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Study of seasonal variation on WUE of eight varieties of lucerne plants exposed to drought

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SUMMARY – Water stress, especially during the warm periods, is one of the main factors limiting plant growth. Eight varieties of exclusively atmospheric N_2 fixing alfalfa plants were grown in 7 litres white plastic pots filled with sand in a greenhouse. The experiment was conducted during 6 months including spring and summer periods. Control plants were grown in optimal water availability conditions whereas droughted plants were watered at 50% of control. *Sicilano Magali* and *Sardi* were the varieties with larger DM production as a consequence of their larger CO_2 fixation rates. Drought induced stomatal closure phenomena with the consequent reduction on photosynthesis and plant growth (60%). Elevated temperature decreased DM of control and droughted plants. Compared to the corresponding droughted plants, during the warm period, control plants were not able to adjust stomatal closure. In both water regimes, elevated temperatures induced the death of several nodules with its consequent effect on N_2 fixation.

Keywords: Alfalfa, greenhouse, water stress, photosynthesis.

RESUME – *"Etude des variations saisonnières sur la WUE de huit variétés de luzerne exposées à la sécheresse". Le stress hydrique est, particulièrement pendant les périodes chaudes, un des facteurs principaux limitant la croissance des plantes. Huit variétés de luzerne fixant exclusivement le N_2 atmosphérique ont été cultivées en serre dans des pots de plastique blanc de 7 litres remplis de sable. L'expérience a été conduite pendant 6 mois incluant des périodes de printemps et d'été. Les plantes témoins ont été cultivées dans des conditions de disponibilité optimale d'eau tandis que des plantes en conditions de stress hydrique ont été arrosées à 50% des témoins.* Sicilano Magali *et* Sardi *étaient les variétés avec la plus grande production de MS comme conséquence de leur fort taux de fixation du CO_2. La sécheresse a induit des phénomènes de fermeture des stomates avec une réduction conséquente de la photosynthèse et de la croissance (60%). La température élevée a diminué la MS des témoins et des plantes en conditions de stress hydrique. Comparées aux plantes stressées, les plantes témoins n'étaient pas capables d'ajuster l'ouverture des stomates pendant la période chaude. Dans les deux régimes d'eau, la température élevée a induit la mort de plusieurs nodules avec un effet conséquent sur la fixation du N_2.*

Mots-clés : *Luzerne, serre, stress hydrique, photosynthèse.*

Introduction

Mediterranean climate is characterized by dry summers with high temperature episodes. Yield of plants cultivated in the Mediterranean area is mainly constraint by water scarcity and elevated temperature (Volaire *et al.*, 1998; Araus *et al*., 2002; Aranjuelo *et al*., 2006, 2007). The inhibitory effects of elevated temperatures on plants, target photosynthesis, water status, and N_2 fixation in the case of N_2-fixing plants (Aranjuelo *et al*., 2007). Alfalfa (*Medicago sativa*) is a temperate perennial forage frequently exposed to low water availability specially during the summer.

The main goal of this study was the physiological characterization of 8 varieties of southern Europe and North Africa alfalfa plants (with different tolerance to drought conditions) exposed to drought and elevated temperature conditions to determine the characteristics that enable those plants to adapt their growth efficiently/inefficiently to the Mediterranean environmental conditions.

Materials and methods

Experimental design

Alfalfa (Magali, Ameristand, Manuntanas, Gabes, Rich, Sardi, Tamantit and Siciliano varieties) seeds were sown in 7 l white plastic pots filled with sand. The experiment was conducted during 7 months (February 2006 until the end of August 2008) in a greenhouse at 25/15ºC (day/night) with a photoperiod of 14 hours under natural daylight. Plants were watered twice a week with Hoagland N-free nutrient solution and once a week with tap water to avoid salt accumulation in pots. During the first month (February 2007), plants were inoculated three times with *Sinorhizobium meliloti* strain 102F78. At the end of May the aboveground area was cut in order to study the regrowth capacity of those plants and to determine the biomass production. When plants were four months old (June 2006), they were randomly assigned to two water availability regimes. Half plants continued growing under optimal (maximum soil volumetric water content, θv) water availability (CONTROL) conditions whereas the other half were exposed to low water availability (DROUGHT) conditions. In case of droughted plants, θv was decreased gradually (first until 80% and after two weeks until 60% of θv during June). In July, θv was reduced until 40% θv and maintained until the end of the month. In August θv of all the plants (control and drought) was set at 40% cc until September. At the end of June, July, August and September, we proceeded to the harvesting (total dry mass, DM) of plants together with the water state analyses (leaf relative water content, RWC; water use efficiency WUE) and gas exchange (photosynthetic rates, A; stomatal conductance g_s) measurements. After such determinations the aboveground area was cut in order to analyse the regrowth capacity of the different varieties.

