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Performance potential and biomass production of *eucalyptus camaldulensis* under saline condition, Pakistan

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ABSTRACT

Eucalyptus camaldulensis is a fast-growing species that produces large amount of biomass in a span of short time. The aspect of high biomass production is very important especially in the arid and semi-arid climate condition of Pakistan to meet the growing timber requirements of the country. In Pakistan, about 6.3 million lands is salt affected soil which is nearly 10% of the total land area. In this scenario, the solution is to increase the eucalyptus plantation. However, to harness the production potential of this species need careful evaluation of its production potential and survival capacity under saline condition. Therefore, present study is designed to evaluate the growth potential and biomass production of eucalyptus under saline condition. One-month old seedlings were procured from the local nursery. Thirty earthen pots were filled thirty earthen pots were filled with sand-peat mixture (2v/Iv). After one month of growth, 30 seedlings of uniform size were selected and randomly assigned to any of the three treatments; 10 in control condition (CC), 10 moderate salt trees (MS) and 10 high salt stress (HSS) (2, 8 and 16 dSm^{-1}) respectively. Various growth parameters were measured during the experiments and assimilation rate, transpiration rate and stomatal conductance were measuring by suing IGRA at the end of the experiment. Results showed that biomass production (Plan height, stem diameter, of leaves and branches, chlorophyll II contents, fresh and dry weight of leaves, stem, roots) and physiological parameters (Assimilation rate, transpiration rate and stomatal conductance) and moisture contents and biomass allocation percentage were highly affected under high saline soil. Therefore, it can be concluded that *Eucalyptus camaldulensis* perform better under control the medium saline condition but also tolerate high saline.

Keywords: Growth potential and Biomass production of Eucalyptus species under saline condition

INTRODUCTION

Eucalyptus is an exotic species in Pakistan that originated from Peford and Gibb River, Australia. In spite of being native to Australia, it has shown good growth potential under plantations in the regions of Central

America and South Western America. When *Eucalyptus* stems is matured, the bark turns reddish-brown, rounded scales and often falls off. Heart wood is silvery greyish in colour with leaves elongated and leaf length varies from 15 to 22cm. *Eucalyptus camaldulensis* is naturally a fast-growing tree species that can attain 84 to 101ft height and 3.37ft in DBH (diameter in breast height) in a short time (few years) as compared to other species. Individuals show maximum growth under temperatures ranging from 20 to 28^{o}C and at altitudes ranging up to 3370ft (Flexas *et al*., 2004). It thrives best in the regions having precipitation range between 600 and 2900mm. However, it is an evidenced that it can tolerate 4 to 8 months long drought period along with strong winds. The species also has the ability adapt to survive on poor periodic flooded plains. Moreover, it flourishes well in compact soils or a soil having low annual precipitation although under such conditions growth is not optimum. *Eucalyptus* productivity varies considerably with changes in soil type i.e. less fertile soils may result in extreme negative impact on the growth. In compacted soils, radial growth gets severely delayed (Nieto and Rordriguez, 1990). It has been demonstrated that *Eucalyptus* growth can be increased significantly under suitable microclimate with sample water availability. Therefore, various tree establishment techniques have been identified and practiced in various ways under different environmental conditions (Robinson, 1988). Studies have shown that the technique such as tree shelter is very important to enhance growth of young plants during establishment stage (Tuley, 1985).

