

## Original Research Article

### Variation in phenological parameters of Alfalfa *Medicago sativa* in response to water stress

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#### A B S T R A C T

Lack of water is a major constraint responsible for the loss in crop yield and deterioration of vegetation. The development of a vegetation naturally resistant to aridity and salinity is a promising approach for the rehabilitation and recovery of degraded environments. Alfalfa is a forage very answered in the Mediterranean countries including Tunisia. The importance of this culture is its broad adaptation to different environmental conditions (salinity, drought and cold), this plant also has a very significant nutritional value which makes him a good quality feed for sheep and cattle. Tunisian oasis representing the main production sites of this culture have long climate damage, economic and vegetable. In this context, this work has focused on the assessment of phenological responses during the flowering stage at different levels of water stress of alfalfa *Medicago sativa*, forage legume highly adapted to the oasis conditions. The results showed that production in fresh material (MFT) and dry (MST) is variable between the different treatments applied and the plants in 100% of field capacity have produced an MSF about 80 g on average, this weight is high compared to the production of plants subjected to water stress 25% of field capacity (from an average of 25g of order). The increase in water deficit resulted in a reduction of the number of inflorescences formed, number of pods and weight of seeds produced. The weight of seed recorded for the highest stress level is of the order of 2 g while the weight of seeds for recorded control plants is 30g.

#### Keywords

North africa,  
*Medicago sativa*,  
Water stress,  
Biomass,  
Agricultural development,  
Production,  
Population

#### Introduction

Water stress is the main environmental factor that constrains the productivity and stability of plants in the Mediterranean area. Thus, with increasing frequency of dry years in arid and semi-arid, rainfall is low and often irregular, 40 % of cultivated land are affected each year by drought.

Water stress induces a disruption of many morphological processes, phenological, physiological and metabolic (Pérez -Pérez *et al.*, 2009; Carmen Antolín *et al.*, 2010) as the change in net photosynthesis rate of the solids formed, the protein biosynthesis and accumulation of solutes (Huang *et al.* 1997;

Salekdeh *et al.*, 2002; Jaleel *et al.*, 2008; Bouizgaren *et al.*, 2013).

Acclimatization to water deficit results from a series of integrated events increased to morphological, physiological and biochemical levels that help retention or acquisition of water and the protection of plant functions. A better understanding of the physiological mechanisms involved in resistance to water stress is closely connected with research and the selection and development of plants naturally resistant to aridity and salinity. This is a promising approach for the rehabilitation and recovery of degraded environments.

The perennial alfalfa (*Medicago sativa*) is one of the main crops in the South Tunisian -Is mainly in coastal oasis of Gabes. The dispersion of this culture in Tunisia and in the world is mainly due to its strong adaptation to different climatic conditions and high nutritional value. In addition, this plant has a taproot system that can reach deep levels of soil to help recover water losses. In addition, according to (Mauriès, 1994), alfalfa is rich in protein, essential element in livestock nutrition compared to other crops such as corn and soybeans.

This ability to grow in different climates indicates that alfalfa can develop different mechanisms of resistance to a broad range of stresses, particularly in drought.

It is in this framework that incorporates this study to see the effect of four levels of water stress (25%, 50 %, 75% and 100% of field capacity) over three perennial alfalfa populations (population Tebelbou, Gannouch population and population Chenini), grown in the coastal oases of southern Tunisia, mainly the yield of total fresh and dry matter, pods and seeds.

## Materials and Methods

To conduct this study, a seedling was conducted in spring in plastic pots 10L each. Each pot contains a mixture of 2/3 sand and 1/3 of soil of the oasis. The plant material used consists of three alfalfa populations (Population Tebelbou (BER), Gannouch population (GAN) and population Chenini (CHEN) collected from local farmers. Plants have suffered four water stress levels 25, 50, 75 and 100 % of capacity in the field with three replicates for each treatment. The trial was conducted in the experimental plot of the Institut of Arid Regions in Medenine glass greenhouse in controlled conditions.

At harvest, for each population and for each treatment, we conducted the following measures:

- The total number of pods (NTG)
- The total number of inflorescences (NTF)
- The total seed weight in grams (PTG)
- Production in total above ground fresh weight in g (MFT).
- The Production of total dry matter in g (MST)

The results under went statistical analysis by SPSS 16.0 software. An analysis of variance is applied by the LSD test to compare the differences between means. The results are significant at the 5% threshold. A classification of the population is made using the Duncan test to identify the degree of tolerance. The relationship between the parameters studied is performed by the index of Pearson.

