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Glycine Betaine and Proline Production in *Eucalyptus* Plant under NaCl Harassing Environment

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ABSTRACT

An investigation has been carried out to study the production of Proline and Betaine by applying Abscisic acid (ABA) treatment under NaCl and water stressed conditions. The seeds of four provenances of Eucalyptus camaldulesnis were obtained from the University of Agriculture, Faisalabad (Provenance I), Punjab Forest Research Institute, Faisalabad (Provenance II), Bio-saline Research Station-I, Lahore (Provenance III) and Bio-saline Research Station-II of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad (Provenance 1V). It was observed that Proline and Betaine accumulation increased significantly in all the provenances with increase in drought or salt stress, ABA alone and in combination with drought. Provenance II and III species remained successful in maintaining higher Proline and Betaine accumulation as compared to Provenances I and IV. From the results it can be concluded that ABA treatment remains successful in enhancing Proline and Betaine production and maintaining the physiological parameters necessary to enhance plant growth both under salt and in combination with drought condition.

1. Introduction

Eucalyptus camaldulensis (Dehn.) plantation is widespread throughout Pakistan from hilly to marine climate and work on natural production of osmolytes in plant is increasing day by day. Glycine Betaine and Proline production in Eucalyptus under saline and drought conditions is vital in regulating nutrients movement. Glycine Betaine and Proline are for the most part vital organic osmolytes that increase in numerous plant species as a result to environmental stresses such as salt stress, drought and severe temperatures, and heavy metals. Not all plant geneses are capable of normal production or accretion of these osmolytes in reply to stress. Eucalyptus camaldulensis is thought to be a producer of Proline and Betaine that involves in enzymatic bustle and membrane stability along with adaptive contribution in arbitrating osmotic tuning in plants grown under stressful environments. It has been estimated that about **e**very year 120 million tonnes of salts are added to Pakistani land and **o**nly about 20% of this salt finds its ways to the sea [1]. The leftover accumulates in soils and causes continuous decrease in the development and endurance of crops. Almost 10% of the entire land area is covered with different types of salt-affected soils. At present, there are nearly 954 million hectares of excessive soluble salts containing soils on the earth's surface which are scattered all over the world. More than 80 million hectares of such soils are in Africa, 50 million hectares in Europe, 357 million hectares in Australasia, nearly 147 million hectares in Central, North and South America. In the same way, a large bulk of about 320 million hectares land in South and South East Asia is under the grasp of excessive soluble salts [2]. In Pakistan, about 6.30 million hectares of land are salt-affected of which 1.89 hectare is saline having electrical conductivity $\geq$ 4 dS m^{-1} (deci Siemen per meter) and pH from 7.5 to 8.5. Around 1.85 million hectare is permeable saline-sodic, 1.02 million hectare is impermeable saline-sodic having electrical conductivity > 4 dS m^{-1} and pH 8 ± 0.5 and 0.028 million hectare is sodic having electrical conductivity < 4 dS m^{-1} and pH >8.5 in nature. Out of these saline soils (1.89 million hectares), 0.45 million hectares are located in Punjab, 0.94 million hectares in Sindh and 0.5 million hectares in Khyber Pakhtonkhwa provinces. The enormity of the dilemma can be estimated from the fact that the area of productive land is being damaged, annually by salt accumulation at a rate of about 40000 hectares [1].

The increasing global demand for timber, fuel wood and its raw materials necessitates future studies to optimize utilization of soil resources efficiently and urgently. These soils have low productivity for agricultural crops. It appears that considerable scope

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exists to grow trees on adverse land both under dry and saturated conditions adapting tree based strategies to productively utilize salt containing waste lands [3]. This could help rural economics both directly, through the economically valuable products provided to the local people (specially firewood and fiber), and indirectly by helping initiate or advance the land's environmental rehabilitation and sustainable agro-redevelopment of environmental ecosystem in need of innovative attention and care [4].

