

Influences of Salinity, Light and Temperature on Seed Germination of *Leucaena leucocephala* (Lam.) de Wit.

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ABSTRACT

The experiment was investigated to study evaluate the seed germination of *Leucaena leucocephala* (Lam.) de Wit. under salinity, temperature, light and their interaction on the rate and final percentage of germination. The experiment was carried out in plant physiology laboratory at College of Science, King Saud University, Riyadh, Saudi Arabia. The collected seeds of *Leucaena* was treated with boiling water for 3 minutes then left to germinate in Petri dishes in growth chamber under the investigated treatments. Seven concentrations of sodium chloride solution had EC of (0, 50, 100, 300, 400, 500 and 600 mM) were applied to seeds as irrigation water and exposed to three temperature degree (20, 30 and 40 °C). Seeds untreated with NaCl germinated well in a wide range of temperatures and in both full light and complete dark. Seed germination decreased significantly with the increase in both NaCl concentration and temperature. Optimal germination percentage occurred at 30 °C. The inhibitory effect of high salinity on final germination percentage was greater at 40 °C than at 20 and 30 °C. However, germination rate was significantly greater at 40 °C than at 20 and 30 °C in lower salinity and the reverse was true at higher salinity. Germination in light was significantly greater than in the dark at lower salinity levels and high temperature.

Keywords: Salinity, Seed germination, Temperature, Light, *Leucaena leucocephala*.

INTRODUCTION

Studies of environmental stresses for trees at different stages of development are very important for regeneration and consequently for successful establishment of plantations. Seed germination, as a critical stage in plant life is the most vulnerable to such stresses (Catalan *et al.*, 1994). Salinity is one of the most important factors affecting seed germination in the arid regions. Studies showed that sodium chloride is the most important salt affecting seed germination. Its high concentrations inside the soil have an adverse effect on seed germination (Mayer and Mayber, 1982). The adverse effect is attributed to ionic toxicity and the decline in osmotic pressure which do interact under saline conditions (Greenway and Munns, 1980). The principal effects of salinity on seed germination could be summarized in the delay of seed germination, and the decline in its rate and percentage (Villagra, 1997). In

Saudi Arabia, saline water is one of the most frequent environmental stresses that face growing plants. This because groundwater represents more than 90% of water used in agricultural irrigation and it classified as very saline water (Falatah *et al.*, 1999). They found that the salinity (EC) of groundwater from 400 samples represent eight agricultural regions of Saudi Arabia ranged between 0.2 and 29.3 dS m⁻¹. Recently, Al-Matroud (2003) reported that salinity of groundwater from Riyadh Region (Central part of Saudi Arabia) has electrical conductivities (EC) ranged between 1.34 and 7.84 dS m⁻¹ and dominated by sodium chloride cations.

Leucaena leucocephala is a fast-growing, leguminous tree (Mimosaceae) that can be used for reforestation, for firewood, and as a forage crop. *Leucaena* is valued as an excellent protein source for cattle fodder, consumed browsed or harvested, mature or immature, green or dry. The nutritive value is equal to or superior to alfalfa. *Leucaena* has gained a favorable reputation in land reclamation, erosion control, water conservation, reforestation and soil improvement programs, and is a good cover and green manure crop. The leaves, used as mulch around other crops, are said to significantly increase their yields. It is said to possess the power of extracting selenium from the soil and concentrating selenium in the seed. This could be used to ameliorate seleniferous soils if the feed were discarded or used for some purpose other than feed. Seeds yield about 25 percent gum worthy of commercial investigation. Seeds after softening are strung as beans into various items of jewelry for tourists in Puerto Rico and the Virgin Islands. In the Philippine Islands, young pods are cooked as a vegetable and seeds are used as a substitute for coffee. Ripe seeds are sometimes eaten parched like popcorn (N.A.S. 1977). Wood is hard and heavy (specific gravity 0.7), the sapwood light yellow, the heartwood yellow-brown to dark brown, used for fuel or charcoal. Plants are used in some countries for shade for black pepper, coffee, cocoa, quinine, and vanilla and for hedges. In many places, however, renegade seedlings have created a noxious weed situation. The depilatory chemical mimosine has been used, experimentally at least, to shear sheep (Duke, 1981a, b). Medicinally, the bark is eaten for internal

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pain. A decoction of the root and bark is taken as a contraceptive, ecboic, depilatory, or emmenagogue in Latin America. However, in experiments with cattle, leucaena had no effect on conception.

