Effect of Salinity on Chlorophyll & Carbohydrate Contents of Calotropis Procera Seedlings

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Abstract :

The effect of salinity concentrations (0, 5, 10, 20, 40, 80, 160 and 320 mM NaCl) on the leaf content of chlorophyll a and b and carbohydrate (soluble and insoluble) contents of shoot and root of Calotropis procera (Ait.) Ait. seedlings were investigated. The results showed clear effects of salinity on the chlorophyll content especially at high salinity concentration (160 and 320 mM NaCl) and with increasing plant age.

The total soluble and insoluble carbohydrates content in the shoot and root tended to increase with increasing salinity stress in the solution culture and also with plant age which considered playing an important role in the osmotic adjustment.

Introduction:

It has been generally recorded that salinity adversely affects seedling growth and some relevant metabolic processes of glycophytic plants (Shaddad and Zidan, 1989; Hampson and Simpson, 1990 and Zidan and Al-Zahrani, 1994). However, the direction and magnitude of these changes varied according to the level and duration of salinization treatment as well as the plant species used. Seeman and Critchley (1985) and Sharkey et al. (1985) reported that salinity can seriously change the photosynthetic carbon metabolize, leaf chlorophyll content, as well as photosynthetic efficiency. Carbohydrates are accumulated in plant tissues under saline stress, and these substances are suspected of contributing to osmotic adjustment (Munns and Termaat, 1986 & Delaumey and Verma, 1993).

Calotropis procera (Ait.) Ait. a plant species that has a reputation of wide amplitude in Saudi Arabia (Chaudhary and Al-Jowaid, 1999). The effect of salinity on growth (Al-Zahrani, 2002) and some physiological activities (Al-Zahrani et al, 2002) were studied. The present study was conducted to study the effect of NaCl salinity concentrations on the photosynthetic pigment in different plant ages.

Materials and Methods :

Seeds of Calotropis procera were collected locally from the freshly gathered ripe fruits, obtained from various shrubs growing in Jeddah area. Seeds were germinated on two layers of filter paper in flat trays, and irrigated with distilled water until they were large enough to handle (after 16 days). The seedlings were then transferred to boxes (3 liters) which had been painted black containing culture solution (Hogland and Arnon, 1950). Seedlings (30/box) were suspended above the solution by supporting them with non-absorbent cotton inside holes in the small depressions in the box lid.

The seedlings were grown in growth cabinet with a 12-hr photoperiod, the temperature ranging between 25^{o}C during the day and 20^{o}C during night periods and the relative humidity of about 70 %. Salinity treatments were imposed (when the seedling were 21 days old) by adding salt (NaCl) gradually at the rate of 50 mM or less per day up to the specific level of each treatment. The gradual addition of salt was to avoid instantaneous stressing of the seedlings by high salinity levels. The boxes were divided into 8 groups of three boxes and each group has one of the salinity treatments as follows: 0, 5, 10, 20, 40, 80, 160 and 320 mM NaCl. The solutions were aerated constantly by compressed air.

Four harvests were taken, at 7-days intervals after salinization. Five samples were taken for each measurement. The photosynthetic pigments (chlorophyll a and b) were determined according to Mitzener et al (1965). Fresh plant material (0.5 g) was extracted twice with 5 ml of 80% acetone/water solution (V/V), the combined extracts filtered through Whatman filter paper (N0 1) and made to 100 ml with 80% acetone. Chlorophyll was then determined using a pye Unicam series 2 UV and visible spectrophotometer and then the concentration of chlorophyll a, b and total chlorophyll were calculated using Mitzener's equations.

Soluble and insoluble carbohydrates were determined by the method of Fales (1951). Fresh weight (0.5 g) of the leaf tissue from each sample was used using the youngest fully expanded leaf.

Results and Discussion :

The results showed that there was clear effect of salinity concentration on the leaf pigment content of Calotropis procera seedling (Table 1 and Figure 1). It can be observed that the high levels of salinization (160 and 320 mM NaCl) induced a significant decrease in the contents of pigment fractions

(Ch. a and b) and consequently of the total chlorophyll content as compared with control plants. The total chlorophyll content of the leaves of Calotropis procera seedlings exhibited a little increase when grown at 5 and 10 mM NaCl. While the pigment contents increased at the first three treatments (0, 5 and 10 mM NaCl) with increasing plant age and then decreased at the last five treatments. Generally, chlorophyll contents were reduced markedly at high salinity concentration treatments especially with aged plants. It may be due to the reason that the total chlorophyll and the proportion of its components depend on the biological process and development stages of the plant and also on the type and concentration of the salt. Ahmed et al (1978) and Hajar et al (1993) also obtained similar findings. It is also clear from Table (1) that chlorophyll a predominated over chlorophyll b but the values become closer with increasing salinity which is in agreement with other results for some plants (e.g. Hajar et al, 1993). The ratio of chlorophyll a/b (Figure 1) showed a reduction with increasing salinity concentration (especially from 40 mM NaCl and up). The decreased in chlorophyll content under stress is a commonly reported phenomenon and in various studies, this may be due to different reasons, one of them is related to membrane deterioration (Ashraf and Bhatti. 2000).