## Result and Discussion

Analysis of variance showed that water stress (S), the population parameter (POP) and their interactions (S \* POP) have highly

significant effects (1 %) on all parameters are studying (Table 1).

The application of water stress led to a sharp reduction in the total fresh and dry matter produced during the spring mate. This reduction is mainly observed in the higher stress levels (25% and 50% of field capacity) (Fig. 1A). Values spend a production of around 80g as maximum average under treatment 100% of field capacity to a value of around 18g as recorded minimum value under treatment 25% of field capacity for the MFT. For the MST, the results show that the values decrease worth (as recorded maximum value) in the control treatment to a value below 75% of field capacity under treatment at a value 50% of field capacity to reach a value under treatment 25% of field capacity (Fig. 1b).

The number of inflorescences produced cloves and was also influenced by water stress, this effect is highly significant ( $p < 0.0001$ ) when Tougher water stress levels. (25% to 50% of field capacity. For the number of inflorescences, the values recorded under treatment 100% of field capacity exceeding 300 inflorescences as total number, however under treatment 25% of field capacity, values recorded are less than 20 inflorescences.

Reducing the number of inflorescences entails reducing the number of pods formed. Thus, production pods for under control treatment plants is worth up to 22 pods compared to the number registered by the plants under treatment 25% of field capacity (Fig. 2 and 3).

The effect of water stress is highly significant on the total weight of seeds

produced. It goes from a 28g weight value as maximum production plants under treatment 100% of field capacity while this production is of the order of 3 g for plants under treatment 25% of field capacity (Fig. 4).

The results show very highly significant differences between populations for all parameters studied. Moreover, Gannouch population registers the highest values for all parameters studied and even under the most severe stress levels while the population saves Tebelbou lowest values under the same conditions according to the test Duncan (Table 2).

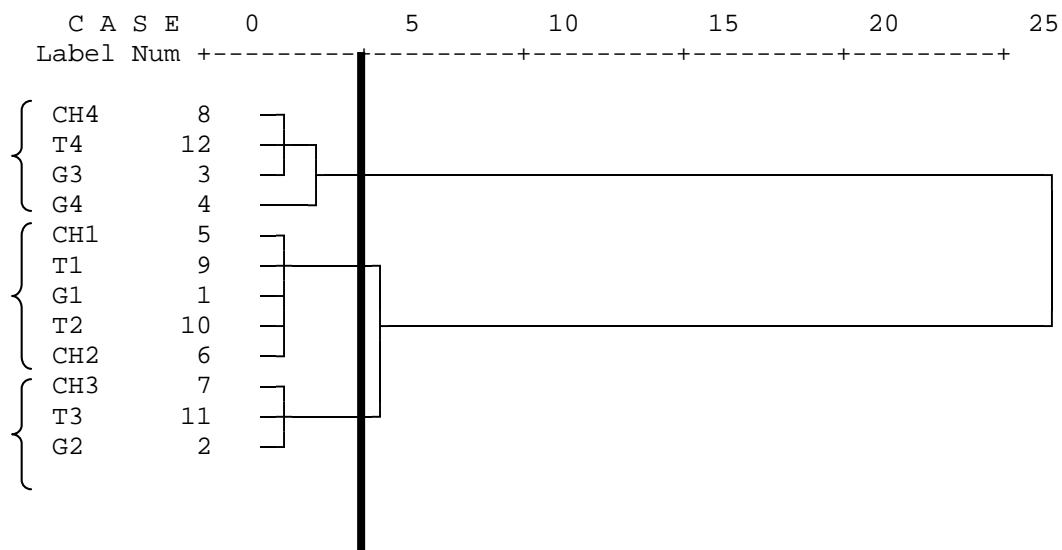
The hierarchical classification permits the classification of the population taking into account the stress level applied and the first group is the three populations subjected to treatment 4 (100% of field capacity) in addition to the Gannouch population (G) subjected to treatment 3 (75% of field capacity) and are characterized by the highest production in NTI, NTG, PTG, MST and MSF.

The second group contains the three populations subject to the most severe treatment (25% of field capacity) in addition to the Tebelbou population (T) and chenini (CH) 2 subjected to the treatment (50% of field capacity) and which are characterized by the lowest productions for all the parameters studied. The third group consists of people with average values.

This classification allows to visualize the treatment 25 and 50% of field capacity as the most severe treatment resulting in weaker productions.

