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Effect of water stress on plant growth and nodulation of some Algerian local population of *Medicago truncatula*

Abderrezak Chebouti^{1*}, Nassila Meziani², Mohamed Laib¹

1. National Institute of Agronomic Research of Algeria, Plant Genetic Resources Research Division, BP 200 Hacén Badi, El-Harrach, Algiers, Algeria.
2. University of Science and Technology Houari Boumediene, Faculty of Biological Sciences, BP 32 Bab Ezzouar, Algiers, Algeria.

*Corresponding author: cheboutiabderrezak@yahoo.fr

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Abstract

The objective of the present research was to evaluate the response of five local populations of *Medicago truncatula*, collected from different sites of Djelfa (Algerian central steppe), to water stress. Two water levels were applied during experiment: normal irrigation treatment (control) and severe stress treatment (20% of pot capacity). At the beginning of the flowering stage, the following traits were measured: Stem length which has the first flower (SL), aerial biomass weight (ABW), root weight (RW), number of nodules (NN) and nodule weight (NW). Drought response index (DRI) was calculated. Analysis of variance showed significant variation among populations and between treatments for the measured traits. Significant differences between DRI values of aerial biomass weight, number of nodules and weight nodule were found too. In general, all measured characters were affected by water stress. The Reductions were more significant for all traits. The highest effect was observed for nodulation traits (number and weight of nodules). Based on DRI values, population from Mliliha region (Algeria) recorded the highest DRI values for plant growth and nodulation traits. This population seems to be drought tolerant and can be used as parental material in *Medicago* species breeding program for drought tolerant.

Key words: drought respons index; *Medicago truncatula*; nodulation; plant grought; water stres.

Introduction:

Biosphere's continued exposure to abiotic stresses, for example, drought, salinity, extreme temperatures, chemical toxicity, oxidative stress, etc., cause imbalances in the natural status

of the environment (Lisar et al., 2012). Agricultural production worldwide is severely limited by these abiotic stresses. The main one of these abiotic constraints is drought, which not only creates gaps between average yields and potential yields, but which also causes variations from one year to another (yield instability) (Ruivenkamp and Richards, 1994). Drought is multidimensional in nature, and it affects plants at various levels of their organization. In fact, under prolonged drought, many plants will dehydrate and die (Lisar et al., 2012).

Drought risks being one of the major consequences of climate change for large part of the planet, whether in the tropics or the temperate regions of Europe or North America. But the drought is also the heaviest consequences constraint that most of the world's farmers face each year (Gaufichon et al., 2010).

The water deficit constitutes a major constraint for agricultural production in the world. Drought stress is considered as one of the crop performance limiting factors and a threat for successful crop production (Fathi and Tari, 2016). Drought or soil deficit can be chronic in climatic regions with low water availability or random and unpredictable due to changes in weather conditions during the period of plant growth (Alizadeh et al., 2014). Therefore, the development of plants with increased survivability and growth during water stress is a major objective in the breeding crops (Osakabe et al., 2014).

Algeria represents one of the richest areas of genetic diversity, where a wide variety of agro-ecological environments can be identified; nevertheless, the random characteristics of annual precipitation and unpredictable and severe droughts often aggravate the situation of Algerian agriculture, which recognize a great fodder deficit, where animals are often subjected to periods of frequent food shortages (Abdelguerfi, 1994).

Legumes of the genus *Medicago* are of special ecological and agro-pastoral importance in the Mediterranean regions. They include forage species expressing high levels of N-fixation and protein production per hectare (Huguet et al., 1994). Their high levels of hard seededness makes them well adapted to ley-farming systems and to persistence in regions of unreliable rainfall (Nichols et al., 2007). Most of agronomical traits of interest such as the tolerance to drought stress can be found within annual species of *Medicago* L. (Badri et al., 2006). *M. truncatula* is closely related to many economically important legumes and therefore its investigation is of high relevance for agriculture (Colditz and Braun, 2010). *M. truncatula*

present a considerable variability genetic, resulting in many differences in phenotype and growth characteristics between ecotypes (Barker et al., 1990). Key attributes of *M. truncatula* include its self fertile mature, its prolific seed production and its rapid generation time (Cook, 1999).

