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The effect of salinity and high temperature on biomass production of some alfalfa landraces

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SUMMARY – In Morocco, local populations of alfalfa (*Medicago sativa* L.) are being used in the traditional farming systems, which mainly consist of mountains and pre-saharian oasis. Although, these materials are adapted to biotic and abiotic stresses (salinity, high temperature and drought), the detailed agronomic and physiological characterization of those lines has to be carried out. Therefore, the present study aimed to know the effect of the abiotic stresses (salinity and high temperature) on some agronomic and physiological characteristics of four alfalfa landraces, namely Demnate, Dades, Rich and Gabes. The study revealed that high temperature, salinity and their combined effect significantly reduced shoot and root growth compared to control treatments. Under the combined effect of these stresses, plant height, internode number and distance were higher in Demnate and Rich landraces, compared to the control population (Gabes). The landrace Dades was sensitive to all the three stress treatments. The physiological studies showed that the combined effect of salinity and high temperature decreased Na^+ and Cl^- penetration, increased Mg^{2+} absorption and maintained adequate levels of chlorophyll a and b in the cells, thus indicating a normal process of photosynthesis in all the 4 landraces in response to stress. Relative water content (RWC) in the leaf was decreased in all the landraces as a result of the combined effect the two stresses. However, the decrease was very small in the case of Demnate. Our study showed that, in general, salt tolerance in alfalfa seems to be quiet linked to heat tolerance, suggesting that selection against one stress (high temperature or salinity) could lead to tolerance to the other stress.

Keywords: Alfalfa, salinity, high temperature, growth.

***RESUME –** "Effet de la salinité et des fortes températures sur la production de biomasse de populations locales de luzerne". Au Maroc, les populations locales de luzerne (*Medicago sativa *L.) des montagnes et des oasis présahariennes sont utilisées dans les systèmes agricoles traditionnels. Bien que ces populations soient adaptées aux stress biotiques et abiotiques (salinité, température élevée et sécheresse), leur caractérisation agronomique et physiologique nécessite une étude approfondie. Par conséquent, la présente étude vise à connaître les effets des stress abiotiques (salinité et température élevée) sur certaines caractéristiques physiologiques et agronomiques de quatre cultivars de luzerne, à savoir Demnate, Dadès, Rich et Gabès. L'étude a révélé que la température élevée, la salinité et leur effet combiné ont significativement réduit la croissance du système racinaire et de la partie aérienne par rapport au témoin. Sous l'effet combiné (salinité et température élevée), la hauteur de la plante, et la distance entre les entre-nœuds étaient plus élevées chez les populations Demnate et Rich en les comparant à la population témoin Gabès. La population Dadès était la plus sensible aux trois traitements étudiés. Les études physiologiques ont montré que l'effet combiné de la température élevée et de la salinité a diminué la pénétration excessive des ions Na^+ et Cl^-, en augmentant l'absorption de Mg^{2+} et en maintenant une teneur en chlorophylle a et b assez adéquate pour le déroulement du processus photosynthétique. L'effet du stress combiné température-salinité sur la teneur relative en eau, s'est traduit par une perte d'eau au niveau des feuilles de toutes les populations étudiées. Toutefois, la population Demnate se distingue des autres populations par une meilleure teneur. Notre étude a montré que, en général, la tolérance de la luzerne au sel semble être liée à la tolérance à la température élevée, ce qui suggère que la sélection contre l'un des deux stress (température élevée ou salinité) pourrait conduire à la tolérance aux autres stress.*

