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Plant physiology

Interaction of salinity and temperature on the germination of alfalfa cv CUF 101

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Summary — Alfalfa, *Medicago sativa* L is a major forage legume in the arid climate of the Gulf countries where salinity is a serious production problem. A laboratory experiment was initiated to evaluate the effect of salinity – temperature interactions on the germination of alfalfa cv CUF 101. The alfalfa seeds were sown in Petri dishes with saline solutions of varying concentrations (electrical conductivities of 0.01, 6.4, 12.2, 17.4, 22.6, 27.2, 32.1 and 37.2 dSm^{-1}) prepared with NaCl. The germination response of the seeds were determined over a wide range of temperatures (15, 20, 25, 30, 35 and 40 °C) for a period of 8 d. Germination counts, taken every 2 d, were used to compute germination rate index. Generally, germination percentage decreased with increasing salinity and was severely limited at 40 °C. Salinity–temperature interactions were highly significant. The optimum germination temperature was 20–25 °C. Based on these results, the most favourable period to establish alfalfa cv CUF 101 in the Sultanate of Oman and other areas with similar climatic conditions has been proposed.

salinity–temperature / interaction / optimum germination / germination rate index / *Medicago sativa* L

Résumé — Interaction de la salinité et de la température sur la germination de la luzerne cv CUF 101. *La luzerne* Medicago sativa *L représente une culture fourragère importante dans les régions arides des pays du golfe arabo-persique où la salinité constitue un problème majeur pour la production. Un travail de laboratoire a été initié pour évaluer l'effet des interactions température x salinité sur la germination des semences du cv CUF 101. Des solutions de NaCl (ayant pour conductivités électriques 0,01, 6,4, 12,2, 17,4, 22,6, 27,2, 32,1 et 37,2* dSm^{-1}*) sont utilisées pour étudier la germination des semences en boîtes de Petri à des températures variées (15, 20, 25, 30, 35 et 40 °C). L'index de germination est défini par le nombre de semences germées tous les 2 jours. Le pourcentage de germination diminue en fonction de l'augmentation de la salinité pour atteindre une valeur très faible à 40 °C. Les interactions température x salinité sont hautement significatives. La température optimale de germination est comprise entre 20 et 25°C. La meilleure période pour l'établissement de la luzerne cv CUF 101 dans le sultanat d'Oman et dans les zones à climat similaire est suggérée d'après les résultats.*

interaction / salinité et température / germination optimale / indice de germination /* Medicago sativa *L

INTRODUCTION

Germination is one of the most critical periods for a crop subjected to salinity (Fowler, 1991). Germination failures on saline soils are often a result of high salt concentrations in the seed planting zone because of the upward movement of soil solution and subsequent evaporation at the soil surface (Bernstein and Hayward, 1958). Since the soil surface is normally more saline than lower layers (Uhvits, 1946; Dotzenko and Dean, 1959; Khatib and Massengale, 1966) seed germinaton occurs in a more saline environment than that in which established plants grow.

Several researchers have observed a decrease in germination rate as salinity increases and the osmotic potential of the germination medium decreases (Abel and Mackenzie, 1946; François *et al,* 1969; Greenway, 1973; Redmann, 1974; Sharma, 1976). The effects of temperature–salinity interactions on the germination of some crop plants have also been reported (Ungar, 1967; Tadmor *et al,* 1969). In alfalfa, salinity and temperature stresses are primary limiting environmental conditions which restrict its successful establishment in irrigated and semi-arid regions (Stone *et al,* 1979). Although it has been shown that salinity is increasingly detrimental to alfalfa germination at high temperatures (Ahi and Powers, 1938; Uhvits, 1946) and that low salt concentrations tend to stimulate its germination (Mulwani and Polland, 1938), little is known about the interaction of salinity and temperature on germination of this forage species.