Eucalyptus camaldulensis has been identified as a tree species tolerant to salinity and water logging, and has more than 85% survival rate under saline soil conditions [3]. Hence, it is the most successful tree species under a variety of saline conditions [3, 4]. *Eucalyptus* has been planted successfully under a variety of ecological conditions of Pakistan and its survival in nutrient solution up to 50 dSm^{-1} in aerobic conditions [5-7] and up to 4 dSm^{-1} in both aerobic and water logged conditions [3, 7, 8] have been reported. In an adaptation trial near Faisalabad, *Eucalyptus camaldulensis* performed well in saline soil for over seven and half years [3, 4]. Literature is available regarding the salinity and water logging effects on *Eucalyptus camaldulensis* [5, 7, 8]. However, comparatively little information is available on the combined effect of salinity and drought in spite of frequent and simultaneous occurrence of these two stresses in arid and semi arid regions [8, 9]. Salt tolerant species significantly adapt to salinity by Proline accumulation [10-13], and the ability of salt-tolerance depends on salt concentration and plant species [14, 12].

Abscisic acid is an important phytohormone regulating seed dormancy, germination, seedling growth, and plant transpiration [15]. In many salt-stressed plants abscisic acid seems to be involved with the formation of compatible solutes and the ability of salt tolerance depends on salts concentration, plant species and plant variety [11, 12].

Keeping in view the importance of utilization of salt-affected land, the current project has been conducted to study the effects of abscisic acid on the production of Glycine Betaine and Proline in this plant. Different provenances of *Eucalyptus camaldulensis* were selected on the basis of their growth performance under saline conditions. The research work was aimed to identify biochemical markers for salt tolerance in *Eucalyptus.*

2. Material and Methods

2.1 Experimental Site/ Soil Collection:

The work presented in this manuscript has been carried out in green houses of Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The soil used in the experiment was collected from the Biosaline Research Station-II of NIAB located at Pacca Anna, Faisalabad.

2.2 Germplasm Collection

Different seed lines or accession of Eucalyptus camaldulensis (Dehn.) were obtained from the University of Agriculture, Faisalabad (Provenance I), Punjab Forest Research Institute, Faisalabad (Provenance II), Bio-saline Research Station-I, Lahore (Provenance III) and Bio-saline Research Station-II, NIAB, Faisalabad (Provenance 1V).

2.3 Raising of Plant Nursery

The nursery of the Eucalyptus seeds was initially raised in oval shaped plastic tubs (15x30 cm size) filled with sand. These saplings were then transferred to plastic bags filled with soil and allowed to grow for further 12 weeks (3 months).

2.4 Treatments

Treatments applied to plants are mentioned in Table 1. The roots of the seedlings (120 days old) were dipped in ABA solution (10-6 M) for about 2 h. Plastic pots (10x12 cm) were filled with 4 kg soil. Proline concentrations (µmole/g of fresh weight) of upper 3rd leaves of *E. camaldulensis* were measured 10 days before harvesting (110 days after ABA application to roots) and drought stress conditions were continued up to the end of experiment. Moisture levels were maintained by watering every 2nd day. The experiment was continued for 4 month.

Table 1. Treatments applied to the *E. camaldulensis*

Treatments	Abbreviated as/ Symbol
Control	T1
ABA (Abscisic acid)	T2
Salt (20 dS m^{-1} with NaCl)	T3
ABA+ salt	T4
100% F.C (Field capacity)	T5
ABA+100% F.C	T6
60% F.C	T7
ABA+ 60% F.C	T8
Salt + ABA+100% F.C	T9
Salt + ABA+ 60% F.C	T10

The analyses were performed according to the methods described by U. S. Salinity Laboratory Staff [16], unless otherwise mentioned. The pH and conductivity of the soil samples were obtained using a pH meter (8520, Hanna) and electrical conductivity meter (LF 538, WTW, Germany) respectively. All the reagents and chemicals used were from reliable sources and of high analytical grades. Salinity (20 dS m^{-1}) was induced in soil using NaCl which was mixed well using soil mixer. Experimental soil was characterized with pH of saturated soil paste and electrical conductivity of saturated soil extract. Proline content of leaves was estimated according to Bates method [17] and Betaine with method given by Grieve and Gratan [18].

Table 2. Proline concentration (µmole/g of fresh weight) of upper 3rd leaves of *E.camaldulensis*.