***Mots-clés :** Luzerne, salinité, température élevée, croissance.*

Introduction

In the Mediterranean basin, salinity and high temperature are the major environmental factors limiting the establishment of several crops in the arid and semi-arid areas (Mauromicale and Licandro, 2002). In Morocco, local populations of alfalfa (*Medicago sativa* L.) are being used in the traditional farming systems which mainly consist of mountains and pre-saharian oasis. Although, this crop is adapted to various stresses including abiotic stresses such as salinity, high temperature and drought, detailed agronomic and physiological characterization of these populations yet to be studied. The effect of salinity is reflected in a reduction in the aerial and roots growth (Mezni *et al.*, 1999), resulting in a lower rate of the dry matter production (Kavari and Chapazadeh, 1998; Rogers, 1998). The tolerance to salinity stress involves several mechanisms which includes selective transport of ions as well as the accumulation of osmoregulating substances (Zid and Grignon, 1991). High temperature results in reducing plant growth and thereby a relative decrease in plant biomass (Karim *et al*., 2000). Similarly, Aranjuelo *et al.* 2001, showed that the dry matter content of shoot and root and the leaf area decrease with increasing temperature above optimum level. Adaptation to heat stress is done either by acclimatization to the high temperature (Talwar *et al*., 1999) or by stabilizing properties of the thylakoïdienne membrane following a de-epoxidation of xanthophylls (Havaux *et al.*, 1996). The combined effect high temperature and salinity was also shown to reduce a large extent growth of aerial parts and roots. The present study aimed to know the effect of the abiotic stresses (salinity and high temperature) on some agronomic and physiological characteristics of four alfalfa landraces, namely Demnate, Dades, Rich and Gabes.

Material and methods

Plant material and experimental conditions

Four populations of alfalfa studied were chosen based on the basis of results of germination at high temperature and salinity. The alfalfa landraces are Demnate 203/III (population 1), Dades (population 2), Rich (population 3) and Gabes (population 4). The latter was used as a control. About 30 seedlings were transplanted in a tray (34 cm x 24.2 cm x 7.5 cm) containing soil and peat moss mixture (2 parts of soil and 1 part of peat moss). Once the seedlings established successfully, only 20 seedlings were retained in the tray for imparting treatments. Uniform spacing between the 20 seedlings were maintained and the rest were rouged out. After a month of culture at 25 ℃ and normal soil moisture conditions, the salt and high temperature treatments were given in a factorial fashion with two levels of each factor (salt and temperature). The salt treatment was imposed gradually by irrigating with NaCl solution every alternate day, as follows: First, the treatment was initiated by irrigating the tray with low 4g/l NaCl solution, followed by 4 times irrigation with 8 g/l NaCl solution and followed by one time irrigation with normal water. Untreated trays irrigated with normal water every alternate day acted as control. Two levels of temperature treatments were imposed: first level consisted of day and night temperatures of 25℃ and 18℃ respectively. The second level consisted of gradual rise of temperature to 36℃ for 6 hours followed by gradual decrease of temperature to 25℃ in a day. The night temperature was maintain ed at 25℃. The experim ent was conducted in a phytotron with photoperiod of 16 hr day and 8 hr night light conditions. The light intensity of 425 µ E/m^2.S was maintained during the treatments period. The treatments were imposed for 30 days and various parameters were recorded on weekly basis, after one week of treatments initiation.

The measured parameters

In order to evaluate the effect of salt and high temperature as well as their combined effect on growth of alfalfa, some agronomical parameters such as the number of internodes (NETR), the distance between the internodes (DETR), diameter of the stem (DIAS), the final height of plants (FH), the shoot (SDW) and root (RDW) dry weight, and total dry weight (TDW), were measured. In addition to above, we measured various mineral elements (Benmiloud and Poloezancka, 1977) namely sodium and potassium (by flame spectrophtometry), magnesium and calcium (by atomic absorption), and phosphorus (by colorimetry). The amount of chlorine was estimated by volumetric method (Homer and Parker, 1961) and total nitrogen content was determined by the Kjeldahl method. The relative water content (RWC) in the leaf was determined 20 days after restoration of the stresses. Analysis of variance (ANOVA) and treatment comparisons were carried out using SAS.

Results and discussion

The performance of plants were measured in terms of growth, biomass and quality and their reduction due to the stresses were estimated. Indeed, the results of the analysis of variance (Table 1) revealed a very significant effect of salinity on the shoot dry weight (SDW), the final height of the plant (FH), the number of internodes (NETR), the distance between the internodes (DETR), the diameter of the stem (DIAS), the root dry weight (RDW) and on the total dry weight (TDW). Similar significant effects were observed for factors temperature and populations. The interactions effects of population-salinity, population-temperature population-temperature-salinity were also highly significant for SDW, RDW and TDW.