Salinization of land is gradually increasing throughout the world (Kozlowski, 1997). Saline affected areas are dividing into inland and coastal salinity areas, which are a serious problem in many regions of the world, Africa, Asia, Europe, Latin America, Near East, Australia and North America. The salinity areas are widely spread over 397 million hectares worldwide, which are three times larger than agricultural area (Anonymous, 2000). The inland salinity area is one of the major problems which are continuously expanding, depending on natural underground salt, unsustainable agricultural cultivation, low quality irrigation, industrial waste and human-induced Stalinization (Pitman and Auchli, 2002). Saline soil is an area comprising of various mineral salts in both cat ions, Na+, Ca2+, Mg2+, and K+, and anions, Cl-, SO42-, HCO3-, CO32-, and NO3- (Tanji, 2002). The ions directly affect plant growth and development causing either osmotic effect or ionic effect. A primary response of plant exposed to salt stress is a decrease in plant water potential, resulting in detrimental of water use efficiency (Glenn and Brown, 1998). The defence response of higher plants to salt stress is a complex system, which depends on a stage of morphological and developmental processes, salt tolerant ability (halophyte or glycolphyte) and environmental effects (Ashraf and Harris, 2004). The responses of each genotype are displayed as the cascades of their genetic background and phenotypic expressions. Salt tolerant or halophytic species seem to lack the unique metabolic machinery, which is sensitive to or activated by high toxic-ions, especially Na+ and Cl-. Defence responses of halophytic species comprise of many mechanisms such as osmotic regulation, ion homeostasis, antioxidant and hormonal systems (Hasegawa *et al*., 2000). Therefore, there are many reports that depict that salt-tolerant species can be categorized using physiological criteria such as chlorophyll content and chlorophyll fluorescence (Percival and Fraser, 2001). In that case, net-photosynthetic rate (NPR) is one of the candidate physiological parameters, which is simple, rapid, and sensitive method to establish the salt-tolerance index (Ashraf, 2004).

Species adapted to specific climatic conditions must have adapted some structural adaptations (Nawazish *et al*., 2006) and leaf anatomical feature are the representative of such environmental adaptations. Low stomata density and presence of two or more layers of palisade cells in leaves are the xenomorphic features (Cao, 2000). Trachoma density is generally higher in plants from dry season than those from wet seasons and in plants from sun-exposed areas than in those from shaded area (Pérez and Estrada *et al*., 2000). Leaf thickness, amount of clarification in leaves and other organs of plants, shape and orientation of stomata on both leaf surfaces and clarification and size of vascular tissue, in particular vessels in meta xylem (Hameed et *al*., 2009), and nature and density of salt hairs, trachoma's and salt glands (Naz *et al*., 2009) are good indicators of environmental stress like salinity and drought. The present studies were, therefore, focused on the anatomical adaptations of leaves in the genus Eucalyptus (family Myrtaceae) from the Faisalabad region. Exploitation of these useful genetic variations in salinity tolerance of crop plants / trees is an economical approach for revegetation of salt-affected lands. Plant characteristics such as salt and water logging tolerance, adaptation, uses, propagation and management, and productivity are important factors to be considered while selecting tree species for the rehabilitation of salt affected lands. Eucalyptus is an important tree species tolerant to salinity, thus has great potential for rehabilitation of salt-affected wastelands. In Pakistan, out of the total salt-affected area, approximately half is wastelands, and is extremely saline and saline sodic in nature. These soils are mostly unfit for agricultural crops. *Eucalyptus camaldulensis* has been identified as a tolerant tree species to salinity and waterlogging, and has more than 85% survival rate under saline soil conditions (Sandhu and Qureshi, 1986). Hence, it is the most successful tree species under a variety of saline conditions (Qureshi *et al*., 1993). It is assessed that half of the land is present in the semiarid and the coastal areas is affected by

Stalinization. About 10 million ha irrigated lands are becoming uncultivable annually due to salinity (Epstein *et al*., 1998). In Pakistan, about 6.3 million ha area is affected by salinity and sodality. Several strategies including use of chemical (Gypsum) to neutralize salt concentration in the soil and biological approaches (growing trees on affected soils) are being practiced for reclamation and re-vegetation of saline waste lands (Ashraf, 1994; Flowers, 2004). In some cases, deforestation has identified as one of the reasons for salinity increase as it involves removal of natural water pumps that help leaching down underground water level (Morris and Jenken, 1990). Salt stress provides several plant responses ranging from inhibition of growth and variations in the rate of photosynthesis (Niu *et al*., 1995). Cell differentiation, elongation, and photosynthesis are the basic plant processes that are negatively affected by salinity (Munns *et al.,* 2006). The duration, rate of progression and intensity of salinity influences the magnitude of tree responses, because these factors accelerated several extenuation processes depending upon the species (Ort, 2001). Photosynthesis is a vital plant process by which plant prepares the various sugars for its growth. Reduction in stomata conductance (gs) or inhibition under sever salinity has been evidence in many species (Flexas *et al*., 2004).