The contents of soluble and insoluble and total carbohydrates in the shoot and root of the treated seedlings of Calotropis procera plants are given in tables (2-4). It can be seen that the contents of carbohydrates (soluble and insoluble) in the shoot and root tended to increase with increasing salinity level and age (Tables 3 & 4). Many plants, which are stressed by NaCl salinity, accumulate starch and soluble carbohydrates (Greenway and Munns, 1980 and Rathert, 1984). This accumulation has been attributed to impaired carbohydrate utilization (Munns and Termaat, 1986). It is apparent from the results that the soluble carbohydrate content in the shoot was higher in salt stress plants compared with control. In contrast, the total carbohydrate in the shoot was much higher than in the root of the treated seedlings (Table 2).

There is strong evidence indicating that photosynthesis is the main source of accumulation of organic solutes under water stress. Meyer and Boyer (1981) showed that cutting the photosynthetic cotyledons from soybean seedlings prevented solute accumulation and osmotic adjustment as also concluded by Kutachera and Kohler (1994). The accumulation of organic solutes (soluble and insoluble carbohydrates) might play an important role in increasing the internal osmotic pressure (Zidan and Al-Zahrani, 1994) which is widely regarded as response to salinity stress condition. While that the photosynthesis is the main source of carbohydrates accumulation, Munns (1993) has been reported that the concentration of sugars (and reserve polysaccharides) always rise after plants are exposed to salinity in both growing and fully expanded tissues. This is consistent with a blockage in utilization of sugars in the growing tissues and a subsequent build-up in the rest of the plant. A reduction in photosynthesis could be due to feedback inhibition by the high sugar concentrations in the mesophyll cells. It is appear in the begging of growth that Calotropis procera seedlings are not deficient in carbohydrates, and that the supply of carbon compounds are not limiting their growth, so, after prolonged periods (days or weeks) of exposure to salinity the levels of reserve carbohydrates increased, particularly in the shoot.

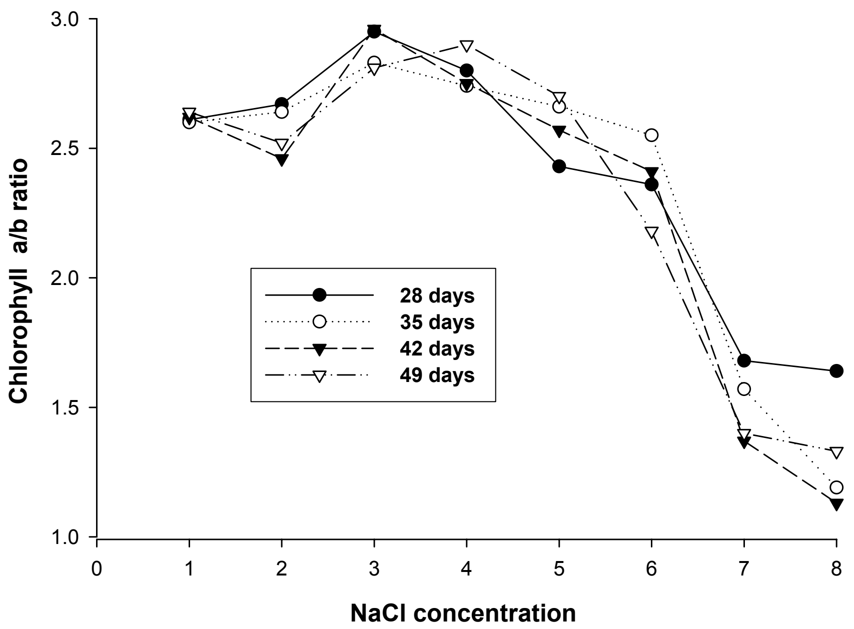

Figure (1): Effect of NaCl on the chlorophyll a/b ratio of *Calotropis procera* seedlings.
(1-8 are NaCl concentrations 0, 5, 10, 20, 40, 80, 160 and 320 mM respectively)

Table (1)

Effect of NaCl on the leaf content of chlorophyll a, b and total chlorophyll (mg/g/fwt) of *Calotropis procera* seedlings