Leucaena leucocephala shows great potential for use as a multipurpose tree in different parts of the world, in comparison to several native and exotic species (Deans *et al.*, 2003). In these regions, germination occurs during rainy seasons when soil salinity levels are usually reduced (El-Keblawy, 2004). Optimal germination in seeds of halophytes often occurs under fresh-water conditions and germination of most species is reduced and delayed with salinity increase, so the response may vary greatly depending on species. Whereas several perennial halophytes could germinate in 860mM NaCl or more (Lyndlee and Kleindorfer, 2009). Other halophytes showed little germination above 125mM NaCl (*Zygophyllum simplex*, Khan and Ungar, 1996).

Temperature and salinity can interact in determining salinity tolerance during germination. Although higher salinity decreases germination, the detrimental effect of salinity is generally less severe at optimum temperature. The detrimental effect of salinity was found to be severe at higher temperatures in some species, including *Atriplex cordobensis* (Aiazzi *et al.*, 2002), but at lower temperatures for other species.

Finally, salinity tolerance did not depend on temperature in some other species, such as *Arthrocnemum indicum* (Khan and Gul, 1998). Salinity, temperature interaction may have significant ecological implications in terms of time of germination under field conditions (Ungar, 1995); it has been attributed to both osmotic and toxic effects (Khan and Ungar, 1998).

Despite the number of extensive studies that report the interactive effects of salinity and temperature on germination, the influence of light in these responses is not clear (Gulzar *et al.*, 2001).

The aim of this study was to evaluate the effect of salinity, temperature and light, and the interactions between these factors on final germination percentage of *Leucaena leucocephala*, grown under conditions of the arid environments of the Riyadh Region, Saudi Arabia.

MATERIALS AND METHODS

Plant material

Seeds of *Leucaena leucocephala* (Lam.) de Wit. Fam. (Mimosaceae) were collected from botanical garden of Department of Botany and Microbiology, College of Science, King Saud University. The collected seeds were cleaned then placed in boiling water for three minutes, as a well known pre-germination treatment for most leguminous seeds.

Germination conditions and treatments

Seeds used in germination experiments were not scarified. The germination was conducted in 9-cm Petri-dishes containing two disk of Whatman No. 1 filter paper, with 5ml of test solution. Dishes were wrapped with parafilm.

Germination experiments were conducted in growth chamber set at 20, 30 and 40 °C in either continuous light or dark. Seeds were germinated in double distilled water (0), and in 50, 100, 300, 400, 500 and 600mM NaCl solutions under these temperature and light treatments. Four replicates of 25 seeds each were used for each treatment. The dishes were wrapped in aluminum foil to prevent any exposure to light (for dark treatment). Seeds were considered to be germinated with the emergence of the radicals. Germinated seedlings were counted and removed every alternate day, for 15 days following seed sowing.

The rate of germination was estimated using a modified Timson index of germination velocity = $\Sigma G/t$; where G is the percentage of seed germination at 2d intervals and t is the total germination period (Khan and Ungar, 1984).

Statistical analysis

RCBD was used for the experimental as described by **Snedecor and Cochran (1968)**. A three-way analysis of variance (ANOVA) was carried out to test effects of main factors (salinity, light, temperature) and their interactions on the final percentage of germination. The effects of light on germination rate were not assessed as the germinated seedlings were counted only once, at the end of the experiment. Two-way ANOVA were performed to evaluate the effects of salinity and temperature on germination rate and the effects of light and temperature on germination percentages of non-salt treated seeds. All statistical methods were performed using SAS, (1990).

