change, and provide essential nutrients to soils and other crops. Growing legumes can help to restore soil health and support soil biodiversity. It's the key to sustainable management of soil nutrients [4]. Annual legumes of Mediterranean zone are known to be adapted and survive hot dry summers and severe intermittent drought. These species developed suited strategies that enabled them to survive the rigors of the climate. Such strategies include seed dormancy and delayed germination and morphological traits of seeds dispersal by grazing animals or soil burial [5]. Research work have proposed the uses of some annual self-reseeding pasture species (lupin and subterranean clover) to enhance the capacity for herbage and seed production under water stress [6].Therefore, such species area necessary component of sustainable agricultural production systems.

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In this regard, subterranean clover (*Trifolium subterraneum* L.) is a widely distributed, self-seeding annual in the Mediterranean area. Periods of drought may become more pronounced because of global climate change [6] and sustaining total production, productivity and quality of herbage may become more difficult. Increased knowledge of the mechanisms underlying whole-plant responses to soil water depletion may help to improve management practices and identify characteristics for drought. The objective of this present research work is to study the effects of water stress on plant growth and some physiological traits of *T. subterraneum* L. associated with resistance to drought.

2. Material and methods

2.1. Plant material and growth conditions

Trials were carried out to examine drought tolerance of subterranean clover (*Trifolium subterraneum* L. cv. 45C). Seeds were provided by the Tunisian National Institute of Agronomic Research (INRAT). The experiment was carried out under greenhouse conditions at Ecole Supérieure agriculture du Kef research station. Subclover seeds were sown in plastic pots of 5 Kg containing a mixture of sand and soil (1:3). Pots were arranged in complete randomized design with six replications and each replication had six plants. Seedlings were allowed to establish for seventy days after sowing (at the second-leaf stage), before treatment started. Three water regimes (100%, 50% and 25% of field capacity) were applied to subterranean clover plants. Soil field capacity (FC) was estimated according to the technique of Bouyoucos.

2.2. Vegetative growth analysis

Growth parameters were evaluated at the end of the experiment. Plants were harvested and divided into roots and shoots. Dry weight (DW) measures were obtained by weighing the plant material after drying at 80 °C until a constant weight was reached. Water content (WC) was calculated as (FW-DW) / DW. Leaf area (LA) was measured as carried out by direct measurements using a LI-3000A (LICOR Inc., NE, and USA). A chlorophyll content measurement was performed according to [7], and total chlorophyll concentration was calculated as in [8].

2.3. Statistical analysis

Data collected was analyzed statistically using analysis of variance techniques (ANOVA). Differences among treatments means were compared by using Duncan test. Significant differences were accepted at $P < 0.05$ and represented by different letters. All stats analyses were performed using "Statistical Package for Social Sciences" (SPSS, 20) program.

3. Results

3.1. Growth response and water status

Water regime imposed at the vegetative stage, significantly (P < 0, 05) decreased stem and root length compared with that of the control plants (Table 1). The Subterranean clover plants showed a higher value of stem elongation (11.6 cm) under well-watered conditions. In the moderate and (50% FC) severe (25 % FC) water stress treatment, height was reduced compared with the water-sufficient treatment by 26.62% and 39.57%, respectively (Table 1). The Subterranean clover plants showed significant difference between the root lengths of plants under water restriction treatments (Table 1). Under deficit irrigation, plants of *T. subterraneum* L. cv. 45C plants showed a significant decrease in root length. This reduction was about 30% and 38%, respectively.

Water treatments were applied at the second-leaf stage. At this stage, the initial root and shoot dry weights were (0.013 ± 0.004) g and (0.012 ± 0.007) g, respectively. The changes in the root and shoot dry weights (DW) and in shoot-to-root ratio in root were investigated after 70 days period of deficit water treatment. With reduced water supply, the root and shoot dry weight accumulation decreased in both the moderate water stress and severe water stress treatments. Results shows that the plants subjected to 50% and 25% of FC produce 42.86% and 64.29% less root dry weight in comparison with the non-limiting water regime, respectively. In fact, the SDW in the two deficit water treatments was reduced of similar magnitude (72.5%).

The root to shoot ratio of DM was significantly increased for plants subjected to water deficit compared to in the well-watered plants. When water was a limiting factor, this ratio increased by 2.29 and 1.19 under 50% and 25% of FC, respectively in comparison with the treatment without water stress (100% of FC) (Table 1).

In response to a water stress treatment, the leaf area was significantly reduced (P≤0.01). At the end of the water stress period, the decrease was marked in the severe water stress treatment (FC 25%) and in the moderate stress (FC 50%) with a reduction of 67.42 % and 47.32%, respectively (Tab. 1).

Table 1 Average values of growth parameters of *Trifolium subterraneum* under three soils - water levels: 100% (control), 50 and 25% of field capacity (FC)

Growth parameters	Water regime			Sig. P<0.05
	25 % FC	50% FC	100 % FC	
Stem elongation (cm)	7.00b	8.50b	11.60a	**
Root elongation (cm)	7.50b	8.58b	12.25a	**
Shoot DW / g–1 plant	0.120b	0.100b	0.40a	**
Root DW / g–1 plant	0.05c	0.08b	0.14a	***
Root-to-shoot ratio	0.42b	0,8a	0,35c	***
Leaf area(mm2/leaf)	228c	368,67b	699,8a	***

3.2. Water status and Chlorophyll concentration

Drought treatments caused a significant (p<0.01) decrease in leaf relative water content (RWC) of *Trifolium subterraneum* plants. Under severe level of water stress treatment, the decrease in RWC was 66%. No differences were observed between full irrigation treatment and the moderate stress treatment (FC 50%) (Tab. 2).