About one-third of developed agricultural land in arid and semi-arid regions is affected by salinity (Allison, 1964). In most of the Gulf countries, saline soils and saline irrigation constitute a serious production problem for alfalfa. In this region, especially the Batinah Coast of Oman, where ocean spray and ocean water intrusion are additional sources of salinity, alfalfa plants affected by salinity are generally stunted and have smaller leaves than normal plants. They are usually dark-green or the leaves may have a bluish-green cast, probably due to wax accumulation. Thus, apart from yield reduction, salinity also affects the quality of alfalfa. Therefore, a better understanding of the effects of salinity and temperature, as well as their interactions on germination of this forage crop is important in the development of cultural practices for its establishment under saline conditions. The objective of this study was to investigate the germination response of alfalfa cv CUF 101 to a wide range of salinity levels and temperature and to determine their interactions.

MATERIALS AND METHODS

Several alfalfa seed lots were obtained from the Yates Seed Company in Australia and subjected to preliminary laboratory germination tests. Seeds for the current study were selected from the lot with the highest germination rates (95–100%).

A stock solution was prepared with reagent grade NaCl and from this, different concentrations (60, 120, 180, 240, 300, 360 and 420 mmol.l^{-1}) were prepared by dilution. The electrical conductivities as well as the osmotic potentials of these solutions were determined with a conductivity meter, Model PCM 3 (Jenway Felstead, Essex) and the advanced tigemetric osmometer, Model 3T-II (Advanced Instruments, Boston, MA), respectively (see table I).

Several 90-mm triple vent Petri dishes into which 3 circles of Whatman No 1 filter paper had been placed were prepared. One hundred seeds of alfalfa cv CUF 101 were placed in each of them and 5 ml distilled water or the various NaCl solutions were added. The Petri dishes were covered and arranged in an incubator in a randomized complete block design. Germination response to salinity at 6 temperatures (15, 20, 25, 30 35 and 40 °C) was evaluated by replicating the temperature twice in the same incubator over time. Temperatures were maintained within ± 1 °C of target levels.

Germination counts were taken at 2, 4, 6 and 8 d after sowing (DAS). Seed germination was determined by radicle protrusion through the seed coat, in accordance with the Association of Official Seed Analysts (AOSA, 1970) definition of germination. Distilled water equal to the mean loss of water from dishes with only water was added to each Petri dish on d 2, 4 and 6 to maintain salt concentration near target levels throughout the germination period. A germination rate index (GRI) was determined following the procedure described by Bouton *et al* (1976) as modified by Fowler (1991). According to the formula,

$$GRI = G2/2 + G4/4 + G6/6 + G8/8$$

where G2, G4, G6 and G8 are germination percentages x 100 at 2, 4, 6 and 8 DAS. The data were subjected to analysis of variance and orthogonal contrasts (SAS, 1985).

RESULTS

The germination responses of alfalfa cv CUF 101 seed to a wide range of salinity and temperature treatments are shown in figure 1. Temperature–salinity interactions were highly significant at each counting date (table II). The optimum germination temperature for the control was 25 °C. The cumulative germination percentage for the lower salinities (6.4–12.2 dSm^{-1}) also peaked at 25 °C, while salinity levels of 17.4–37.2 dSm^{-1}

Table I. Description of NaCl solutions used to determine the germination response to alfalfa cv CUF 101 seed to salinity.

Concentration (m mol l^{-1})	*Electrolytic (dSm^{-1})*	*Osmotic potential (MPa)*
0	0.01	–0.007
60	6.4	–0.246
120	12.2	–0.469
180	17.4	–0.693
240	22.6	–0.923
300	27.2	–1.259
360	32.1	–1.376
420	37.2	–1.649