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	0.74	1.16	1.08	0.62	0.90g
T2	1.04	1.25	1.26	0.96	1.10f
T3	6.75	9.33	8.79	5.43	7.58d
T4	7.62	10.05	10.58	6.21	8.62b
T5	0.72	0.94	1.05	0.63	0.84g
T6	1.01	1.21	1.22	0.97	1.10f
T7	5.91	8.79	7.76	5.66	7.03e
T8	6.81	9.07	9.22	6.91	8.00c
T9	6.52	8.87	8.67	6.44	7.62d
T10	7.80	11.62	10.87	8.51	9.70a
Mean	4.49c	6.23a	6.05b	4.23d	

S.E for T=0.024 and for P=0.015 Co-efficient of variation=1.58%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

Table 3. Betaine concentration (µmole/g of fresh weight) of upper 3rd leaves of *E.camaldulensis.*

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	0.80	0.98	0.90	1.50	1.04h
T2	1.24	1.40	1.52	1.22	1.34g
T3	10.25	17.32	16.80	11.25	13.91d
T4	11.75	18.65	18.42	11.75	15.1b
T5	0.81	0.96	0.85	0.93	1.03h
T6	1.24	1.50	1.50	1.17	1.35g
T7	9.54	15.50	16.20	8.68	12.48f
T8	10.44	16.80	16.55	10.20	13.50f
T9	12.12	17.30	18.21	11.80	14.86c
T10	13.44	19.26	18.75	12.11	15.89a
Mean	7.16b	10.97a	10.97a	7.06c	

S.E for T=0.03 and for P=0.019 Co-efficient of variation=1.14%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

Transpiration and stomatal resistance were measured with the help of porometer (LI-1600, Steady State Porometer, LI-COR, Inc., USA) and water use efficiency was determined according to method given by Lindroth et al., [19]. Plant shoots were kept in oven at 70 °C till attaining constant weight. The dried plant samples were then weighed and measurements recorded .The data obtained were subjected to analysis of variance and mean values were compared using Duncan's New Multiple Range Test [20].

3. Results and Discussion

3.1 Proline Concentration of Leaves

The Proline concentration of leaves was obtained for all samples and is given in Table 2. From this table it can be seen that Proline concentration was influenced significantly by ABA, NaCl and drought and its accumulation increased in all the provenances with augmentation in drought stress, salt stress, ABA alone and in combination with drought. Provenance II and III had the highest accumulation while Proline concentration was lowest in provenance IV. Among the treatments T10 (salt+ABA+60% F.C) had maximum Proline accumulation followed by T4 (ABA+ salt), while it was lowest in T2 (ABA) and T5 (100% F.C). The results showed that ABA increased Proline accumulation significantly both under and in combination with stresses of salt and drought.

3.2. Betaine Concentration of Leaves

The Betaine concentrations of leaves were obtained for all samples studied and are given in Table 3. From this table it can be seen that Betaine was maximum in T10 (salt+ABA+60% F.C), while T1 (control) and T5 (100% F.C) showed the lowest accumulation. The means for the provenances also differ significantly (at P=0.05). The maximum Betaine accumulation was recorded in provenances II and III and minimum in provenance IV. The provenances means shows that ABA had stimulatory effect only under stresses that is why the accumulation of Betaine was highest in T10 (salt+ABA+60% F.C) followed by T4 (ABA+ salt), while it was lowest in T2 (ABA) as compared to control. The interaction mean indicates that Betaine accumulated significantly in all the provenances with the application of either salt or drought while ABA further enhanced its accumulation.

3.3 Correlation between Proline and Betaine concentrations

Proline and Betaine increased in all the plant provenances with the addition of salt and in drought conditions. ABA has a significant role in increasing the accumulation of both organic solutes in all the plant provenances. Maximum accumulation of Proline and Betaine was recorded in provenances II and III plants at combined stress of salt + ABA+ 60% FC (T10). Under all the treatments provenances II and III plants maintained higher Proline and Betaine levels. Proline and Betaine amounts were enhanced under salt stress [21]. The increase in Proline is considered to be an index to stress tolerance and reports indicate that plants with higher Proline and Betaine concentrations show better osmotic adjustment in adverse conditions [21, 22]. As provenances II and III plants have higher accumulation of Proline and Betaine they adjust themselves osmotically in severe environmental conditions and maintain higher plant growth [23]. Similar findings have also been reported [24, 8, 25, 14, 12]. The Proline and Betaine concentrations obtained are plotted in Figure 1. This figure shows that Proline and Betaine concentrations correlate for all provenances and for all treatments confirming that the same mechanism produces both these species.

