

Physiological Behaviour and Growth Responses of *Eucalyptus camaldulensis* Dehn. Seedlings to Salt Stress Conditions

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Abstract: Soil salinity is a critical global problem, especially in arid and semi-arid regions. In such regions soluble salts accumulate in the soil since precipitation is greatly outweighed by evaporation. *Eucalyptus camaldulensis* is an important forest tree species, which is of high productivity in a relatively short rotation and is extensively grown in irrigated plantations in many parts of the globe. In the present work our main goal is to evaluate the sensitivity and growth performance of *E. camaldulensis* under salt stress conditions. *E. camaldulensis* seedlings (shoot height 32.4 ± 0.87 cm) were subjected to a relatively wide range of salt stress: 0 (control), 30, 50, 80 and 100 mM NaCl for four weeks under nursery conditions. Growth performance (shoot height, stem diameter and leaf formation rates) was regularly monitored twice a week. At harvest, each plant was separated into root and shoot systems to evaluate the effect of the salt treatment on dry mass production, accumulation and partitioning. Initially, growth was unaffected by salt treatments until the second week. However, significant reductions were observed towards the end of the exposure period. Among the growth parameters analyzed leaf formation rate was the most sensitive compared to stem height and stem diameter growth rates. Dry mass showed significant reductions in both shoot and root especially in plants that received the highest salt concentrations. Based on these results it might be suggested that *E. camaldulensis* is sensitive to salt stress and is rather unsuitable tree species to be used in sites affected by high levels of salinity. Since leaf formation is tightly associated with shoot apical meristem activity, the current findings obviously points to a link between salinity and plant hormone status. Therefore, it would be helpful to conduct further experiments including some hormone analysis to validate these speculations and to find explanations for the physiological events responsible for the poor performance of *E. camaldulensis* under saline soils.

Keywords: *Eucalyptus camaldulensis*, growth, dry mas, soil salinity.

1. Introduction

Salinization of soil is one of the most serious environmental problems in agriculture and universally considered an important limiting factor for plant growth and production. Such a problem is progressively increasing world-wide [1]. Previous reports have estimated that one-third of irrigated lands world-wide and nearly 50% of irrigated as well as non-irrigated lands in arid and semi-arid regions are influenced by excess salinity [2,3]. The problem of salinity is characterized by an excess of inorganic salts and is most common in arid and semi-arid lands, where it has been naturally formed under the prevailing climatic conditions and due to the higher rates of evapotranspiration and lack of leaching water [4].

The salinity of soil generally refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. It has been well documented that excess salinity cause severe effects on plant growth, productivity and survival. Some studies have demonstrated that salinity affects plant growth via its influence on plant-water relations [5]. It has been shown that under restricted water supply under high levels of salinity the leaf expansion is reduced, which might be considered the most important short-term effect of salinity on plants [6]. Besides, previous

studies have also indicated that under high salinity conditions, i.e., presence of high concentration of salts in the soil solution interferes with the balanced absorption of essential nutrients by the plant leading to critical nutritional disorders [7,8]. It is now generally accepted that under salinity stress nutritional disorders cause plant growth reduction by affecting the availability, transport and partitioning of nutrients. Under saline conditions, [9] have demonstrated that plant growth reduction can be related to specific ion toxicities (e.g., Na^+ and Cl^-) and ionic imbalances acting on biophysical and/or metabolic components of plant growth. Studies on several species [10, 11, 12] have reported that increased NaCl concentration induce increases in Na and Cl as well as decreases in N, P, Ca, K, and Mg levels.

Plant species and varieties vary widely in their responsiveness to conditions of elevated salinity where some plants show variable degrees of salt stress tolerance while others are quite sensitive to salinity even at very low levels [13, 14, 15, 16, 17]. In a recent study, [18] investigated the effect of saline water on the growth of three walnut cultivars. Based on the observed significant differences in both fresh and dry weight of the different plant fractions the authors concluded that cultivars were different in response to salinity stress.

Apart from the variation among different species and cultivars in their responses to salt stress, it has been well established that the reaction of plants to salt vary depending the time of exposure to salt, the growth period of the plant, salt concentration, climatic and soil properties [5]. For instance, in some studies [19, 20] it was documented that decrease in vegetative growth parameters was depending on increasing the salinity level.

To date, the effects of salt stress on plant growth and physiology is fairly known. However, the actual mechanisms behind such effects are not perfectly understood. Knowledge of the specific physiological mechanisms in charge of salt stress tolerance would facilitate setting reliable criteria for improving plant tolerance to soil salinity stress. The complexity in analyzing the actual physiological events when plants are subjected to elevated levels of soil salinity might be attributed to several factors interacting with salinity. These factors might include light, temperature, humidity, soil fertility, soil calcium concentrations, flooding, atmospheric CO₂ concentrations, variation in field condition and the type of a potting medium [21, 22, 23, 24, 25, 26].