Table 1. Analysis of variance of salinity and temperature effects on the growth parameters of the alfalfa populations. F- values are presented in the Table

Factors	df	FH	NETR	DETR	DIAS	SDW	RDW	TDW
Populations (P)	3	2.38^{NS}	16.70***	8.91***	32.45***	16.00***	14.03***	16.90***
Salinity (S)	1	17.61***	97.25***	7.61***	54.52***	133.05***	210.16***	190.92***
Temperature (T)	1	0.00***	109.58***	125.36***	58.80***	315.07***	881.27***	655.57***
P X S	3	2.13***	1.36^{NS}	1.39^{NS}	7.10***	3.58**	4.63***	4.47**
P X T	3	1.30^{NS}	4.60***	3.68**	1.00^{NS}	5.73***	9.53***	8.49***
T X S	1	1.51^{NS}	31.05***	10.24***	14.38***	66.45***	192.03***	154.90***
P X T X S	3	0.20^{NS}	1.04^{NS}	2.21^{NS}	3.66**	4.17***	8.19***	6.88***

*** Very highly significant (P=0.001); **Highly significant (P=0.01); *Significant (P=0.05); NS: No significant.

The study revealed that high temperature, salinity and their combined effect significantly reduced the rate of growth of shoot dry weight (Fig. 1A) and all parameters characterizing the shoot growth, and the root dry weight (Fig. 1B) compared to control treatments.

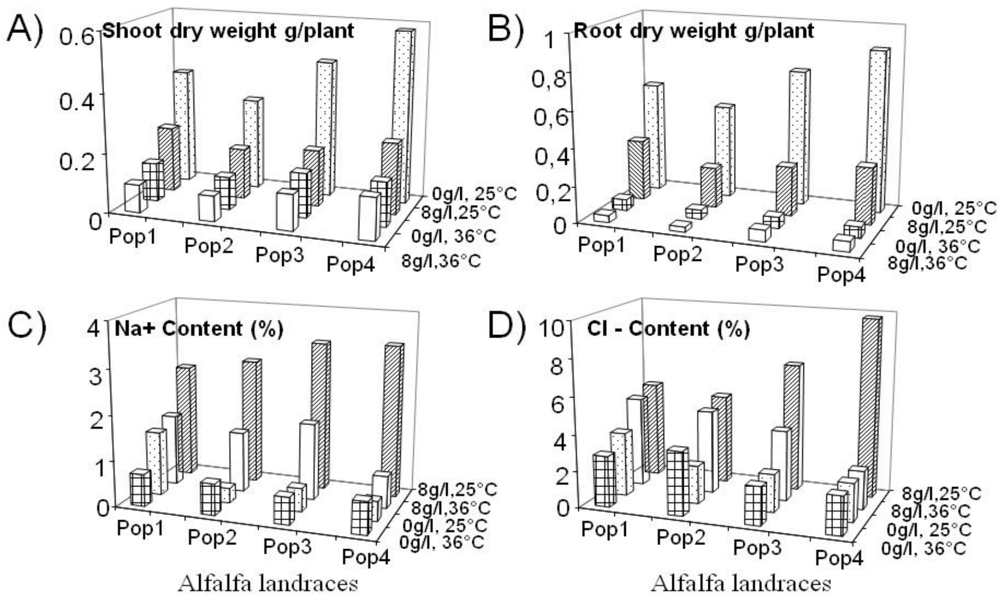

Fig. 1. The effect of salinity, high temperature and their combined effect on shoot (A) and root (B) dry weights, Na^+ content (C) and Cl^- content (D).

Under salt stress (8g/l, 25°C), Na^+ and Cl^- increased in the shoot part of the four populations studied (Figs 1C and 1D) compared to control. In contrast to the K^+, phosphorus and total nitrogen accumulation was reduced compared to control in the populations (reduction of K^+ (%): 27.32, 2.28, 30.07 and 30.08; reduction of P (%): 14.28, 4.76, 5.26 and 21.73; and total nitrogen reduction (%): 26.45, 28.34, 34.63 and 38.77, respectively for populations 1, 2, 3 and 4).