RESULTS AND DISCUSSIONS

Effects on final germination

The results indicate that non-salt treated seeds of *Leucaena leucocephala* germinated well in a wide range of temperatures and in both light and dark. Two-way ANOVA showed significant effects of temperature ($P<0.001$) and interactions between light and temperature ($P<0.05$) on final germination percentage of non-salt treated seeds (Fig. 1).

Results of three-way ANOVA showed significant effects for light, temperature and salinity on final germination % of *Leucaena leucocephala* (Table 1). Seed germination decreased with the increase in both NaCl concentration and temperature. Few seeds

germinated at 600mM NaCl. Optimal germination occurred at 30 °C; seeds at both 20 and 30 °C germination attained significantly greater values than those at 40 °C (Fig. 1).

The interaction effect of temperature and NaCl concentration on final germination was significant ($P<0.001$; Table 1), indicating that the germination response to salinity depended on temperature. The inhibitory effect of high salinity on final germination was greater at 40 °C than at 20 and 30 °C. Despite the fact there were no great differences in final germination between 0 and 400mM NaCl, at either 20 °C or at 30 °C,

germination at 40 °C was greatly reduced in 300mM NaCl and completely inhibited in 400mM NaCl (Fig. 1).

Seed germination of *Leucaena leucocephala* at different temperatures depended also on ambient light conditions. The interaction between light and temperature on final germination was significant ($P<0.01$; Table 1). Whereas no significant difference in final germination was observed between light and dark at either 20 or 30 °C, the germination in light was significantly greater than in the dark at 40 °C (Fig. 1).

Table 1. three-way ANOVA showing influences of salinity, light and temperature on *Leucaena leucocephala*

Source of variation	df	Mean-square	F-ratio	P
Temperature (T)	2	4.937	520.534	<0.001
NaCl conc. (S)	6	2.077	230.531	<0.001
Light (L)	1	0.050	5.936	<0.05
T*S	12	0.247	29.157	<0.001
T*L	2	0.049	6.117	<0.01
S*L	6	0.019	2.025	0.210
T*S*L	12	0.014	1.904	<0.05
Error	126	0.0086		