In the present investigation the main objective was to analyze the growth responses of *Eucalyptus camaldulensis* seedlings exposed to a wide range of NaCl concentration (0 – 100 mM NaCl). *Eucalyptus camaldulensis* has been chosen for this study as it proved an excellent forest tree species for its relative fast growth and thus widely grown in plantation system for the production of wood fuels and constitutes a good source for wood-based industries.

2. Materials and Methods

Preparation of Plant Materials and Growth Conditions

Plant Materials and Experimental Conditions

The current investigation was conducted on copper stress treatments employing young *Eucalyptus camaldulensis* seedlings. Germination and early establishment of the seedlings were performed in a nursery under partial shade. Freshly collected *Eucalyptus camaldulensis* seeds were directly sown in black non-transparent polythene bags (15 cm diameter X 40 cm height), five seeds per bag. The polythene bags were filled with a soil mixture containing silt: sand in a 2: 1 ratio (by volume), leaving the top 5 cm as a margin for irrigation. The bags were also perforated (6 holes/ bag) to ensure good aeration and also to facilitate easy drainage of excess water. Thereafter, the sown bags were placed under partial shade in a nursery. Watering was applied on daily basis by flood irrigation to field capacity for the first two months, then every other day for one month. During this phase of seedlings establishment singling and weeding were timely carried out. A total of 40 seedlings were chosen to run the experiment on the basis of vigour and uniformity in shoot height (mean shoot height 38.6 ± 1.05 cm). These candidate seedlings were grown for two more months for hardening outside the nursery. Watering was similarly maintained close to field capacity every second day.

Experimental Design and Copper Treatments

The seedlings for the experiment were distributed into five groups of seven seedlings each. Each group was assigned for each of the following NaCl treatments: 0 (control), 30, 50, 80 and 100 mM NaCl) identify the range of sub-optimum, optimum and excess NaCl supply. Salt treatments were applied every week by adding a fresh NaCl solution to the seedlings at the same time with irrigation. The salt treatments lasted for four weeks.

Growth Performance

To monitor growth, some growth variables (plant shoot height, number of leaves and stem diameter at the stem-root interface) were regularly determined once a week throughout the entire experimental period. At harvest, the final measurements of these growth parameters were also taken. During copper treatment, the growth rates of these growth variables were calculated for the last two weeks of the Cu treatment.

Dry Mass Production and Partitioning

After four weeks exposure to copper treatments, the experiment was terminated by destructive harvesting of the whole plants. Each seedling was separated into shoot and root. The shoot was further divided into leaves and stem. Similarly, the root system was carefully rinsed with distilled water immediately after harvest then divided into coarse and fine roots. The fresh mass of each plant fraction was determined and for dry mass determination all plant materials were oven-dried to a constant weight at 60°C for seven days.

Statistical Analysis

Results were statistically treated using the statistical programme JMP 5.1 Start Statistics, 3rd edition (SAS Institute, Inc., Cary, North Carolina, USA). The experiment was established in a completely randomized design. Data were expressed as means of eight replicates for each copper treatment. Analysis of variance was performed as One-Way-ANOVA and the separation of the means was performed by Tukey-test. A probability level of $P \leq 0.05$ was chosen to show the statistically significant variations among the means. Means followed by same letters are not significantly different from each other.

3. Results and Discussion

The present work investigated the response of young *Eucalyptus camaldulensis* seedlings to salt stress treatments (0 – 100 mM NaCl for 4 weeks). To analyse the impact of salt stress on the growth of *Eucalyptus camaldulensis* seedlings, growth indicators (plant shoot height, stem diameter and leaf formation) were determined during the course of the experimental period. Additionally, total plant dry mass as well as root-to-shoot ratios were also evaluated.

Growth

The present data showed that exposure of *Eucalyptus camaldulensis* seedlings to low and moderate salt stress (0 – 50 mM NaCl) did not affect leaf formation. However, increasing stress levels was accompanied by significant reductions in the number of leaves of the salt-treated seedlings compared to their respective controls (Table 1). These findings are in conformity with several previous studies [17, 27, 28, 29, 30, 31, 32]. The observed reduction in the number of leaves under exposure to high levels of salt stress might be attributed to the accumulation of NaCl in the cell walls and cytoplasm of the older leaves. Also, the vacuoles sap is likely incapable to accumulate more salt leading to significant drop in salt concentration inside the cell, a situation which eventually leads to quick death and shedding of leaves [33].