In the present study, two different water stress gradients were established, by controlling the irrigation. The objective of this experiment was to assess the effect of the severity of water stress occurring during vegetative stage on plant growth and nodulation in local population of *Medicago truncatula*.

Materials and Methods

Plant material and water stress treatment

Five local populations of *Medicago truncatula* collected were used. These populations were collected in different sites of Djelfa (Algerian central steppe) by the National Institute of Agronomic Research of Algeria (INRAA) in 2008. The latitude and longitude of each site were taken using a portable Global Positioning System (GPS) receiver (Table 1).

Table 1: Geographic coordinates of the sites of *M. truncatula* populations.

Geographical origin	Symbols of Populations	Altitude (m)	Latitude	Longitude
Ain Oussera	MtAO	758	35° 17' 08''	2° 57' 37''
Mliliha	MtMli	806	34° 48' 89''	3° 48' 94''
Charef	MtCh	960	34° 40' 31''	2° 43' 13''
Oued Touil	MtOT	718	35° 16' 43''	2° 33' 23''
Bouiret Lahdab	MtBL	830	35° 12' 66''	3° 02' 88''

The study was conducted during the 2020/2021 cropping season at the experimental farm of the Research Station of National Institute of Agronomic Research of Algeria (Baraki, Algiers). The trial was conducted out in a glass greenhouse in order to control irrigation, in particular to avoid rainfall. Scarified seeds were sown on 23 December 2020 in 5L pots containing 2/3 clay soil and 1/3 loam, at a rate of five seeds per pot. A layer of gravel was

placed at the bottom of each pot to facilitate irrigation water drainage. At emergence, two plants per pot were maintained. Pots were arranged in a completely randomized design with four replications.

Two water levels were applied during experiment: normal irrigation treatment (control), while the second treatment corresponds to severe stress (Stress applied corresponds to a drying rate of 80% of the soil available water). At the beginning of the stress period (branch development stage), the water content of each pot was at field capacity. Plants are irrigated as soon as the threshold of 80% of the drying rate is reached. The control of the drying rate of the soil during the stress period was done by weighing the pots every two days.

Water requirements were determined using the mean maximal evapotranspiration (MET) of five lysimetric pots, according to Mouhouche et al. (1998) method. The difference between the amount of water supplied and that drained was calculate in each lysimetric pots. The maximal evapotranspiration was equal to: $MET = I - D$. where **I** is the irrigation water supply (ml), **D** the drainage lysimetric pots (ml). Lysimetric pots were cultivated under the same conditions as the experimental pots.

At the beginning of the flowering stage, eight individual plants were harvested and used to evaluate the effect of water stress on plant growth and nodulation. The following traits were measured for each plant and for each treatment: Stem length which has the first flower (SL in cm), aerial biomass weight (ABW in g), root weight (RW in g), number of nodules (NN) and nodule weight (NW in mg). Drought response index (DRI) according to Chen et al. (2207) was estimated: $DRI = \text{Value under stress condition} / \text{Value under control condition}$.

Statistical analyses

Data of measured traits and DRI were subjected to analysis of variance (ANOVA) using R software version 3.6.1. Differences between means were performed using the Least Significant Difference (LSD) test at a 5 % probability level. The relationships among traits and DRI values of traits were tested using Pearson correlation coefficients. Principal components analysis (PCA) was performed using Statistica 6.0 software.

Results

Drought effects on plant development and nodulation

Analysis of variance showed significant variation among populations and between treatments for the measured traits, except for root weight and nodule weight, where differences are not significant (Table 2). The mean values of stem length, aerial biomass weight, root weight, number of nodules and nodule weight of the well irrigated treatment were 28.92 cm, 24.8 g, 17.86 g, 44 and 28.23 mg, respectively. In general, all measured characters were affected by water stress. The Reductions were more significant for all traits. Compared to the control treatment, traits decreased significantly by 26.3 %, 36.6 %, 29.6, 51.8 and 59.5 %, respectively. The highest effect was observed for nodulation traits (number and weight of nodules). Furthermore, the highest reduction for growth and nodulation traits were registered in Ain Oussera population, demonstrating its vulnerability to deficit water.