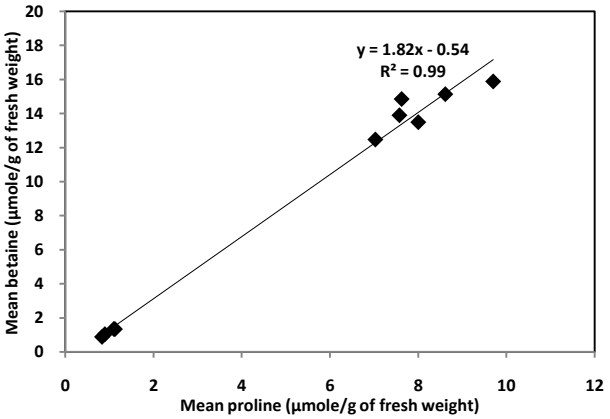

Fig. 1: Correlation between Proline and Betaine concentrations

3.4. Augmenting Parameters

3.4.1 Shoot length

The shoot lengths for the samples studied are given in Table 4. From this table it is clear that ABA has no marked effect on shoot length but salt treatment has significantly increased the shoot length and ABA application made simultaneously with salt also resulted in decrease in shoot length. At 100% field capacity (FC), ABA has no effect but at 60% FC, shoot length was more than the control following ABA application. The inhibitory effect of ABA was pronounced in the presence of salt at 60% of F.C. Provenances III and IV plants showed marked increase in shoot length following different treatments whereas, provenances I and II plants showed lesser increase in shoot length. Shoot length is highest for provenance III and T3 treatment while it is lowest for provenance I and treatment T7.

ABA allows plants to continue to grow and reproduce during periods of low water availability or to survive and recover from dehydration, but may also have an impact on crop yield in the context of agriculture [26]. The observed increase in shoot length due to salt treatment at both phases (120 days after treatment and at harvest stage) could be attributed to the stimulatory effect of salt at low concentration on cell elongation [21]. The reduction in shoot length under drought stress has previously been reported [27, 28] and could be attributed to the reduction in cell division and cell elongation.

Noteworthy, ABA applied alone does not exhibit any significant effect but in combined treatment with drought it nullifies the inhibitory effect of drought at both stages. Perhaps feedback regulation of ABA metabolism operates and drought induced ABA synthesis is suppressed counteracting the effect of applied ABA followed by the simultaneous increase in growth promoting hormones under ABA and drought treatments [29]. ABA decreased the promotory effect of salt when applied simultaneously. Perhaps endogenous ABA production was increased which enhances the effect of ABA under salt stress [27].

3.4.2 Shoot fresh weight

Shoot fresh weights for the samples studied were obtained and are given in Table 5. The shoot fresh weight decreased due to ABA treatment but was greater than control after salt addition. ABA used in the presence of salt further augmented the stimulatory effect of salt. ABA was stimulatory at 60% FC but was inhibitory at 100% FC. In the presence of salt, ABA significantly increased the fresh weight of shoot as compared to control at 100% FC and ABA partially overcame the inhibitory effect of drought on shoot. Provenances III and IV plants showed marked increase in shoot fresh weight following different treatments whereas, provenances I and II plants showed lesser increase in shoot fresh weight. Shoot fresh weight is highest for provenance III and T9 and T4 treatments while it is lowest for provenance I and treatment T7.

3.4.3 Shoot dry weight

Shoot dry weights for the samples studied were also obtained and are given in Table 6. It shows that shoot dry weight also decreased in ABA treatment but salt treatment has stimulatory effect. ABA in the presence of salt has minimized the stimulatory effect of salt when used alone. ABA was effective only at 60% FC but at 100% FC it was inhibitory. In the presence of salt, ABA was stimulatory at 100% F.C. Shoot dry weight is highest for provenance IV and T3 treatment while it is lowest for provenance II and treatment T7.