The results of relative water content (RWC) in the leaf under saline treatment showed a reduction of 20.28%, 20.63% and 6.82% respectively at the populations 1, 2 and 3. While for population 4, the RWC under salt stress was similar to that of the control. Under heat stress and no salinity conditions (NaCl 0g/l, 36℃), the results show ed that an increased amount of Na^+ ions in the shoot part. However, this increase was lower than that recorded under the salt stress. For chloride ions, all the 3 populations sowed higher levels of Cl^- compared to control under salinity stress (at 36℃) except the population 4 (Fig. 1D). Under heat stress and no salinity conditions (NaCl 0g/l, 36℃), phosphorus, total nitrogen and Mg^{++} were also increased in all the four populations compared to control. After 20 days under heat stress, there was a loss of water from the leaves of populations 2, 3 and 4 while for the population 1, there was no change compared to the control. The combined effect of salinity and high temperature resulted in increased Na^+ and Cl^- in shoots in all the populations compared to control (NaCl 0g/l; 25℃). This increase was lower compared with the same salinity treatment at 25℃ (Figs 1C and 1D). The combined effect of salinity and high temperature showed an increase of total nitrogen in population 2, whereas for populations 1 and 4 showed a decrease in the content of this element compared to the control. Reduction of total nitrogen at 25℃ under salt stress was higher compared to the combined effect of salinity and high temperature. The combined effect of high temperature and salinity had also shown to effect on water content of the leaves. Greater loss of water was observed in populations 1 and 2 (44.57 and 35.20%), where as populations 3 and 4 (respectively 11.14% and 2.10%) showed marginal loss of water.

The performance of plants is measured in terms of growth, biomass and quality. However, these parameters can be reduced as a result of abiotic stresses.

Salinity is known to affect morphological and physiological processes and also nutrition of the plants. The result of this study showed significant reduction in response to salinity in various morphological parameters namely shoot dry weight, the final height of the plant, the number of internodes, the distance between the internodes, the diameter of the stem, the root dry weight and on the total dry weight. These findings are consistent with those of Mezni *et al.* (1999) have reported that salinity stress reduces the shoot and root growths in lucerne. Similarly, the results of Rogers (1998) also showed lower dry matter production under increasing salt concentration. The growth reduction was due to decrease of the final plant height, the reduction in the number of internodes, which further resulted in less number of leaves and smaller diameter of the stem. This observation was further supported by positive and significant correlation coefficient (r = 0.565***) observed between the growth parameters and dry matter accumulation. These results are in agreement with those of Ben Naceur *et al.* (2001) who showed that salinity could reduce plant height. The reduction of the growth can also be attributed to an excessive accumulation of Na^+ and / or Cl^- in the shoot. In general accumulation of these ions disturb of mineral nutrition of plants. In addition, the results of this study showed a higher accumulation of sodium and chloride ions accompanied by a reduction in the absorption of potassium, phosphorus and total nitrogen. Several other studies have also showed similar results (Khan *et al.*, 1999; Günes *et al*., 1999). With regard to the relative water content (RWC), the present study showed a 20% reduction in populations 1 and 2, and a slight decline in the population 3. These results are in agreement with those of Khan *et al.* (1999) have reported that the relative water content increases slightly at low salt concentration, but it declines at a high salt concentration treatments. Maintaining water content more or less similar to that of control noted among populations 4 and 3 is probably a sign of tolerance to salt stress. Under heat stress, there was a negative effect of this on all the growth parameters. Indeed, the temperature affects the rate of plant growth and maturation with a loss of stem quality. It also leads to reduction in shoot and root dry weights and leaf surface area (Aranjuelo, *et al.* 2000). In terms of nutrition, the results showed an increase in the absorption of nutrients. Indeed, the high temperature facilitated the absorption minerals that affect membrane permeability (Talwar *et al.,* 1999). For RWC, the results showed that populations 1 and 4 were able to adjust their water potential by limiting the loss of water in their leaves. However, the population 1 maintaining almost the same RWC both at 36℃ and 25℃, whereas, the population 4 showed a slight decrease. The combined effect temperature has been shown by a large reduction in the growth of plant. The results of this study revealed a very highly significant interaction of temperature on all parameters characterizing the shoot growth. With regard to the root system, there has been a significant reduction in the root dry weight due to inhibition of the formation of secondary roots. For the mineral absorption, the combined effect was manifested by an increased uptake of mineral elements in relation to the control. As compared to salt stress, the high-temperature probably corrects the damaging effect of Na^+ and Cl^- under salt stress. At a physiological level, the combined effect of temperature and salinity on the water content has proven to be not significant. However, populations 1, 3 and 4 better adjust to their water potential by limiting the loss of water in their leaves.

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