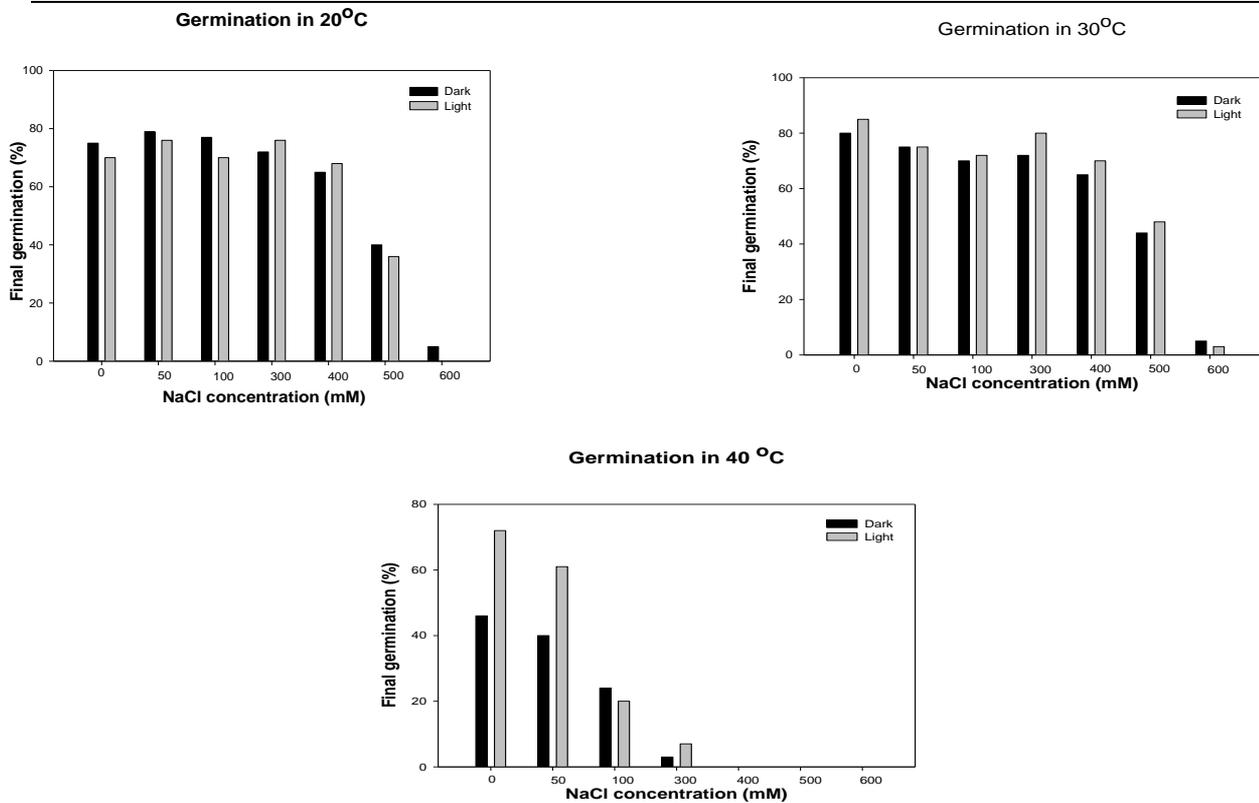


Fig. 1. Influences of salinity, light and temperature on final germination percentage in *Leucaena leucocephala*

The interaction among salinity, temperature and light on final germination was also significant ($P < 0.05$; Table 1). Germination in light did not differ significantly from that in dark in any of the concentrations of NaCl at either 20 or 30 °C. At 40 °C however, germination in light was significantly greater than in dark, for seeds treated with lower salinities (0 and 50mM NaCl). Moreover, at this temperature, few seeds germinated in the 300mM and none in 500mM NaCl. Germination at 0 and 400mM NaCl increased from 49.2% and 41.3% for seeds incubated in dark to 72.4% and 66.2% for seeds incubated in light, respectively (Fig. 1).

Seed dormancy in plants growing in saline habitats of subtropical deserts is often regulated by salinity and temperature. Dormancy reduces the risk of seedling mortality, when moisture is limited and salinity is increased (during summer). For example, in the desert annual *Zygophyllum simplex*, germination occurs only when temperature and salinity were reduced, but this happen either during winter or after monsoon rain (Khan and Ungar, 1997). Germination was also greatly reduced in several perennial halophytes at higher temperatures and salinities (Aiazzi *et al.*, 2002; Khan *et al.*, 2002).

In the present study, the inhibitory effect of high salinity was greater at 40 °C than at 20 and 30 °C. Few seeds germinated in 400mM NaCl and no germination occurred in 500mM NaCl at 40 °C (Fig. 1).

The detrimental effect of NaCl at higher temperatures has been attributed to toxicity of Na^+ that usually causes irreversible damage (Al-Khateeb, 2006). In *Atriplex cordobensis*, however, water stress resulted from either high salinity or polyethylene glycol (Aiazzi *et al.*, 2002) caused germination inhibition at higher temperatures, what suggests that the mechanism of Na^+ toxicity may not be present in some species. The inhibitory effect of salinity at higher temperatures has ecological significance because it prevents seeds from germinating in salt-affected habitats and consequently avoiding seedling mortality during this period when surface soil salinities are extremely high (Khan and Ungar, 1998). This could be true if the damage caused by Na^+ toxicity is not irreversible.

Effects on germination rate

Results of two-way ANOVA showed significant effects for temperature and salinity on germination rate of *Leucaena leucocephala* ($P < 0.001$; Table 2).