Chlorophyll a

Age (days)	Control	NaCl concentration (mM)						
		5	10	20	40	80	160	320
28	1.15±0.08	1.16±0.00	1.21±0.02	0.98±0.06	0.90±0.03	0.85±0.02	0.52±0.07	0.36±0.08
35	1.22±0.07	1.32±0.01	1.30±0.05	1.15±0.01	1.01±0.01	0.88±0.05	0.47±0.10	0.25±0.04
42	1.31±0.07	1.33±0.05	1.39±0.08	1.21±0.06	0.95±0.03	0.72±0.05	0.26±0.10	0.18±0.05
49	1.40±0.04	1.41±0.04	1.46±0.07	1.22±0.11	0.89±0.08	0.63±0.04	0.21±0.07	0.16±0.03
LSD	Salinity stress: 0.015; age: 0.087; interaction between stress and age: 0.207.							

Chlorophyll b

28	0.44±0.03	0.42±0.01	0.41±0.01	0.35±0.02	0.37±0.01	0.36±0.01	0.31±0.05	0.22±0.11
35	0.47±0.03	0.50±0.01	0.46±0.02	0.42±0.01	0.38±0.01	0.34±0.03	0.30±0.02	0.21±0.02
42	0.50±0.02	0.54±0.02	0.47±0.03	0.44±0.01	0.37±0.01	0.30±0.02	0.19±0.03	0.16±0.06
49	0.53±0.03	0.56±0.01	0.52±0.02	0.42±0.04	0.33±0.03	0.29±0.02	0.15±0.03	0.12±0.07
LSD	Salinity stress: 0.050; age: 0.153; interaction between stress and age: 0.208.							

Total chlorophyll

28	1.60±0.12	1.59±0.02	1.63±0.03	1.34±0.09	1.28±0.05	1.21±0.02	0.84±0.12	0.59±0.13
35	1.70±0.10	1.84±0.02	1.76±0.08	1.57±0.04	1.39±0.01	1.22±0.09	0.77±0.13	0.47±0.06
42	1.84±0.09	1.88±0.07	1.87±0.12	1.65±0.08	1.33±0.04	1.02±0.07	0.46±0.13	0.35±0.04
49	1.94±0.07	1.98±0.06	1.98±0.10	1.65±0.15	1.22±0.12	0.92±0.06	0.37±0.07	0.29±0.09
LSD	Salinity stress: 0.033; age: 0.548; interaction between stress and age: 0.804.							

(Mean ±SE)

Table (2)

Effect of NaCl on the shoot and root content of total carbohyd+rate (mg/g/fwt) of *Calotropis procera* seedlings.

Shoot

Age (days)	Control	NaCl concentration (mM)						
		5	10	20	40	80	160	320
28	0.87±0.19	0.70±0.10	0.73±0.17	0.76±0.12	0.98±0.03	1.04±0.10	0.98±0.06	0.97±0.10
35	1.23±0.06	1.00±0.09	0.86±0.05	0.92±0.32	1.28±0.09	1.37±0.13	1.53±0.42	1.23±0.15
42	1.30±0.13	1.12±0.14	0.91±0.05	1.19±0.24	1.19±0.15	2.35±0.05	1.77±0.40	1.49±0.07
49	1.36±0.44	1.18±0.16	1.02±0.13	1.32±0.34	1.48±0.55	2.77±1.27	1.98±0.66	1.77±0.06
LSD	Salinity stress: 0.436; age: 0.262; interaction between stress and age: 0.522.							

Root

28	0.25±0.05	0.30±0.10	0.30±0.04	0.35±0.04	0.37±0.05	0.35±0.07	0.43±0.06	0.54±0.09
35	0.30±0.52	0.39±0.18	0.44±0.04	0.53±0.09	0.56±0.07	0.59±0.05	0.63±0.49	0.57±0.21
42	0.38±0.05	0.51±0.25	0.58±0.04	0.61±0.11	0.66±0.14	0.67±0.06	0.64±o.21	0.62±0.03
49	0.60±0.07	0.87±0.25	0.93±0.13	0.92±0.06	0.93±0.33	1.07±0.18	0.72±0.11	0.59±0.07
LSD	Salinity stress: 0.257; age: 0.095; interaction between stress and age: 0.428.							

(Mean ±SE)

Table (3)
Effect of NaCl on shoot and root content of soluble carbohydrate (mg/g/fwt) of *Calotropis procera* seedlings.