Results and discussion

As it is shown in Table 1 after 3 months of growth (March, April and May) in optimal conditions, the Ameristand, Gabes and Siciliano varieties were the ones that produced a larger aboveground biomass. On the other hand, Tamantit variety was the one that produced less aboveground biomass.

Table 1. Dry matter production throughout the experiment and Water Use Efficiency of Productivity (WUEp) from March to August 2006

Variety	Total Biomass produced (g DM)							WUE_p (g DM/L H_2O added)	
	mar-apr-may-06	june-06		july-06		august-06			
	Control	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Ameristand	8,29	6,40	2,67	2,14	1,01	0,74	0,46	0,97	1,08
Gabes	7,15	6,61	2,51	2,10	1,09	0,89	0,23	0,93	0,95
Magali	4,95	7,13	2,44	2,68	0,76	0,62	0,23	0,85	0,73
Manuntanas	4,94	5,65	2,45	2,02	1,02	0,76	0,67	0,74	0,79
Rich	5,95	6,51	2,56	1,78	1,00	0,46	0,61	0,81	0,88
Sardi	6,83	6,98	2,67	2,24	0,92	0,82	0,77	0,93	0,97
Siciliano	6,91	7,21	3,12	2,82	1,00	0,26	0,64	0,95	1,01
Tamantit	4,03	5,02	1,99	2,04	1,02	0,67	0,52	0,65	0,66

In June, under optimal water availability conditions, the aboveground production (Table 1) of the varieties was slightly different to the values obtained in the previous harvest, being Siciliano, followed by Magali and Sardi the varieties that produced more dry mass (DM). Such increase was caused by their larger photosynthetic activity (data not shown). As it was observed in previous harvest, Tamantit was again the variety with lower DM production. Interestingly Table 1 also revealed that drought decreased DM production around 60% of controls due to reduction of pot soil water content (θv) from 100% to 80% cc and 60% cc. The inhibitory effect of drought on DM was caused by the stomata closure phenomena induced by the reduction on θv (data not shown). Stomata closure leaded out the reduction of photosynthesis that explained their lower growth.

During July, and compared to DM values obtained in June, DM production of control plants during this month, decreased 70% (Table 1) probably as a consequence on the ambient temperature and deficit pressure vapour (DPV) increase registered in Barcelona during this month. Siciliano and Magali were the plants that produced more DM during this month. The larger DM detected in Siciliano was caused by their larger leaf area whereas in case of Magali, such increase was explained by their larger photosynthetic activity. RWC data (Fig. 1) revealed that, especially Magali, Mamuntanas and Sicilano varieties, even under optimal watering conditions, presented low RWC values probably due to the above mentioned temperature and DPV increase which induced certain water stress situation. Diminishment observed on stomata leaf conductance (Table 2) suggests that the DPV increased the water loss through transpiration, bringing out the water regulation problems.

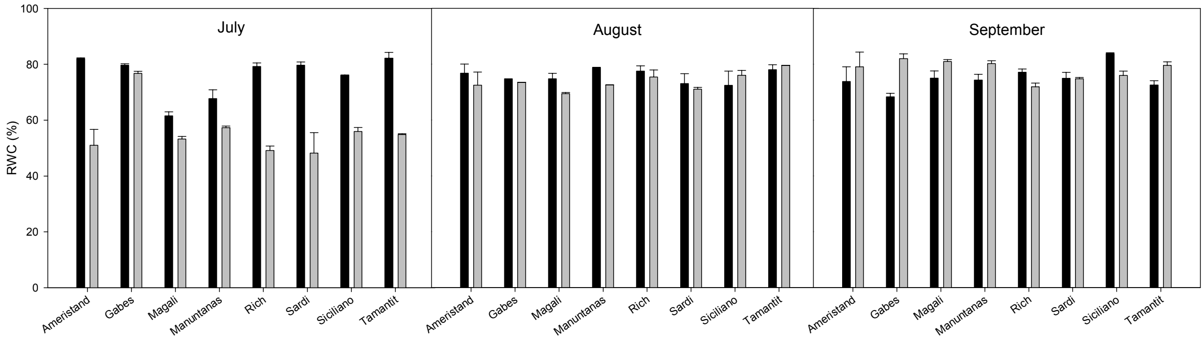

Fig. 1. Relative water content (RWC) of different varieties in July, August and September.