Table 2: Combined analysis of variance for measured traits of *M. truncatula* populations.

Traits	SL	ABW	RW	NN	NW
Population					
Df	4	4	4	4	4
<i>p</i> -value	0.496 ^{ns}	0.001***	0.001***	0.001***	0.001***
Lsd	2.494	2.594	2.113	6.118	5.120
Mean NIT	28.9	24.8	17.8	44.3	28.2
Mean SST	21.3	15.7	12.5	20.9	11.4
RR	26.3	36.6	29.6	51.8	59.5
Treatment					
Df	1	1	1	1	1
<i>p</i> -value	0.001***	0.001***	0.001***	0.001***	0.001***
Lsd	1.577	1.641	1.336	3.869	3.238
Population x treatment					
Df	4	4	4	4	4
<i>p</i> -value	0.448 ^{ns}	0.002**	0.690 ^{ns}	0.001***	0.003**
Lsd	3.527	3.668	2.988	8.652	7.240

SL: Stem length; **ABW:** Aerial biomass weight; **RW:** Root weight; **NN:** Number of nodules; **NW:** nodule weight. **NIT:** Normal irrigation treatment; **SST:** Severe stress treatment; **RR:** Reduction rate. **Lsd:** Least significant difference; Significant levels: ns: not significant; *, **, ***: significant at 5%, 1% and 0.1% respectively.

Relationships among measured traits values were obtained in both treatments (control and severe stress). Similar correlations were found in two water treatments (Table 3). Nodule weight is positively correlated with number of nodules and root weight. The number of

nodules and nodules weight are correlated between them, this suggest that nodulation traits vary in similar ways. Comparisons between the two treatments, specific correlations were noted. In control treatments, the root weight is positively correlated with aerial biomass weight and number of nodules, while Stem length is negatively correlated with root weight in severe stress treatment.

Table 3: Correlation coefficients among measured traits recorded in *M.truncatula* populations under normal irrigation treatment (**above**) and under severe stress treatment (**below**).

	LS	ABW	RW	NN	NW
LS	1.000	-0/045	-0.204	-0.131	-0.273
ABW	-0.105	1.000	0.886*	0.634	0.388
RW	-0.957*	-0.077	1.000	0.907*	0.898*
NN	0.203	0.114	0.602	1.000	0.891*
NW	-0.139	0.438	0.883*	0.879*	1.000

Significant levels, *: p<0.05; **: p<0.01; ***: p<0.001.

SL: Stem length, ABW: aerial biomass weight; RW: root weight; NN: number of nodules; NW: nodule weight.

The principal component analysis was performed to visualize the main sources of variability among populations of *M. truncatula*. For both treatments, the principal components analysis identified two principal components. The two principal components accounted 86.6 % and 77.64 % of the total variability among genotypes for the control treatment and severe stress treatment, respectively (Figures 1, 2). For the control treatment, the first principal component accounted for 65.89 % of the total variability and was associated negatively with aerial biomass weight, root weight, number of nodules and nodule weight. The second component explained 20.17 % of the total variation and was associated only with stem length. The cluster analysis performed on the first two components grouped all of *M. truncatula* populations. Population of Ain Oussera region, negatively correlated with PC1, were characterized by high weight of aerial biomass and root, high number of nodules and high weight of nodules, contrary to Charef and Oued Touil populations plotted on the positive side of PC1. While population from Bouiret Lahdab region, correlated negatively with PC2, presented the longest stem than Mliliha population. For the severe treatment, the first PC explained 40.78 % of the total variation and was correlated positively with root weight and negatively with stem length. Contrary to the population from Charef region, plants of Ain Oussera population were characterized by high weight root and short stem. The second PC

accounted 36.86 % of total variation and was correlated with aerial biomass weight, number of nodules and weight nodule. Populations from Mliliha and Oued Touil correlated positively with PC2, were characterized by high weight of aerial biomass, high number of nodules and high weight of nodule.