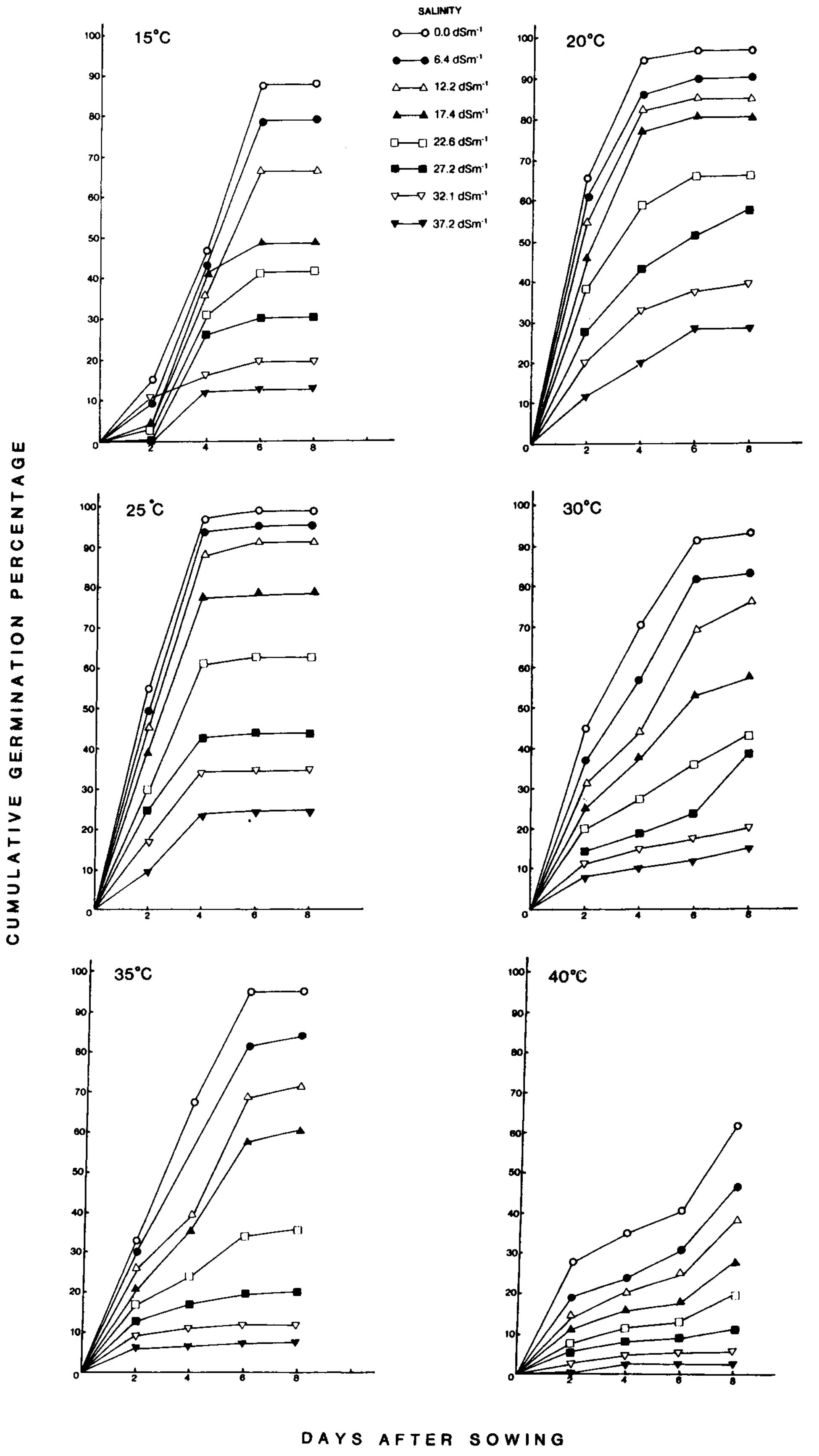

Fig 1. Cumulative germination percentage of alfalfa cv CUF 101 showing response to varied salinity levels at 6 temperature settings.

Table II. Analysis of variance for the cumulative germination of alfalfa cv CUF 101 at 6 germination temperatures and 8 salinity levels over an 8-d germination period.

Source	*df*	*Mean squares*			
		Cumulative germination – days after sowing			
		2	*4*	*6*	*8*
Temperature (T)	5	4 385.50 **	8 943.44 **	7 612.04 **	5 426.92 **
Linear	1	606.00 **	14 030.37 **	18 097.74 **	11 180.68 **
Quadratic	1	12 635.02 **	19 332.86 **	16 343.06 **	13 860.02 **
Cubic	1	6 529.33 **	7 800.46 **	518.06 **	832.13 **
Salinity (S)	7	2 563.18 **	7 336.96 **	12 469.22 **	13 421.30 **
Linear	1	17 908.36 **	51 117.56 **	86 657.19 **	93 488.95 **
Quadratic	1	30.36	0.66	16.30	0.70
Cubic	1	0.15	52.82	553.64 **	411.28 **
T x S	35	96.69 **	207.21 **	196.21 **	111.70 **
Error	96	2.26	11.46	11.80	24.26