Table 2. Net photosynthesis and stomata conductance (g_s) of the different varieties measured throughout the experiment

Variety	Photosynthesis (mmol mol^{-1})						g_s (mmol mol^{-1})					
	July		August		September		July		August		September	
	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought	Control	Drought
Ameristand	21,33	13,58	27,80	21,50	23,65	26,70	0,34	0,26	0,47	0,28	0,45	0,56
Gabes	20,65	19,20	17,45	19,43	29,55	21,40	0,64	0,29	0,34	0,21	0,27	0,57
Magali	20,58	14,27	27,80	24,23	17,90	29,93	0,22	0,29	0,72	0,16	0,24	0,79
Manuntanas	13,60	10,22	14,53	15,47	29,83	24,57	0,22	0,12	0,25	0,18	0,60	0,31
Rich	19,50	11,39	21,65	20,93	26,10	22,23	0,23	0,19	0,40	0,20	0,53	0,30
Sardi	16,46	18,87	29,70	19,08	16,03	20,33	0,35	0,59	0,60	0,30	0,20	0,35
Siciliano	22,98	13,31	21,37	32,93	23,93	24,43	0,40	0,27	0,34	0,81	0,74	0,61
Tamantit	17,98	18,00	25,83	30,50	24,33	19,03	0,37	0,30	0,65	0,36	0,43	0,34

On the other hand, analyses of data corresponding to water stressed plants highlighted that compared to values obtained during June, production in July decreased 45%. There were not large differences between treatments (Table 1). However, when comparing those data with the DM produced in optimal water and temperature conditions (control plants harvested in June) DM production decreased dramatically (85%) highlighting the inhibitory effect of drought interacting with elevated temperature (Aranjuelo *et al*., 2007). Interestingly, RWC (Fig. 1) data showed that plants grown under low water availability conditions reached water content values of control plants. This data, together with the absence of differences on gas exchange data revealed that those plants adapted their metabolism to the available water without suffering any stressful growth conditions (Aranjuelo *et al*., 2006, 2007). Differences on DM production between fully and partially watered plants were caused by the differences on CO_2 fixing surface (mainly leaf area).

In August after growing during one month at 40% θv, aboveground DM of control plants (compared to the corresponding values obtained during July) decreased 50% approximately (Table 1). Absence of large differences between gas exchange data suggest that, compared with July, DM production diminished as a consequence of the probable reduction of storage capacity of those roots. Reduction of plant photosynthetic rates detected in July may cause the reduction of photoassimilates delivered to the roots, diminishing its regrowth capacity in August. Reductions on WUE (Table 1) were caused by their worse regulation of the stomata opening (Table 2). It should be also bear in mind that as observed by Aranjuelo *et al*. (2007), temperature increase inhibits drastically nodule activity and therefore N_2 fixation of alfalfa plants. It is also remarkable the fact that 4 Magali plants died (60% plant survival). Furthermore some plants in Rich and Siciliano varieties grew in poor state during this month.

In case of droughted plants, DM data revealed that during August, even if in case of Sardi, Rich, Siciliano, Tamantit and Mamuntanas, DM production was very similar to the previous harvest, in the case of Amersitand, Gabes and Magali, DM production decreased drastically (Table 1). However, interestingly, due to the previous acclimation to low water availability conditions, water stressed plants maintained a better water state than the control plants. DM of the first 5 varieties was also very close to the values of control plants, revealing that the previous acclimation to low water availability did not improve the DM capacity of plants. During this month, 4 Magali (60% plant survival), 4 Siciliano (60% plant survival) and 2 Gabes (75% plant survival) plants died and some plants in Mamuntanas, Rich, Sardi and Siciliano varieties grew in poor state during this month. Leaf conductance data confirmed that specially in case of water stressed plants with larger mortality (Gabes, Magali and Siciliano), leaf conductance enhanced significantly. Absence of differences on gas exchange parameters reveals that as described in fully watered plants, differences on DM might be explained by the inhibitory effect of elevated temperature on root sink and N_2 fixation capacity (Aranjuelo *et al*., 2007). However in this case, root DM of those plants was smaller, the repercussion was much more dramatic.

Finally we would like to mention the water use efficiency of productivity (WUEp) data corresponding to the period of March-August of all varieties (Table 1) revealed that regardless of water availability, Ameristand, Siciliano, were the varieties with larger WUE. In the opposite site, Tamantit and Mamuntanas were the varieties which had lower WUE. As the amount of water delivered to each water regime was the same, differences on WUE were caused by the different DM production of the plants.

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