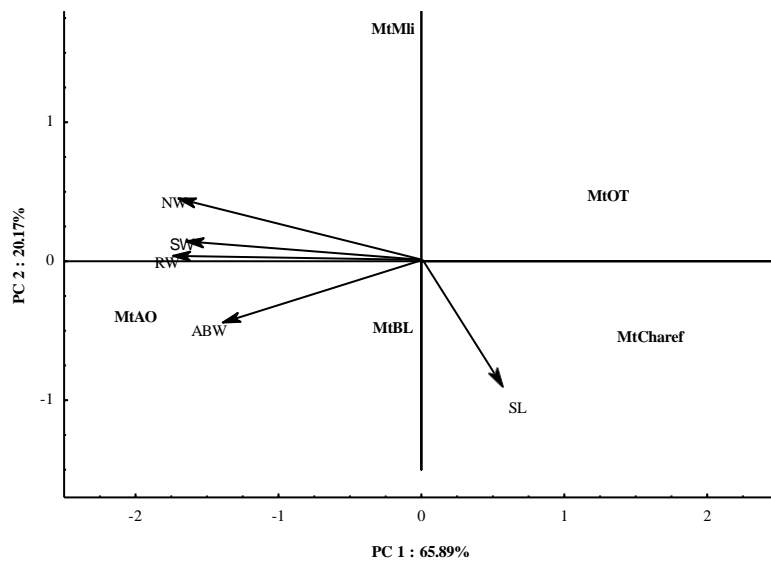


Figure 1: Plot of the two principal components showing dispersal populations of *M. truncatula* grown under normal irrigation treatment.

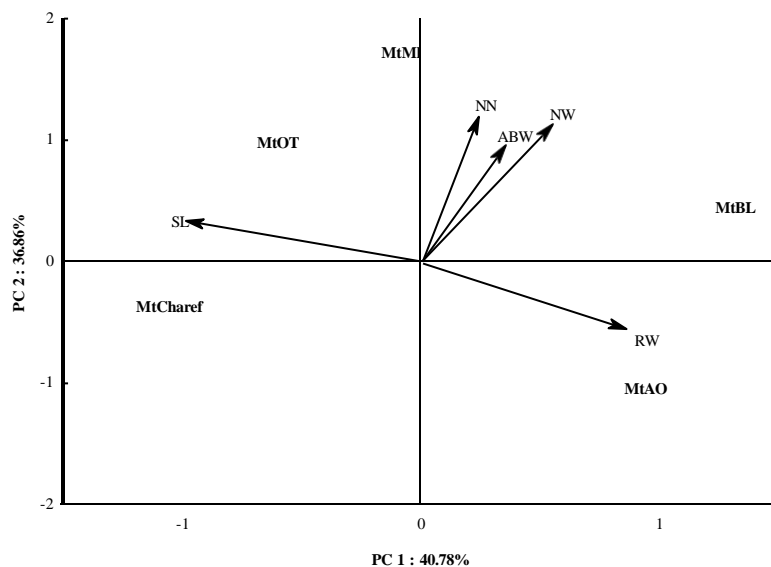


Figure 2: Plot of the two principal components showing dispersal populations of *M. truncatula* grown under severe stress treatment.

Drought response index (DRI) values variation

Generally, analysis of variance showed significant differences between DRI values of aerial biomass weight, number of nodules and weight nodule, with the exception for stem length and root weight DRI, no significant differences were noted (Table 4). The mean DRI varied from 0.51 (MtAO) to 0.75 (MtMli), from 0.33 (MtAO) to 0.68 (MtMli) and from 0.24 (MtAO) to 0.60 (MtBL) for aerial biomass weight, number of nodules and weight nodule, respectively. Compared to all the populations studied, population from Ain Oussera region recorded the lowest DRI values for plant growth and nodulation traits.

Table 4: DRI mean values of measured traits in *Medicago truncatula* populations.

