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Effect of Salt Stress on Germination and Seedling Development in *Leucaena leucocephala*

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ABSTRACT. To determine the possibility of introducing *Leucaena leucocephala* into saline areas of Saudi Arabia, observations were made on the effect of salt stress on germination and seedling development of this species. Germination percentage and rate were both reduced in the presence of various monovalent and divalent ions. Monovalent ions were more inhibitory than divalent ions for germination percentage and germination rate. Germination of *Leucaena leucocephala* was more tolerant to these ions than seedling development. Salinity reduced root growth more than shoot growth and ions accumulated in plant tissues to toxic levels. It was concluded that like other legumes, *Leucaena leucocephala* is salt sensitive and may not prosper in those regions of Saudi Arabia with high salinity.

Leucaena leucocephala is a very productive tropical legume and is one of the best sources of high protein feed. This species originated from Latin America but has spread widely throughout the tropics (Oakes 1968). Its widespread adaptation, gregarious and rapid growth, high protein production capabilities and nitrogen fixing properties have enhanced its economic importance (Brewbaker and Hutton 1979). The possible introduction of *Leucaena* into Saudi Arabia could help to meet the shortage of irrigated forage and reduce grazing pressure on natural rangeland which is already in deplorable condition due to overgrazing and mismanagement.

Soil salinity is of widespread occurrence in Saudi Arabia, hence knowledge of the response of *Leucaena leucocephala* to salinity is essential to determine the advisability of importing this species for commercial use. The present study was conducted to determine the effects of salt stress on germination and seedling growth of *L. leucocephala*.

Material and Methods

Seeds of *Leucaena leucocephala* variety K67 were imported from Tree Seed International Company, U.S.A. Response to salt stress was studied at seed germination and during seedling development.

Germination

Laboratory germination tests were carried out in petri dishes placed in an incubator maintained at a constant temperature of 25°C. Twenty five seeds were placed in a petri dish which was lined with a double layer of filter paper and the desired concentration of salt solution which ranged from 0 to 300 m-equiv/*l* of different salts. Germination in distilled water served as control. Treatments were replicated four times. Germination counts were made on alternate days from the 5*th* to the 20*th* day when the experiment was concluded. Seeds were considered germinated when the radicle had emerged and reached 1 cm long. All seeds were discarded after counting. Germination rate was calculated as the number of days required for 50% germination.

Seedling Stage

Experiments were conducted in water culture in the greenhouse. Seeds were first germinated in sand and when the first trifoliate leaf had emerged, the seedlings were transferred to plastic dishes of 3-liter capacity. Each plastic dish was covered with a thick polythene cover having eight holes each 1 cm diameter. Seedlings were placed in the holes with a small pack of cotton so that shoots stood erect on the polythene cover and the roots were bathed in aerated nutrient solution. Salt treatments were imposed a week after transfer of seedlings to the dishes. Each dish of eight plants comprised a replicate and there were four replicates in each treatment. Salt level in the nutrient solution ranged from 0 to 200 m-equiv/*l*. To avoid osmotic shock, the salt level in the nutrient solution was raised in gradual steps. Hoagland nutrient solution plus 1 mM NaCl was used as control. Plants were harvested one month after treatments began. Dry weight of shoots, roots and the ratio of shoot/root weight were determined. Plants were analysed for their Na, K and Cl content.

Results

Effect on Germination

Effect of NaCl

The germination percentage decreased as the NaCl level in the germination medium increased (Fig. 1). At 200 m-equiv/*l* NaCl, germination was reduced to 50% and then further reduced to 20% at 300 m-equiv/*l* NaCl.

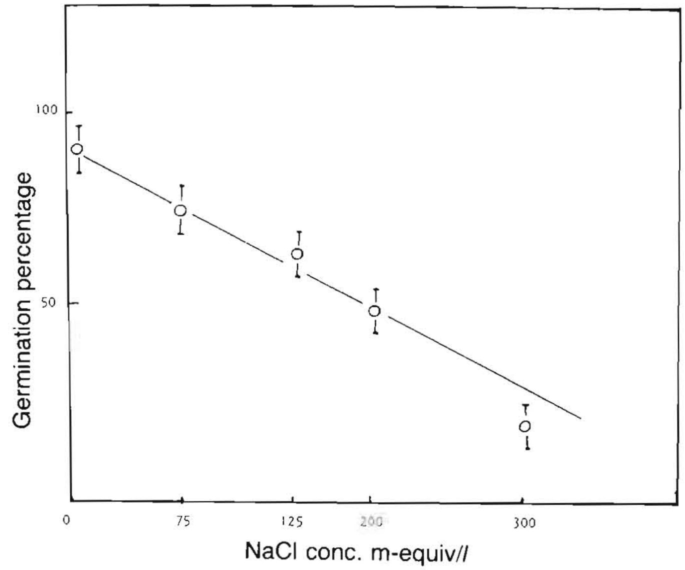

Fig. 1. Germination percentage of seeds of *Leucaena leucocephala* in increasing concentrations of NaCl. Vertical base represents one standard deviation above and below the mean.