Germination rate decreased significantly with the increase in salinity. Overall germination rate at 0 and 50mM NaCl (35.6 and 37.1%, respectively) was significantly greater than in higher concentrations (Fig. 2). The interaction between temperature and NaCl

concentration on germination rate was significant ($P < 0.001$; Table 2). Unlike germination percentage, germination rate at 40 °C attained significantly greater values than at 20 °C, for seeds treated with 0 and 50mM NaCl. The increase in NaCl to 100 and 200mM resulted in a significant decrease in germination rate at 40 °C compared to 20 and 30 °C. Germination rate at 30 °C was less affected by salinity increase than at 20 °C. For seeds treated with 500 and 600mM NaCl, the germination rate at 30 °C was significantly greater than at 20 °C (Fig. 2).

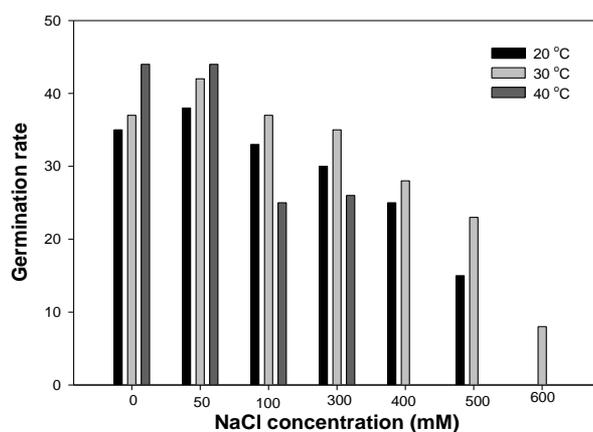
The present study showed that about 57% of the non-salt treated seeds of *Leucaena leucocephala* germinated at 40 °C, suggesting that the temperature in non-saline habitats is not a limiting factor, if moisture is available during summer months. Many seedlings have been observed after summer rainfalls that typically occur in the tropical and subtropical environments, where *Leucaena leucocephala* grows naturally. In arid deserts, surface layers of soils often dry out quickly due to evaporation or rapid penetration, and water is stored below the soil surface, where a considerable amount may remain available to plants (Ghorbanli *et al.*, 2001). Faster germination recorded in *Leucaena leucocephala* at 40 °C (Fig. 2) might enhance seedling emergence shortly after summer germination episodes. Consequently, seedling roots will grow when the surface layer of soil is still wet. This could secure seedling survival through the rapid growth of roots enabling them to reach a moist layer of soil.

Ismail (1998) reported that *Leucaena leucocephala* is equipped with a number of biological characteristics that can facilitate its rapid invasion of new areas. The present study demonstrated that a significant proportion of non-salt treated seeds of *Leucaena leucocephala* germinated under a wide range of temperatures and in both light and dark. This could be another reason behind the successful invasion of this species in several tropical and subtropical countries of the world.

In many plant species, light is one of the most important environmental regulatory signals that interact with temperature to regulate seed germination (Baskin and Baskin, 1998). Light requirement for germination may vary with temperature. No significant difference between light and dark germination at 20 and 30 °C, for both non-saline and saline treated seeds, were found for *Leucaena leucocephala*. However, at the highest temperature (40 °C), germination in light was significantly greater than in darkness (Fig. 1). Similar results were reported for the cosmopolitan annual plant species, *Portulaca oleracea*, (El-Keblawy and Al-Ansari, 2000).

Table 2. Two-way ANOVA showing influences of salinity and temperature on *Leucaena leucocephala*

Source of variation	df	Mean-square	F-ratio	P
Temperature (T)	2	759.148	39.731	<0.001
NaCl conc. (S)	6	2376.861	118.736	<0.001
T*S	12	193.190	9.938	<0.001
Error	63	19.340		

**Fig. 2. Influences of salinity and temperature on germination rate in *Leucaena leucocephala***

Light is abundant only on the soil surface and physiologically active light flux rarely penetrates more than a few millimetres into soil. Shiferaw *et al.* (2004) indicated that *Prosopis juliflora* is characterized by the accumulation of a long-lived seed bank that serves as a source of regeneration of new plants in the event of disturbance. The mean soil seed density, from the top of the litter layer down to 9 cm depth, was estimated to be 1932 seeds/m². Greater germination of *P. juliflora* seeds at 40 °C in light than in dark suggests germination in summer may benefit by more light and seeds in upper layer of litter would have a greater chance for emergence than seeds buried in deeper layers. This would maintain the seed bank of this species, especially as leading to seedling recruitment following summer rainfall very low.