Populations/traits	SL	ABW	RW	NN	NW
MtAO	0.67	0.51 ^c	0.71	0.33 ^c	0.24 ^c
MtBL	0.71	0.58 ^{bc}	0.70	0.48 ^{bc}	0.60 ^a
MtCharef	0.77	0.70 ^{ab}	0.71	0.41 ^{bc}	0.32 ^{bc}
MtMli	0.79	0.75 ^a	0.69	0.68 ^a	0.57 ^{ab}
MtOT	0.77	0.68 ^{ab}	0.72	0.54 ^{ab}	0.54 ^{ab}
GM	0.74	0.64	0.71	0.50	0.45
<i>p-value</i>	0.309	0.001	0.994	0.001	0.001
Sign.	Ns	***	Ns	***	**
Lsd	0.128	0.100	0.134	0.108	0.154

SL: Stem length; **ABW:** Aerial biomass weight; **RW:** Root weight; **NN:** Number of nodules; **NW:** nodule weight. **GM:** Grand mean, **Lsd:** Least significant difference; Significant levels: ns: not significant; *, **, ***: significant at 5%, 1% and 0.1% respectively.

To examine the relationships among DRI values of measured traits, results revealed two positive correlations. Length stem was correlated with aerial biomass weight and nodulation traits (number and weight of nodules) were correlated between them. These relationships indicate that the traits vary in similar ways in control and sever stress treatments (Table 5).

Table 5: Correlation coefficients among DRI values of measured traits.

	SL	ABW	RW	NN	WN
SL	1.000				
ABW	0.912*	1.000			
RW	- 0.216	0.183	1.000		
NN	0.785	0.599	- 0.546	1.000	
WN	0.315	0.355	- 0.190	0.913*	1.000

Significant levels, *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$.

The principal components analysis identified two principal components, which together accounted for 86.6 % of the total variation among populations (Fig. 3). The first PC explained 58.28 % of the variation and was positively correlated with traits stem length, aerial biomass weight, number of nodules and nodule weight. The second PC accounted for 25.32 % of the total variability, mainly due to variation in root weight. The dispersion of populations in the plane defined by the two PCs revealed a strong heterogeneity between *M. truncatula* populations in response to water deficit. In fact, population from Mliliha region, plotted on the positive side of PC1, is distinguished from Ain Oussera population by high DRI values of stem length, aerial biomass weight and nodulation traits. However, the PC2 is negatively associated to Charef and Oued Touil populations. These populations are characterized by high DRI values of root development.

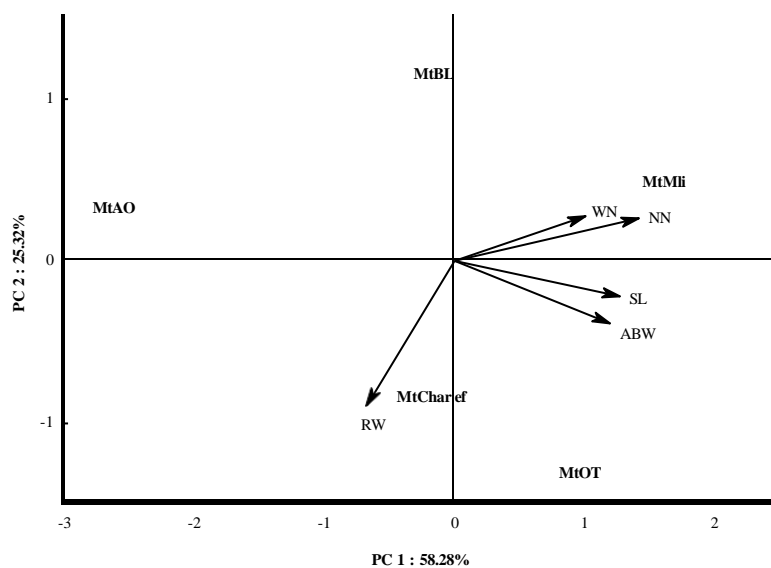


Figure 3: Plot of two principal components showing dispersal populations of *M. truncatula* based on DRI values of measured traits.