Shoot

Age (days)	Control	NaCl concentration (mM)						
		5	10	20	40	80	160	320
28	0.13±0.01	0.19±0.04	0.20±0.01	0.20±0.01	0.20±0.01	0.23±0.02	0.24±0.04	0.26±0.03
35	0.17±0.03	0.23±0.02	0.23±0.02	0.25±0.08	0.30±0.01	0.31±0.02	0.33±0.14	0.48±0.11
42	0.18±0.02	0.23±0.01	0.24±0.01	0.27±0.10	0.29±0.05	0.48±0.18	0.51±0.04	0.68±0.07
49	0.20±0.04	0.23±0.02	0.28±0.02	0.30±0.10	0.40±0.35	0.58±0.04	0.62±0.12	0.84±0.11
LSD	Salinity stress: 0.283; age: 0.254; interaction between stress and age: 0.335.							

Root

28	0.08±0.01	0.12±0.04	0.12±0.01	0.12±0.03	0.13±0.01	0.12±0.01	0.13±0.04	0.23±0.02
35	0.11±0.02	0.12±0.03	0.14±0.02	0.13±0.08	0.13±0.03	0.12±0.02	0.20±0.01	0.24±0.10
42	0.13±0.01	0.15±0.01	0.16±0.02	0.17±0.03	0.18±0.02	0.18±0.02	0.20±0.05	0.23±0.10
49	0.18±0.03	0.19±0.04	0.19±0.02	0.18±0.03	0.17±0.03	0.20±0.02	0.22±0.06	0.18±0.07
LSD	Salinity stress: 0.330; age: 0.660; interaction between stress and age: 0.891.							

(Mean ±SE)

Table (4)

Effect of NaCl on the shoot and root content of insoluble carbohydrate (mg/g/fwt) of *Calotropis procera* seedlings.

Shoot

Age (days)	Control	NaCl concentration (mM)						
		5	10	20	40	80	160	320
28	0.74±0.19	0.51±0.11	0.53±0.16	0.56±0.12	0.78±0.04	0.81±0.11	0.74±0.02	0.71±0.08
35	1.06±0.08	0.77±0.08	0.63±0.07	0.67±0.35	0.98±0.09	1.06±0.15	1.20±0.37	0.75±0.07
42	1.12±0.12	0.89±0.14	0.67±0.05	0.92±0.22	0.90±0.17	1.87±0.11	1.26±0.38	0.81±0.02
49	1.16±0.44	0.95±0.18	0.74±0.11	1.02±0.24	1.08±0.27	2.19±0.71	1.36±0.03	0.93±0.10
LSD	Salinity stress: 0.520; age: 0.358; interaction between stress and age: 0.808.							

Root

28	0.17±0.04	0.18±0.06	0.18±0.02	0.23±0.03	0.24±0.05	0.23±0.06	0.30±0.08	0.31±0.08
35	0.19±0.02	0.27±0.06	0.30±0.05	0.40±0.05	0.43±0.03	0.47±0.07	0.43±0.00	0.33±0.07
42	0.25±0.05	0.36±0.05	0.42±0.04	0.44±0.11	0.48±0.13	0.49±0.08	0.44±0.24	0.39±0.07
49	0.42±0.08	0.68±0.26	0.74±0.12	0.74±0.07	0.76±0.03	0.87±0.14	0.50±0.17	0.41±0.11
LSD	Salinity stress: 0.247; age: 0.100; interaction between stress and age: 0.369.							

(Mean ±SE)

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تأثير الملوحة على محتويات بادرات العشر من الكلوروفيل والكربوهيدرات

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جده - المملكة العربية السعودية

الملخص:

لقد تم في هذا البحث دراسة تأثير تركيزات مختلفة من الملوحة (صفر، ٥، ١٠، ٢٠، ٤٠، ٨٠، ١٦٠ و ٣٢٠ ملي مول كلوريد صوديوم) على محتوى الأوراق من كل من كلوروفيل أ و ب وكذلك محتوى كل من المجموع الخضري والجذري من الكربوهيدرات الذائبة وغير الذائبة لبادرات العشر Calotropis procera (Ait.) Ait.

وقد أظهرت النتائج أن هناك تأثير واضح للملوحة على محتوى الورقة من الكلوروفيل خصوصا في التركيزات العالية من الملوحة (١٦٠ و ٣٢٠ ملي مول كلوريد صوديوم) وكذلك مع زيادة عمر النبات. كما أن المحتوى الكلي من الكربوهيدرات الذائبة وغير الذائبة في المجموع الخضري والجذري اتجه للزيادة مع زيادة الجهد الملحي في المحلول الخارجي وكذلك مع زيادة عمر النبات والذي يعتقد أنه يلعب دورا مهما في الضبط الأسموزي.