Light and salinity interact during germination in a number of other halophytes. For example, the increase in NaCl concentration progressively inhibited seed germination in *Allenrolfea occidentalis*, and this inhibition was greater in the dark than in light (Gul and Weber, 1999). Also, both darkness and high salinity inhibited germination of *Limonium stocksii* (Jie *et al.*, 2008). The interaction effect of salinity and light on germination of *Leucaena leucocephala* was not

significant (Table 1), indicating that the effect of salinity may not necessarily depend on light conditions in this species.

The present study showed that the optimal germination for non-salt treated seeds (90%) of *Leucaena* occurred at 30 °C and in continuous light (Fig. 1), a high germination percentage for seeds did not receive any pre-treatment. High germination for untreated seeds of *Leucaena leucocephala* at optimal conditions could be attributed to seed storage that broke any seed dormancy. Several studies have shown that *Leucaena leucocephala* has a physical dormancy (Scifres and Brock, 1969). This dormancy can be broken by seed pre-treatment with sulphuric acid (Agus, 2007), by passing through the digestive track of animals, and by the treatment with boiling water.

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الملخص العربي

تأثيرات الملوحة والضوء ودرجات الحرارة علي إنبات بذور أشجار اللوسينيا

مُجد عمر باصلاح، هيثم مُجد علي

بالمعاملة بالضوء أو الظلام التام. وقد انخفضت نسبة إنبات بذور اللوسينيا معنويا وذلك بزيادة كلا من تركيز املاح كلوريد الصوديوم و درجات الحرارة.

وقد كانت نسبة الأنبات الامثل في درجة حرارة 30° م وكان التأثير المثبط للأنبات من خلال نسبة الملوحة المرتفعة وذلك علي النسبة المئوية للأنبات النهائي وكانت أعلاها في 40°م ثم 20، 30° م. وعلي ذلك فإن معدل الانبات زاد معنويا عند 40°م ثم 20، 30° م بالمعاملة بتركيزات الملوحة المنخفضة والعكس صحيح في معدلات الملوحة المرتفعة، وقد وجد ان الأنبات في الضوء كان اعلي معنويا مقارنة بالأنبات في الظلام عند معدلات الملوحة المنخفضة ودرجات الحرارة العالية.

أجريت هذه الدراسة لتقييم إنبات بذور أشجار اللوسينيا وذلك تحت ظروف الملوحة، الحرارة، الضوء وكذلك التداخل بين هذه العوامل وتأثيرها علي معدل نسبة الأنبات النهائية لبذور اشجار اللوسينيا.

أجريت هذه التجربة داخل معمل فسيولوجيا النبات بكلية العلوم جامعة الملك سعود- الرياض- المملكة العربية السعودية. عوملت البذور بالماء المغلي لمدة ثلاث دقائق وذلك قبل وضعها في اطاق بتري داخل غرف النمو في ظروف بيئية متحكم بها.

وقد أستخدم سبع تركيزات مختلفة من أملاح كلوريد الصوديوم (0،50،100،300،400،500،600 مليموز) وقد أستخدمت كماء لري البذور، كذلك أستخدمت ثلاث مستويات لدرجات حرارة (20،30،40° م) وذلك تحت الضوء او في الظلام التام.

وقد أعطت البذور الغير معاملة بكلوريد الصوديوم نسبة عالية من الأنبات علي نطاق واسع من درجات الحرارة المختلفة وذلك سواء