Table 1 Influence of salt stress [0 (control), 30, 50, 80 and 100 mM NaCl] on the growth rate (GR) of some plant growth indicators (shoot height, stem diameter and leaf formation) of *Eucalyptus camaldulensis* seedlings. Salt treatments were applied for four weeks. Growth measurements were performed once a week following salt addition. Data presented are the mean growth rate of seven plants per treatment (n =7, ± SE). Means separation was performed by Tukey-test at P ≤ 0.05 and the means connected by similar letters are not significantly different from each other.

| Salt (NaCl) treatment (mM) | Plant shoot height (cm/week) | Stem diameter (mm/week) | Leaf formation (number of leaves/week) |
|----------------------------|------------------------------|-------------------------|--|
| 0 (control) | 11.27 ± 0.98 a | 1.24 ± 0.85 a | 7 ± 0.83 a |
| 30 | 10.44 ± 1.02 a | 1.13 ± 0.74 a | 6 ± 0.77 a |
| 50 | 9.51 ± 0.81 a | 0.84 ± 0.79 a | 5 ± 0.24 ab |
| 80 | 6.72 ± 1.13 b | 0.53 ± 0.48 b | 3 ± 0.35 bc |
| 100 | 4.81 ± 0.92 bc | 0.37 ± 0.36 bc | 1 ± 0.84 c |

Similar response pattern was also observed for the plant shoot height which was significantly reduced under the higher concentrations of salt (Table 1). Our results corroborate similar findings of some authors who indicated that increasing the concentration of sodium chloride was accompanied by proportional reductions in plant shoot length [30, 34, 35, 36, 37, 38, 39].

The current results showed that the response of stem diameter growth under different levels of salt stress was similar to that of leaf formation and plant shoot height where the stem diameter was significantly reduced under the highest levels of salt stress treatment (Table 1). These results are in conformity with previous studies [40] which reported significant reductions in plant stem diameter under high levels of salt stress.

Dry Mass Production, Accumulation and Partitioning

Exposure of *E. camaldulensis* seedlings to salt stress has resulted in gradual reduction in total plant dry mass. The effect was most pronounced under the highest concentration of salt. Similar response patterns were also observed for the shoot-to-root ratios for the salt-treated seedlings compared to their respective controls (Table 2). Our present results might be in accordance with [18] who reported significant reductions in both fresh and total plant dry mass in walnut cultivars with increasing irrigation water salinity.

Table 2 Influence of salt stress [0 (control), 30, 50, 80 and 100 mM NaCl] on the total plant dry mass and root-to-shoot ratio of *Eucalyptus camaldulensis* seedlings. Salt treatments were applied for four weeks. Data presented are the mean seven plants per treatment (n =7, ± SE). Means separation was performed by Tukey-test at P ≤ 0.05 and the means connected by similar letters are not significantly different from each other.

| Salt (NaCl) treatment (mM) | Total plant dry mass (g) | Root-to-shoot ratio |
|----------------------------|--------------------------|---------------------|
| 0 (control) | 13.768 ± 0.44 a | 0.19 ± 0.05 a |
| 30 | 12.024 ± 0.51 a | 0.17 ± 0.07 a |
| 50 | 9.819 ± 0.23 b | 0.12 ± 0.05 a |
| 80 | 7.005 ± 0.65 bc | 0.08 ± 0.04 b |
| 100 | 5.013 ± 0.37 c | 0.06 ± 0.02 b |

The suppressive effect of high salt stress concentrations on plant growth and biomass production has been reported in numerous early studies which indicated significant reductions in plant biomass under imposed saline conditions [16, 41, 42, 43, 44]. These findings are in well agreement with our present results. However, our findings with regard to the response pattern of plant biomass under salinity stress conditions are in contrast to other investigations which showed a stimulatory effect of salt stress on the growth and production of plant biomass under low levels of salt concentrations [17].

In the light of the present findings it might be speculated that elevated levels of salinity had inhibitory effect on the growth and physiology of *E. camaldulensis* seedlings as attested by the dramatic reductions in growth and total plant dry mass production. Since plant growth is the resultant of photosynthetic performance of the plant under tight hormonal control, it would be logical to link the intensity of salt stress treatment with the photosynthetic capacity and the hormonal status of the plant. Therefore, more experimental work at the molecular level involving hormonal analysis under saline conditions is required to elucidate the mechanisms assumed by the plant under unfavourable soil salinity. Information provided from such investigations would no doubt help improve our understanding for the behavior of plant under salt stress conditions.

4. Conclusions

Salinization of soil is a genuine environmental problem which causes dramatic losses of agricultural crops worldwide. To address such a problem, scientific research has recently been focused to find practical answers to alleviate the impact of salinity on plant growth and productivity especially in agricultural countries. On one hand, some progress has been achieved so far in improving plant tolerance to high salt stress conditions and on the other hand several innovative approaches for reclamation of salt-affected soils have been adopted, which realized some success. The challenge ahead is to maximize the output from the breeding programs and other relevant disciplines towards screening for genes with excellent plant tolerance to salinity stress. Currently, great efforts are exerted by the scientific community towards improving plant tolerance to salt stress via advanced breeding programmes in support with the molecular and biotechnological approaches for screening genes responsible for high salt stress tolerance. Recently, the possibility of identification of special genes that control salt tolerance in plants has led to development of transformation techniques in genetically modified plants which are considered a great breakthrough for salt-stressed plants in acquiring adequate levels of salt stress tolerance.

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