Total chlorophyll concentration and chlorophyll a to chlorophyll b ratio are shown in Tab. 2. Water treatment decreased leaf chlorophyll content of plants under 50% FC and 25 % FC by5.54 % and 16.80 %, respectively. Total chlorophyll concentration and chlorophyll a to chlorophyll b ratio are shown in Tab. 2. Water treatment decreased leaf chlorophyll content of plants under 50% FC and 25 % FC by 34% and 21%, respectively. Chlorophyll a–to–chlorophyll b ratio was decreased by 51.72% and 32 % fewer than 50% FC and 25 % FC of field capacity relative to control plants, respectively.

Table 2 Total leaf chlorophyll concentration, chlorophyll a to chlorophyll b ratio in *Trifolium subterraneum*L. leaf under three soils - water levels: 100% (control), 50 and 25% of field capacity (FC)

Growth parameters	Water regime			Sig. P<0.05
	100 % FC	50% FC	25 % FC	
Relative Water content	3.95a	3.66a	1.25b	***
Total chlorophyll / mg (g FW)$^{-1}$	38.48a	36.35b	38.48c	***
Chl a–to–Chl b ratio	2.77a	1.88b	1.37c	***

4. Discussion

Soil and atmospheric drought are two processes constraining grassland ecosystems productivity. Water scarcity disturbs and damages the growth and development of grassland vegetation, where growth and biomass production parameters decrease progressively in response to water deficiency. The results from this study demonstrated that the water stress throughout plants of *Trifolium subterraneum* L. cv. 45C significantly affected trefoil plants growth processes, resulting in a decrease in plant height, leaf area, root length and biomass accumulation (Table 1). These results are congruent with previous reports, which demonstrated that by increasing moisture stress, plants showed a progressive decrease in leaf area, growth rate, height and shoot dry matter [9,10 and 11].

For the *Trifolium subterraneum* L. plants, plant height decreased significantly in response to soil water availability compared with control plants. According to [12], the reduction in plant height is resulting in reduction of leaf and stem expansion due to restriction of stem cell division and elongation caused by the lower turgor pressure. Our results are in accordance with those reported previously for other crops such as soybean [13]and Lucerne [14]. Under moderate and severe water deficit, leaf area was affected by water stress and was reduced by 67.5% and 47.5% compared with the control, respectively. The reduction in leaf area may be due to a decrease in the epidermal cell mitotic activity which results of reduction in the total number of leaf cells per leaf [15]. It may also be due to a reduction in cell size due to anatomical changes [16]. According to [17], the decrease in LA is a strategy to improve survival ability under drought conditions through reduced energy consumption and increased resource storage. The leaf area plays *an important* role in plant strategies [18]. [19] have also shown that the decrease in leaf area under the limiting water regime is an adaptive mechanism of plants aimed at limiting their leaf transpiration when water conditions become unfavorable.

In this study, we found that shoot and root DM of *Trifolium subterraneum* L. plants were negatively affected by different water-stress levels. It indicates that the two treatments reduced the growth of shoots rather than roots, relative to the control treatment. Thus, shoots were more sensitive than roots because their DM reduction was greater [20] indicated that plants in drought often decrease their biomass production and allocate more biomass to roots, to reduce consumption and increase absorption of water. As suggested by the optimal partitioning hypothesis, greater biomass would be distributed to roots in both cold and dry climates because plants react to environmental gradients by adjusting their allocation pattern to optimize resource use [21]. Our results agree with previous findings with other species, including those of [11] on *Lotus ornithopodioides* L. plants,[22] on *Medicago laciniata* [10] vetch.

Trifolium subterraneum L. plants grown under severe water treatment (25 % FC) showed lower shoot RWC compared to well-irrigated plants and moderate stress treatment (FC 50%), where RWC decreased by to 24% under severe treatment. Previous studies have found that water content reduced by 13%, 5% and 6% under 40% FC treatment for *V. narbonensis*, *V. sativa* and *V. villosa*, respectively [10]. [23] 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. A higher RWC indicates that the plant is better adapted to water stress [24]. 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.

A decrease in chlorophyll concentration under drought has been reported for many plant species [10,11]. In our study, water treatment decreased leaf chlorophyll content of plants under 50% FC and 25 % FC by 16.80% and 5.5%, respectively. Regarding photosynthetic pigments, current results revealed a decrease with an increase in the severity of drought stress. Our current results support the findings of [25]. [11] Who also reported a reduction in chlorophyll contents under drought stress conditions in *L. ornithopodioides* L. The reduction in chlorophyll content has been attributed mainly to the disruption of chloroplast caused by the up regulation of reactive oxygen species (ROS) during stressful conditions such as drought [26]. The increase in Chl b ratio under drought for plants indicates greater damage to Chl b than to Chl a [27].

5. Conclusion

Water deficit also reduced chlorophyll contents of Subclover plants compared to non-limited water treatment at 100% of field capacity. The water deficiency also caused an increase in Chl a to Chl b ratio in of *Trifolium subterraneum* L. plants. Studied specie as adapted to the constraint of water deficit mainly through avoidance mechanisms. They were able to retain water in their leaf tissues by reducing growth.

Our data confirmed the basis for the ecological distribution of Subclover specie. The tolerance of *T. subterraneum* explains it's adapted to dry areas. Further studies are needed to determine whether this specie involves tolerance strategies to cope with water stress. Such data would be essential for promoting the economic and environmental sustainability of crop livestock systems in water-limited regions.

Compliance with ethical standards

Disclosure of conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication between the authors.

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