Discussion

This study investigated plant growth and nodulation of local populations of *M. truncatula* subjected to severe water stress. The populations exhibited significant variation in drought tolerance. The results obtained showed the existence of high level of variability among populations of *M. truncatula* in terms of their tolerance to water stress. Such variability can

be exploited in different breeding program of forage species to select adapted material (Chebouti et al., 2019). Populations of *M. truncatula* respond to water deficit with significant reductions in all the characters of the plant. Results obtained are in accordance with those reported by Badri et al., (2013) on the effect of water stress on *Medicago laciniata* and *Medicago truncatula* lines sampled from Morocco sympatric populations and Chebouti et al., (2018) on local populations of *M. aculeata*, *M. orbicularis* and *M. truncatula*. Slama et al., (2006) reported that water deficit reduced the growth and productivity of plants. Ohashi et al., (2000) indicated that water stress also reduced the dry weight of leaf, stem plus petioles, roots, and nodules in soybean.

In the present study, plant and root development was significantly affected by water stress. The reduction ranged from 25.3% to 49.4% for aerial biomass weight and from 26.9% to 32.5% for root weight. The decrease in plant biomass consequences from the water deficit in crop plants, mainly due to low photosynthesis and plant growth and leaf senescence during the stress conditions (Lisar et al., 2012). Severe water stress on plants results in a reduction of growth is associated with a decrease in the number of leaves (Badri et al., 2016) and of root volume flux (Siemens and Zwiazek, 2004). The decrease of plants growth under water deficit is also due to an inhibition of photosynthesis (Cornic, 2000). According to Verslues et al. (2006), water deficit reduces the growth of leaves more compared to roots because the growth of roots and leaves exhibits different sensitivities to reduced water availability. In contrast to the stem length, biomass and root development, the nodulation traits (number and weight of nodules) are the most affected trait. The reduction varies between 32.3% and 68.0% for number of nodules and it varied between 41.8% and 74.3% for weight nodules. This result is linked to a greater sensitivity of the nodules compared to other organs of the plant in the event of water deficit. Chebouti (1999) reported that water stress applied during the vegetative phase reduced the number as well as the fresh and dry weight of nodules in populations of *M. aculeata*, *M. orbicularis* and *M. truncatula*. This can be attributed to the weak infection process or to the partial development of the nodules. Felix (1981) indicated that a moderate desiccation of the soil irreversibly causes the loss of 20% of the fresh weight of the nodules in *Phaseolus vulgaris* L. According to Ohashi et al.(2000), water stress tended to decrease the nodule weight more severely compared to other plant parts. Graham (1992) reports that water stress modifies the rhizobial cells and leads to a reduction in infection and nodulation of legumes.

In the current study, positive correlations were recorded between the root weight and nodulation traits for studied populations under both treatments (control and severe stress). The reduction of root growth is associated with an inhibition of nodule establishment, estimated by the number of nodules. This behavior would be linked to a decrease in potential infection sites resulting essentially from an inhibition of the emergence and elongation of root hairs and of root system growth in general (Saadallah et al., 2001).

The principal component analysis was used to characterize and identify drought resistant and susceptible populations. Results of PCA based on drought response index (DRI) values, population from Mliliha region had the highest DRI values of plant growth and nodulations traits. This population seems to be drought tolerant and can be used as parent in *Medicago* species breeding program for drought tolerant. According to Ouk et al. (2006), if genotypic variation in DRI is not due to water availability among the genotypes, then genotypes with a high DRI would indicate the possession of trait combinations contributing to drought tolerance, and could be used as donors for development of genotypes adapted to the target environments of the region. The tolerance of *Medicago truncatula* lines to water deficit seems to depend, in particular, on their aptitude to maintain a good photosynthetic activity. Knowledge obtained by studying *M. truncatula* can be transferred to other crop and forage legumes (Badri et al., 2016).

Conclusion

It is essential to develop cultivars of *Medicago* spp. with large genetic variability that can tolerate water deficit in order to exploit in pastures in Algeria. From the results obtained in this study, it can be concluded that water stress strongly affected the plant growth and nodulation in *M. truncatula* populations. Compared to the control treatment, severe stress reduced significantly the length stem, aerial biomass weight, root weight and nodulation traits. The highest reduction was observed for nodulation traits (number and weight of nodules). *Medicago truncatula* population from Mliliha region exhibited the highest DRI values. This population seems to be drought tolerant and can be used as parental material in *Medicago* species breeding program for drought tolerance and contribute to development of cultivars adapted for arid and semi-arid environments.

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