Similarly, assimilation rate (A) is also affected negatively under salt stress condition depending on the severity of the salinity (Flexas *et al*., 2007). Many other studies on various species have shown contrasting physiological responses under salt stress. Such variations in the stomata conductance (gs) and assimilation rates (A) within the species may provide an opportunity to select better adapted clones without conducting extensive field trials and glass house (Niknam and McComb, 2000). Therefore, understanding plants response to salt stress can play a vital role in stabilizing plant growth performance and sustaining productivity under saline conditions. *Eucalyptus camaldulensis* trees can be found in saline areas of Faisalabad, Pakistan, and studies have shown that the fully mature trees have the ability to tolerate salt stress (Mahmood et al*.,* 1996). It has been observed that the fully mature individual can survive in soils with EC up to 30 dSm^{-1} conductance (309mM NaCl) which has been attributed to a higher accumulation of NaCl in plant tissues as compared to other tree species. Khan *et al*.,1999 found that dry and fresh weight of shoots and roots were highest for the individuals growing at 90mM NaCl, but growth was reduced at higher salinities and all the individuals died at 360Mm NaCl. In the above mentioned studies, trees growth performance was examined under the saline conditions and DBH (diameter at breast height) was used to assess the response of species to salinity. However studies evidencing effect of salinity on young individuals are scarce. Such studies are important to evaluate the survival and production potential of *Eucalyptus* as plant growth and survival is most vulnerable to salt stress during early stages of plant growth.

Major objective of the planting on NaCl affected land uptake the salt and lower the ground water table. Therefore, capability of species to use water through the mechanism of transpiration and distribution of root growth in the soil profile, both horizontally and vertically, is vital. It is also vital that selected plant species that are grown in the saline soils will provide fuel wood and timber wood and would be of immense advantage to the timber suppliers as well as fuel wood supplies for the indigenous people (Van der Moezel *et al*., 1991). In contrast to the previous studies focusing on tree morphological parameters such as species leaf area and leaf size in this study, we will enlarge the spectrum of response variables and focus will be to isolate variable that can be used as an index to determine the salt tolerance i.e. physiological parameters and other plant growth parameters.

MATERIAL AND METHOD

In this study, we studied the effect of salinity on seedling and plant physiology in relation to salt stress of *Eucalyptus camaldulensis* at the Research Area, Department of Forestry, University of Agriculture, Faisalabad. Earthen pots were filled with soil mixture composed of (peat/sand, 2v/1v). In control (C) pots initial EC was at 2.2dS m^{-1} and no additional salt was added. For high salinity treatments EC was adjusted by adding appropriate amount of NaCl. Saline condition in each pot was maintained on weight basis by checking EC at the beginning of the experiment using EC meter. In moderate (MSS) and high salt stress (HSS) treatment EC were maintained at 8 dS m^{-1} and 16 dS m^{-1} respectively. For doing so, 3.160g and 7.37g of salt was added to 3Kg of soil on weight basis and to adjust soil EC to 8 dS m^{-1} and 16 dS m^{-1} respectively. Commercial salt (NaCl) were used to control the EC in each pot to study effect of salinity at on plant growth.

Salinity effect on plant growth

Plant growth and physiology was measured on two-month-old seedlings already left to grow in control pots under natural environmental conditions of the University of Agriculture Faisalabad. Prior to the application of salt stress, 30 individuals of uniform stature were selected and were transplanted in the pre-prepared pots with EC at 2, 8,dS m^{-1} and 16 dS m^{-1} representing control (C), moderate salt stress (MSS) and high salt stress (HSS) treatment respectively. Individuals were randomly distributed to minimize position effects within the green house. Total duration of the experiment was four months.