** Significant at $P = 0.01$.

peaked at 20 °C (fig 2). Germination percentages at the upper salinities (32.1–37.2 dSm^{-1}) were very poor, with none attaining 50%. At the lower salinities, however, germination percentages were > 50% at all temperatures with the exception of 40 °C where depressed germination percentages were obtained.

Germination rate expressed as germination rate index (GRI) was influenced by salinity and temperature in a similar pattern to final germination (fig 3). GRI at 20 and 25°C were similar throughout the range of salinities, but were significantly higher than all other temperatures. Germination temperature of 40 °C had the least GRI, ranging from 26 at 6.4 dSm^{-1} to 2.0 at 37.2 dSm^{-1}.

Generally, salinity levels at which 50% reduction in germination occurred varied between temperatures. At 20 °C, 23.5 dSm^{-1} salinity gave 50% reduction in germination, while 21.5 dSm^{-1} salinity caused a similar reduction at 25 °C. At 30 and 35 °C, salinity levels at which 50% reduction in germination occurred were 11.0 and 8.0 dSm^{-1}, respectively.

DISCUSSION

The optimum germination temperature for the control was 25 °C, which was in agreement with the recommended temperature for measuring germination of legumes (AOSA, 1970). Brar *et al* (1991), working with 20 forage legumes, including several alfalfa cultivars, obtained germination temperature optima of 15–25 °C, but suggested that cultivars within the same species could respond differently to changes in temperature regimes, possibly due to genetic variability among cultivars.

Germination was greatly depressed at 40 °C throughout the range of salinity, 46% being the maximum percentage obtained at 8 DAS even at the lowest salinity of 6.4 dSm^{-1}. This relatively low germination response at 40 °C was an indication that this temperature is outside the maximum germination temperature for alfalfa cv CUF 101. Earlier workers (Ahi and Powers, 1938; Tadmor *et al,* 1969; Sharma, 1976; Stone *et al,* 1979) observed that higher temperatures increased the detrimental effect of salinity during germination. Na^+ and Cl^- are potentially toxic and their entry into the seed in high concentrations will have an adverse effect on embryo viability, thus reducing germinability. It has been shown that Na^+ frequently enhances membrane leakage (Epstein, 1972) and it is more likely that this phenomenon is aggravated by high temperatures. The entry of Na^+ and Cl^- into the seed is therefore facilitated. Denaturation of nucleic acids, protein and membranes at high temperatures are some factors contributing to germina-