Effect of Different Salts

To compare the effect of different salts on germination, NaCl, KCl, $MgCl_2$ and $CaCl_2$ at 200 m-equiv/*l* were used. Germination was inhibited more by monovalent than by divalent cations (Table 1). All added salts delayed germination, but monovalent cations, delayed germination more than divalent cations.

Table 1. Germination percentage and rate* of *Leucaena leucocephala* in different salts (200 m-equiv/*l*).

Salt Type	Percentage	Rate
Control	95 ± 2.8	5 ± 1
$CaCl_2$	49 ± 2.4	8 ± 1.5
$MgCl_2$	68 ± 3.1	9 ± 2.0
KCl	47 ± 3.0	13 ± 2.5
NaCl	45 ± 3.3	15 ± 1.8

* No. of days required for 50% germination.

Effect on Seedling Growth

The seedlings grown in control nutrient solution were green and healthy. Development of trifoliate leaves was normal and roots developed profusely. Salt injury symptoms appeared in the treatment with 75 m-equiv/*l* NaCl and became quite pronounced in the 125 m-equiv/*l* and 200 m-equiv/*l* NaCl treatments. Roots became brown and brittle and leaves became yellow and abscised prematurely.

In the highest concentration of NaCl used (200 m-equiv/*l*), the seedlings were completely leafless and were virtually dead at harvest. The condition of seedlings in different treatments is shown in photographs a to e (Fig. 2). Table 2 presents the dry weight of seedlings for the various treatments. The dry weights of both roots and shoots were highest in the control with an almost linear reduction in dry weight of seedlings corresponding to the increase in the NaCl level of the growth medium.

Shoot/Root Ratio

To determine whether roots or shoots were more reduced by salinity stress, the shoot/root ratio was calculated. It was found that in the three highest concentrations of NaCl, *i.e.*, 75, 125 and 200 m-equiv/*l* NaCl, there was an increase in the

a

b

c

d

e

Fig. 2. Photographs showing seedling growth of *Leucaena leucocephala* in different NaCl treatments. (a) 1 m-equiv/*l* (b) 50 m-equiv/*l* NaCl, (c) 75 m-equiv/*l* NaCl, (d) 125 m-equiv/*l* NaCl, (e) 200 m-equiv/*l* NaCl.

Table 2. Dry weight (gm/plant) and shoot/root ratio of *Leucaena leucocephala* grown at different NaCl level in water culture.

NaCl Conc.	Shoots	Roots	Shoot/Root
Control	6.0 ± 1.2	1.95 ± 0.04	3.06
NaCl 50 m-equiv/*l*	4.0 ± 1.1	1.32 ± 0.02	3.03
NaCl 75 m-equiv/*l*	3.7 ± 1.0	0.95 ± 0.01	3.90
NaCl 125 m-equiv/*l*	2.6 ± 0.50	0.60 ± 0.01	4.50
NaCl 200 m-equiv/*l*	1.35 ± 0.20	0.30 ± 0.009	4.70

Table 3. Ion contents (m-equiv/100 g dry weight) of *Leucaena leucocephala* grown at different NaCl levels in water culture.

NaCl Conc.	Chloride		Sodium		Potassium	
	shoots	roots	shoots	roots	shoots	roots
Control	10 ± 1	11 ± 1.11	0	0	26 ± 3	71 ± 4
NaCl 50 m-equiv/*l*	30 ± 3	32 ± 4	20 ± 2	13 ± 2	25 ± 4	51 ± 6
NaCl 75 m-equiv/*l*	50 ± 5	53 ± 5	35 ± 3	25 ± 2.5	27 ± 3	55 ± 5
NaCl 125 m-equiv/*l*	80 ± 6	75 ± 5	55 ± 4	43 ± 5	29 ± 2.5	33 ± 4
NaCl 200 m-equiv/*l*	170 ± 8	165 ± 7	150 ± 7	57 ± 5	27 ± 4	15 ± 2

shoot/root ratio relative to control values, indicating that roots were more adversely affected than shoots by NaCl (Table 2).