Dendrogram using Ward Method

Rescaled Distance Cluster Combine



The Pearson correlation established according to the index can distinguish positive and highly significant correlations between the different parameters and exceed 0.9 (Table 3)

The application of water stress causes the reduction of the total dry matter and fresh in alfalfa seedlings. This reduction is observed very significant under the most severe conditions of stress applied (25 and 50% of field capacity) and in some cases, the reduction reached 50% compared to that recorded in the control conditions. These results are consistent with results obtained for alfalfa (Mauriès 1994) (Laouar, 1996) and (Erice *et al.*, 2010). The same results were obtained in several species such as *Pennisetum glaucum* L. (Kusaka *et al.*, 2004), *Phaseolus vulgaris*, *Sesbania aculeate* (Ashraf and Iram, 2005) and for plant corn (Chimenti *et al.*, 2006) and sesame (Hassanzadeh *et al.*, 2009).

The total number of inflorescences (NTI) and produced pods (NTG) and the total weight of the grains (PTG) were also negatively influenced by water stress. These results are consistent with the results

obtained by Chebouti and Abdelguerfi (1996) which indicate that the number and total weight of pods produced and the total weight of seeds per pot has been reduced in seedling of *Medicago truncatula* stressed during phase vegetative and flowering stage under the effect of water stress. In the same context, Vidal *et al.* (1981a), studying the influence of drought on yield components in soybean, report that the number of pods per plant is the most affected component. These studies revealed that the strain effect depends on its position in the cycle of development and intensity. Although some species such as maize (Lauer, 2005), the reduction in yield occurs both during the vegetative phase reproductive, the flowering phase is the most sensitive to the application of water stress. This is consistent with our results and with others obtained in several species namely *Sorghum* (Mastorilli *et al.*, 1999), the legume *Vigna radiata* (Ney *et al.*, 1994), Bean (Lizana *et al.* 2006) and in

sesame (Boureima *et al.*, 2011). Reducing these parameters is highly significant ( $p < 0.001$ ) during the application of the most severe water stress levels (25% to 50% of field capacity). However, and in contrast to several species, with a strong ability to adapt to water stress, performance is affected as

from a water stress corresponding to 74% of the satisfaction of the overall needs in water culture Sunflower resulting in improved efficiency of water use for the synthesis of total dry biomass (Merrien and Grandin, 1990).

**Table.1** Squares means from the analysis of variance performed for the effect of water stress (S), population (POP) and their interactions (S \* POP) on production total dry and fresh material (MST and MFT), the total number of pods (NTG) and inflorescences (NTF) and the total weight of the grains (PTG)

|              | df | MFT                  | MST      | NTG        | NTI                   | PTG                  |
|--------------|----|----------------------|----------|------------|-----------------------|----------------------|
| <b>S</b>     | 3  | 464.00***            | 1.807*** | 398.694*** | 102362.32***          | 524.963***           |
| <b>POP</b>   | 2  | 333.083***           | 2.162*** | 112.583*** | 2635.861***           | 223.694***           |
| <b>S*POP</b> | 6  | 46.639 <sup>NS</sup> | 2.162*** | 7.139***   | 456.046 <sup>NS</sup> | 14.546 <sup>NS</sup> |
| <b>CV(%)</b> |    | 53                   | 8        | 73         | 69                    | 60                   |

\*\*\*: hautement significatif au seuil de (1‰); NS: non significatif.

**Table.2** Classification des populations [Gannouch(GAN); Chenini(CHEN) at Tebelbou (TEB)] selon le test de Duncan

|             | MSF | MST | NTI | NTG | PTG |
|-------------|-----|-----|-----|-----|-----|
| <b>GAN</b>  | a   | a   | a   | a   | a   |
| <b>CHEN</b> | b   | b   | b   | b   | b   |
| <b>TEB</b>  | c   | c   | c   | c   | c   |