Table 1a. Analysis of variance for effect of salt stress on plant height (cm) of Eucalyptus

SOV	DF	SS	MS	F-value	P-value
Intercept	1	61551.8	61551.8	843.6	0.001
Treatment	2	298.9	149.5	2.04	0.148
Error	27	1970.0	72.96		

Table 1b. Comparison of treatment means for plant height (cm) of Eucalyptus

Treatment	Plant height of Mean	Standard error
Control	48.1	40.8
Moderate Salt Stress	46.8	3.48
High Salt Stress	40.8	1.79

$P < 0.001$ highly significant $P = 0.148$ non significant

Growth measurements

One month after the onset of salinity stress following growth parameters was measured on all the individuals present in all the three treatments.

Plant height (cm)

Stem was marked at collar with a permanent marker and each time, stem height was measured from this mark up to the plant tip using a measuring tape in centimetre. Measurements were taken after an interval of three days.

Stem diameter (mm)

Stem diameter was measured at the collar (marked previously for all the individuals), using the digital vernier caliper. All measurements were taken after an interval of three days throughout the duration of the experiment.

Number of leaves

Number of leaves was manually counted from each individual throughout all the treatment carefully. Mark the first leaves with permanent marker, and then repeated this activity with regular interval of three days.

Gas exchange measurements

For each individual in both treatments, leaf gas exchange measurements was performed on a preselected, fully developed, and well-lit leaf using a leaf gas exchange system under natural condition. The CO_2 concentration at the inlet will be adjusted at 380µmol mol^{-1} and block temperature will be set 27°C. CO_2 assimilation rate (A, $\mu mol\ m^{-2}\ s^{-1}$), stomata conductance (g_s, $mol\ m^{-2}\ s^{-1}$), and transpiration rate (E, $mol\ m^{-2}\ s^{-1}$) was measured under natural irradiance for all the individuals in all the treatments.

Statistical analysis

The data collected for various characteristics was subjected to Analysis of Variance and the means obtained was compared by LSD at 5% level of significance (Steel et al., 1997). The samples were analyzed in the laboratory and statistical analysis of the data was performed using Completely Randomized Block Design (CRBD).

RESULT AND DISCUSSION

The study was conducted to evaluate the effects of salinity on seedling and plant physiology of *Eucalyptus camaldulensis.* Plant growth and physiology was measured on two-month-old seedlings already left to grow in control pots under natural environmental conditions of the University of Agriculture Faisalabad. Prior to the application of salt stress, 30 individuals of uniform stature were selected and were transplanted in the pre-prepared pots with EC at 2, 8,dS m^{-1} and 16 dS m^{-1} representing control (C), moderate salt stress (MSS) and high salt stress (HSS) treatment respectively. Individuals were randomly distributed to minimize position effects within the green house. Total duration of the experiment was four months. There are following different parameters showing significant results about saline soil.

Plant height (cm)

The ANOVA Table 1(a) shows that treatment effect was not significantly different from each other (P=0.143). Mean plant height along with SE is represented in Table 1(b). Maximum plant height was recorded in control condition (48.1cm) and minimum was recorded for

Table 2a. Analysis of variance for effect of salt stress on stem diameter (mm) of Eucalyptus

SOV	DF	SS	MS	F-value	P-value
Intercept	1	132.7	132.7	396.1	0.001
Treatment	2	0.29	0.14	0.43	0.649
Error	27	9.05	0.33		

Table 2b. Comparison of treatment means for stem diameter (mm) of Eucalyptus

Treatment	Stem Diameter Mean	Standard error
Control	2.10	0.16
Moderate Salt Stress	2.22	0.17
High Salt stress	1.98	0.20

$P < 0.001$ highly significant $P = 0.649$ non significant

Table 3a. Analysis of variance for effect of salt stress on number of leaves (g) of Eucalyptus

SOV	DF	SS	MS	F-value	P-value
Intercept	1	15686.5	15686.5	339.5	0.001
Treatment	2	440.2	220.1	4.76	0.001
Error	27	1247.2	46.1		

Table 3b. Comparison of treatment means for number of leaves (g) of Eucalyptus

Treatment	Number of leaves Mean	Standard error
Control	26.6	2.40
Moderate Salt Stress	17.6	1.89
High Salt stress	24.4	2.11

$P < 0.001$ highly significant $P < 0.01$ highly significant

individuals in moderate saline condition (46.8cm) and individuals under remained high saline condition at (40.8cm) in Table 1(b). Maximum reduction in plant height was evidenced under high saline condition which was 15.0% and under moderate saline reduction in plant height remained at 12.7% as compare to control condition in Table 1(b). Similar increase in plant height under control condition and progressively decrease with increasing salt concentration was evidenced in *Eucalyptus camaldulensis* species (Qureshi *et al.,* 1993). Reduction in plant height has been demonstrated due to exerted loss of water potential due to excessive ex-osmosis due to the high salt concentration present in soil (Saqib *et al*., 2005).