Na Content

In controls, there was no accumulation of Na either in roots or shoots of the seedlings. In the NaCl treatments, Na in the shoot increased gradually and became very high at 200 m-equiv/*l* NaCl. Initially, Na was low in the roots but it also increased with the increase of NaCl concentration in the nutrient solutions. The Na content of roots was always lower than that of the shoots (Table 3).

K Content

The pattern of K content in the seedlings was the reverse of that of Na in that the latter was high in roots and low in shoots. NaCl treatment did not appreciably change the K content of the shoots but K in the roots was reduced at higher NaCl levels in the growth medium (Table 3).

Cl content

An appreciable amount of chloride was present in the control seedlings. This content increased with increasing NaCl level of the nutrient culture. In all the treatments, Cl content was higher than Na content both in roots and shoots (Table 3).

Discussion

Legumes, in general, are more salt sensitive than other crops (Bernstein and Hayward 1958). Similarly, the findings of the present study suggest that *Leucaena leucocephala* is also a sensitive species, at least in the seedling stage, as salt injury symptoms developed even at a NaCl concentration of 75 m-equiv/*l* in the rooting medium. However, salt tolerance during germination was greater than in the seedling stage because at 200 m-equiv/*l* NaCl only 50% germination was reduced (Fig. 1). In comparison, however, this concentration was lethal to seedlings (Table 2).

Variability to salt tolerance at different stages of growth have been reported in many crops (Bernstein 1964). Difference between mono and divalent cations in delaying and inhibiting germination may be explained partly on the basis of the role of these elements in metabolism. Calcium is important in maintaining membrane integrity (Jones and Lunt 1967) and Mg acts as a coenzyme (Galston 1964). These cations, at the concentrations used, were less damaging than Na and K which are known to create ionic imbalance at high concentrations, thus inhibiting germination.

It is speculated that an adverse effect on growth of seedlings may be due to a combination of two factors (1) greater reduction in root than in shoot growth (Table 2) and (2) accumulation to toxic levels of Na and Cl ions in the seedling tissues (Table 3).

It is obvious that reduced root growth cannot sustain normal plant functions. Also plants tend to lose metabolites in saline environments. Loss of amino acids from barley roots subjected to high NaCl treatment has been reported (Hyder 1971). In the present experiment, gradual loss of K from the roots of NaCl treated plants indicates the possibility that other important metabolites might also have been lost from the roots.

As for the detrimental effect of excess salt accumulation in plant tissues, Greenway (1973) has emphasized that salt sensitive plant species collapse when ions accumulate beyond certain concentration. The adverse effect of high Na and Cl concentrations on seedling health in the present experiment was also noted in soybean (Abel and Mackenzie 1964, Wilson *et al.* 1970), *Trifolium subterraneum* (Hyder 1970) and other crops (Richard 1954).

The conclusion drawn from this study is that *Leucaena leucocephala* may not prosper if introduced into saline areas of Saudi Arabia. However, in the Tihama plains, in the south, where there is less salinity and the climate is nearly tropical, this species may succeed. We hope to investigate this possibility in the next phase of our program.

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تأثير إجهاد الأملاح على الإنبات وطور البادرات لنبات *لوسينيا ليكوسيفالا*

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أخذت بعض المشاهدات على تأثير الأملاح على الإنبات وتطور بادرات أشجار «*اللوسينيا ليكوسيفالا*» وذلك بهدف معرفة إمكانية إدخالها إلى الأراضي المتأثرة بالأملاح في المملكة العربية السعودية. وقد حدث انخفاض في نسبة الإنبات وكذا في سرعة تطور البادرات وذلك في وجود أملاح مختلفة. على أن الأملاح أحادية التكافؤ كانت أكثر تثبيطاً لسرعة تطور ولنسبة إنبات البادرات من الأملاح ثنائية التكافؤ. كذلك لوحظ أن طور الإنبات لنبات *اللوسينيا* كان أكثر تحملا للملوحة من طور البادرة. لقد تأثر نمو جذور النبات سلبيا أكثر من نمو مجموعه الخضري بنتيجة الملوحة، كما وأن الأملاح قد تجمعت داخل أنسجة النبات إلى مستويات التسمم. وعليه يمكن الاستنتاج بأن «*اللوسينيا ليكوسيفالا*» شأنها شأن الأشجار البقولية الأخرى، هي حساسة للملوحة، ومن غير المتوقع نجاحها في مناطق المملكة التي تعاني من درجات الملوحة العالية.