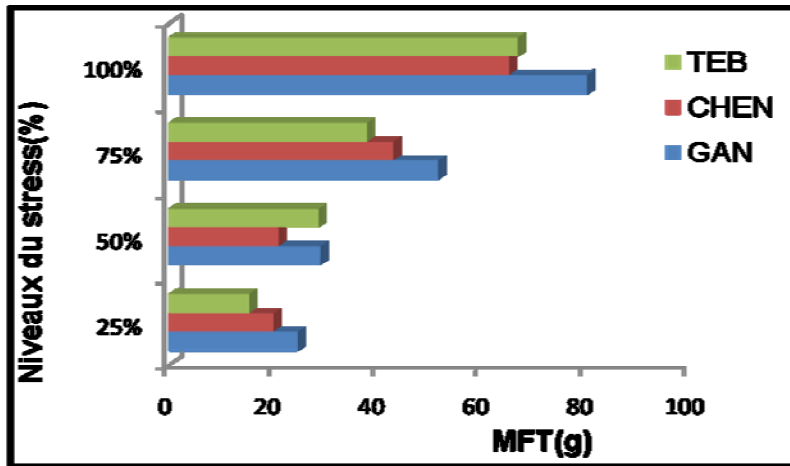
Les valeurs portant la même lettre ne sont pas significativement différentes au seuil de 5%

**Table.3** Correlation according to Pearson index

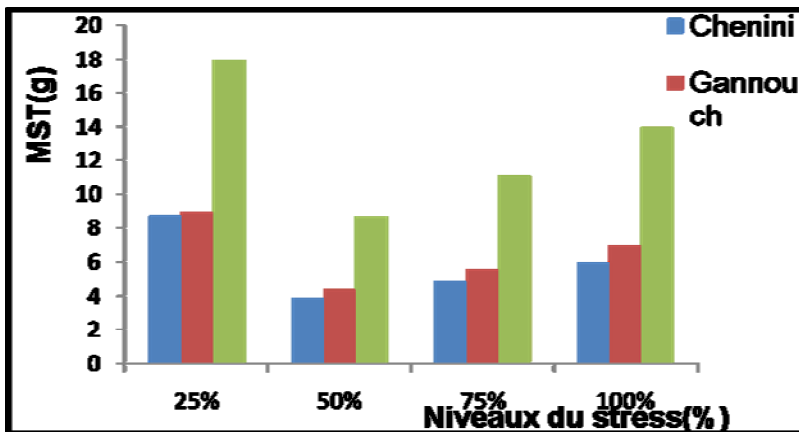
|            | NTF | NTG            | PTG            | MST            | MFT            |
|------------|-----|----------------|----------------|----------------|----------------|
| <b>NTF</b> | -   | <b>0,918**</b> | <b>0,894**</b> | <b>0,999**</b> | <b>0,981**</b> |
| <b>NTG</b> |     | -              | <b>0,965**</b> | <b>0,936**</b> | <b>0,945**</b> |
| <b>PTG</b> |     |                | -              | <b>0,916**</b> | <b>0,908**</b> |
| <b>MST</b> |     |                |                | -              | <b>0,985**</b> |
| <b>MFT</b> |     |                |                |                | -              |

\*\* La corrélation est significative au seuil de 1%.

**Fig.1** Variation of fresh material (MFT) (a) and dry (TSM) (b) Total under water stress among three populations of *Medicago sativa* (GAN, CHEN and TEB)

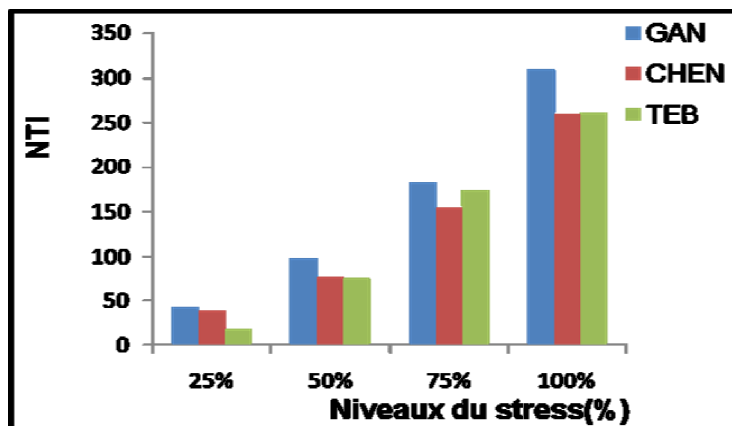


(a)