Stem diameter (mm)

The ANOVA table 1(a) shows that treatment effect was not significantly different from each other (P=0.649). Mean stem diameter along with SE is represented in Table 2(b). Maximum stem diameter was recorded in moderate saline condition (2.22mm) and minimum was recorded for individuals in control condition (2.10mm) and individuals under remained high saline condition at (1.98mm) in table 2(b). Maximum reduction in stem diameter was evidenced under high saline condition which was 10.8% and under control condition reduction in stem diameter remained at 5.40% as compare to moderate saline condition in table 2(b). Similar decrease in stem diameter under saline condition stimulates in moderate saline condition and high growth stem diameter in control condition was evidenced in *Dalbergia sisso* species (Rawat and Banerjee, 1998). Reduction in stem diameter has been demonstrated due to exerted loss of water potential due to excessive ex-osmosis due to the high salt concentration present in soil (Saqib *et al*., 2005).

Number of leaves

Maximum number of leaves was recorded in control condition (26.6) and minimum was recorded for individuals in high saline condition (24.4) and individuals under moderate saline condition remained at (17.6) in Table 3(b). Maximum reduction in the number of leaves was evidenced under moderate saline condition which was 33.8% and under high saline reduction in the number of leaves remained at 8.27% as compare to control condition in Table 3(b). Similar decrease in the number of leaves under saline condition and increase the number of leaves when salt concentration decrease was evidenced in *Eucalyptus camaldulensis* species (Ali *et al*., 2006). Reduction in the number of leaves has been demonstrated due to exerted loss of water potential due to excessive ex-osmosis due to the high salt concentration present in soil (Saqib *et al*., 2005).

CONCLUSION

The present research work was conducted to evaluate potential adaption in *Eucalyptus camaldulensis* against salt stress in a part trail. Various treatment Consisting of the application of 2, 8 and 16 dSm^{-1} at different time interval were applied to individuals data was collected during and at the termination of experiment and was subjected to analyze using statistical analysis. As of treatment application variation in the performance was exhibited as the m value of the studied traits varied significantly with some time highly significantly. Most significantly, data regarding growth parameter after analysis showed variation in their performance the effect of all the treatments on the plant height was not significant. The plant height was maximizing in control condition and when the plant height gradually decreased when the salt stress level increased the stomata conductance was decreased. Stem diameter progressively growth in moderate saline condition maximum in control condition and decreased when the saline stress increased. It was also clear that the number of leaves and the number of branches increased in normal condition and reduced growth in saline condition lengths also varied drastically against the influence of salt stress it was exhibited from the results that minimum root length was produced when maximum stress level. As the same process of salt stress interval increased the shoot fresh weight dramatically decreased leading to minimum value for the shoot fresh weight. The stomata conductance is also increased when maximum saline concentration applied. The chlorophyll content is reduced when the maximum saline condition applied. It is also cleared that among all the treatment the growth parameter is reduced when maximum salt concentration applied and some time growth parameter adapted, few species that have salt tolerance maximum in saline condition *Eucalyptus camaldulensis*. The various growth parameters were measured during the experiment and Assimilation rate, the transpiration rate and stomata conductance were measuring by suing IGRA at the end of experiment. Results showed that biomass production (Plant height, stem diameter, numbers of leaves and branches, chlorophyll contents, fresh and dry weight of leaves, stem, roots) and physiological parameters (Assimilation rate, transpiration rate and stomata conductance) and moisture contents and Biomass allocation percentage were highly affected under high saline soil. Therefore, it can be concluded that *Eucalyptus camaldulenis* perform better under control and medium saline condition but also tolerate high saline.

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