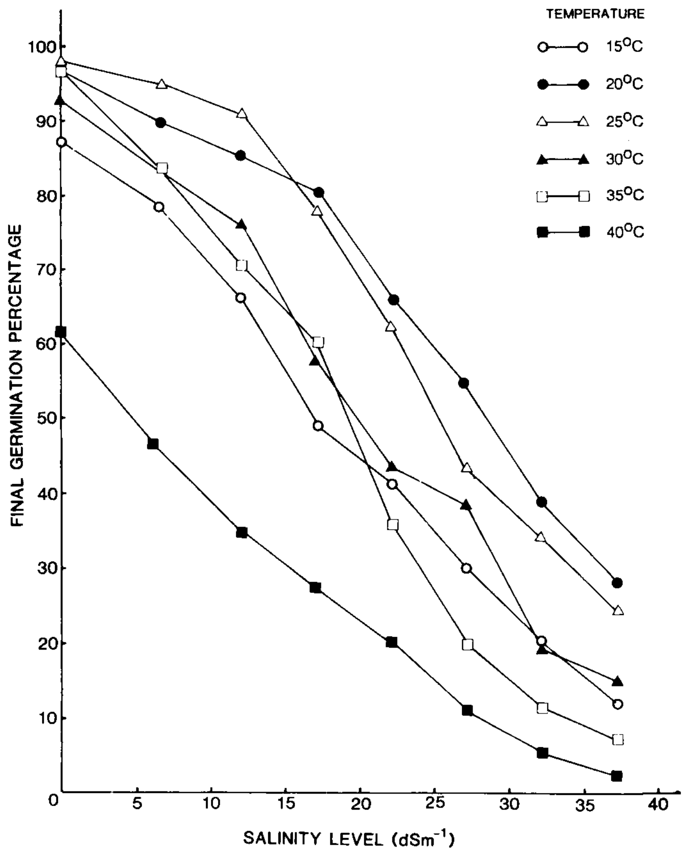

Fig 2. Final germination percentages of alfalfa cv CUF 101 at 8 d after sowing as a function of salinity and temperature.

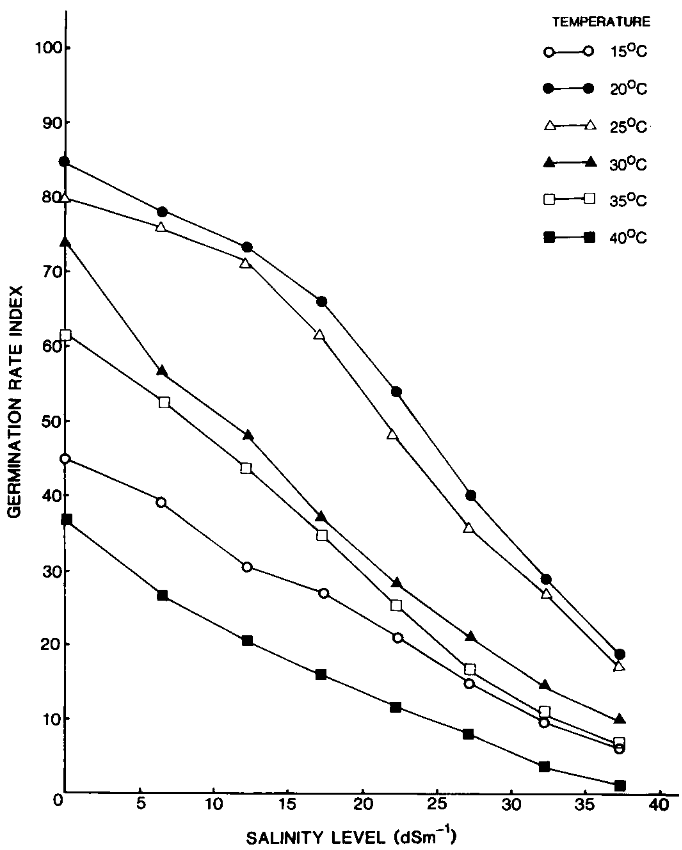

Fig 3. Germination rate index of alfalfa cv CUF 101 at 8 d after sowing as a function of salinity and temperature.

tion failure, as has been observed by Cardwell (1984). Obviously factors which increase evaporative demands such as temperature would tend to aggravate the effects of salinity, as has been reported by Hoffman and Jobes (1978).

At the optimal germination temperatures (20–25 °C), the salinity levels that reduced germination by 50% were in the 21.5 to 23.5 dSm^{-1} range. Based on the salt tolerance categories established by Maas and Hoffman (1977), these salinity levels fall within the moderate salinity range. Accordingly, alfalfa cv CUF 101 would be classified as moderately tolerant to salinity.

However, GRI which is a measure of seedling vigour should involve not only germination but emergence characteristics. Therefore, it seems likely that vigour includes the capacity to mobilize seed resources (improved protein synthesis in the embryo) for the process of germination and subsequent plumule elongation. Faster plumule elongation and perhaps longer final length are important factors contributing to high germination percentages in maize (Riley, 1981) and this may also hold true for alfalfa.

The soils in the Gulf countries, especially the Batinah Coast of Oman, are very saline. The winters are relatively short, while the summers are long and hot. Therefore, it seems that the most ideal period to establish alfala cv CUF 101 in this region is about March to early April when soil temperatures are ≈ 20–25 °C. After this period, soil temperatures increase rapidly due to the summer heat, thus aggravating the detrimental effect of salinity.

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