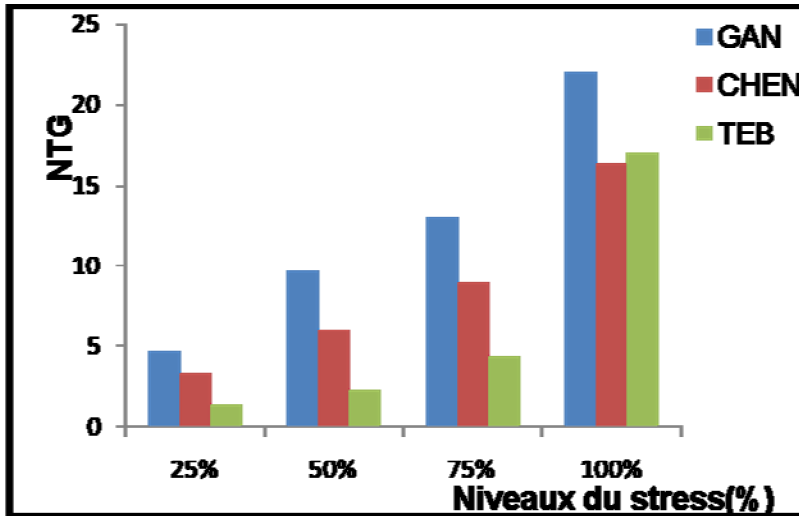


(b)

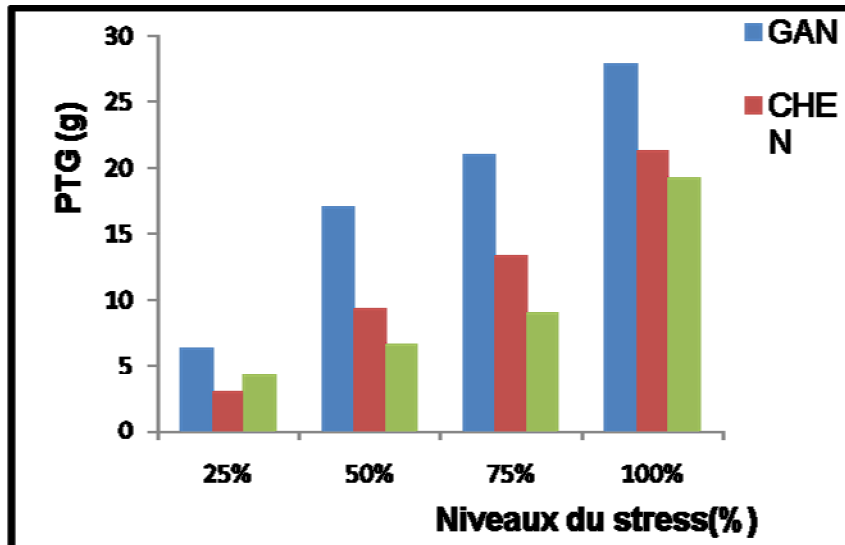
**Fig.2** Change in total number of inflorescences under water stress among three populations of *Medicago sativa* (GAN, CHEN and TEB)



**Fig.3** Change in the total number of water-stressed cloves in three populations of *Medicago sativa*. (GAN, CHEN and TEB)



**Fig.4** Change in the total weight of seeds under water stress among three populations of *Medicago sativa* (GAN, CHEN and TEB)



This decrease in yields was explained by several causes ente other concomitant decrease in plant height, number of branches, and by the fall flowers, in addition, it may be the result of the effect of water stress on biomass production and accumulation of nitrogen (Jeuffroy etWarembourg 1991; Guilioni *et al.*, 2003).

This explanation is reinforced in this study by the coefficients of students and significant correlation between NTI, NTG and PTG one hand and production MST (0.999, 0.936 and 0.916) in MFT (0.981, 0.945 and 0.908) d another part. Similarly, Bidinger *et al.* (1987) attribute the decrease in performance constrained situation in

particular loss of dry matter stems, which probably corresponds to the mobilization of part of the dry matter of stems for filling the ears. In other crops such as millet, Setter and al.2001) explain the yield reduction by increasing the content of ABA. They attribute this acid important in yield loss, since its accumulation inhibits photosynthesis by stomatal closure (Mugo *et al.*, 2000). Furthermore, recent studies have shown that invertase is the enzyme responsible for the inhibition of the grain filling during water stress (Boyer and Westgate, 2004).

The difference between populations can be explained by inconsistent application of ecological factors

Water stress is the major constraint responsible for the loss of crop yields and damage to the canopy years the Mediterranean area. In this study, the water deficit caused significant reductions of different parameters studied. However, these results are a first step in the study of the effect of this stress on the development of alfalfa (*Medicago sativa*), a plant that plays an important role in improving forage production of superior quality and can resistant to different environmental conditions.

The difference between populations can be explained by inconsistent application of ecological factors.

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