Table 4. Shoot length (cm) of *E. camaldulensis* measured at harvesting time

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	83	78	131	64	89.08b
T2	62	85	105	105	89.41b
T3	84	94	102	102	95.50a
T4	88	86	101	76	87.75b
T5	83	78	131	64	89.08b
T6	62	85	105	105	89.41b
T7	67	85	96	72	80.16d
T8	63	101	95	101	89.75b
T9	88	86	101	76	87.75b
T10	98	64	109	62	83.41c
Mean	77.87c	84.10b	107.67a	82.90b	

S.E for T= 0.71 and for P=1.42 Co-efficient of variation=2.79%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

Table 5. Effect of salinity, ABA, and drought stress on shoot fresh weight (g) of *E. camaldulensis* at the time of harvesting

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	28	13	55	31	31.96c
T2	24	35	30	28	29.37d
T3	35	33	40	41	37.16b
T4	26	27	51	55	40.01a
T5	24	35	30	28	31.96c
T6	24	35	30	28	29.37d
T7	31	23	28	20	25.51e
T8	30	29	32	37	32.14c
T9	26	27	51	55	40.01a
T10	41	17	47	13	29.47d
Mean	29.46e	25.69d	42.63a	33.99b	

S.E for T= 0.57 for P=0.36 Co-efficient of variation=6.02%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

Table 6. Effect of salinity, ABA, and drought stress on shoot dry weight (g) of *E. camaldulensis.*

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	13	5	24	14	14.03c
T2	8	16	15	13	12.73d
T3	14	15	21	17	17.09a
T4	10	12	21	19	15.66b
T5	13	5	24	14	14.03c
T6	8	16	15	13	12.73d
T7	12	11	12	9	11.08f
T8	7	13	15	16	13.08d
T9	10	12	21	19	15.66b
T10	18	7	21	2	12.00e
Mean	11.47c	11.82d	19.70b	13.67a	

S.E for T= 0.15 for P=0.09 Co-efficient of variation=3.67%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

3.4.4 Root fresh weight

Root fresh weights for the samples studied were obtained and are given in Table 7. Root fresh weight decreased due to ABA treatment but was higher in salt treatment. ABA in combination with salt maintained increase in root fresh weight. At 100% FC, ABA was inhibitory. At 60% FC, ABA overcame the marked reduction in root fresh weight as compared to that of ABA application made alone. Salt treatment showed stimulatory effect both at 100% FC and at 60% F.C but the magnitude of stimulation was greater at 100% FC. Root fresh weight is highest for provenance III and T4, T3 and T9 treatments while it is lowest for provenance II and treatment T7.

Table 7: Root fresh weight (g) of *E. camaldulensis* measured at harvesting stage

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	8	4	39	9	15.27b
T2	9	14	17	6	11.82e
T3	18	19	21	19	18.95a
T4	22	4	26	24	18.74a
T5	8	4	39	9	15.27b
T6	9	14	17	6	11.82e
T7	13	10	8	5	9.18f
T8	16	10	17	8	12.9d
T9	22	4	26	24	18.74a
T10	24	7	22	6	14.57c
Mean	14.94b	9.18d	23.27a	11.69c	

S.E for T= 0.71 and for P=0.44 Co-efficient of variation=2.79%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

Table 8. Effect of salinity, ABA, and drought stress on root dry weight (g)

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	17.01	0.61	15.57	0.59	8.44a
T2	16.71	5.49	6.13	2.10	7.60c
T3	6.12	6.70	7.53	7.08	6.95d
T4	8.03	1.00	9.16	9.35	6.88d
T5	17.01	0.61	15.57	0.59	8.44a
T6	16.71	5.49	6.13	2.10	7.60c
T7	17.06	3.93	2.10	1.44	6.13e
T8	18.91	3.42	6.80	2.76	7.97b
T9	8.03	1.00	9.16	9.35	6.88d
T10	9.72	1.27	8.36	1.54	5.22f
Mean	13.53a	2.95d	8.65b	3.69c	

S.E for T= 0.07 and for P=0.04 Co-efficient of variation=3.58%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

3.4.5 Root dry weight

Root dry weights for the samples studied are given in Table 8. Root dry weight decreased both due to salt and ABA either used alone or in combination. ABA alone was stimulatory and overcame the inhibitory effect of 60% FC but the value was still less than the control but in the presence of salt the fresh weight was markedly lower both at 60% and at 100% FC, though the magnitude of inhibition was greater at 60% FC. Root dry weight is highest for provenance I and T1 and T5 treatments while it is lowest for provenance II and treatments T10 and T7.

3.4.6 Shoot and root weight analysis

In the presence of salt, ABA was not inhibitory to shoot fresh weight or dry weight. Combination of two stresses, i.e., salt and drought had not modulated ABA effect on shoot fresh and dry weight for ABA applied, simultaneously. ABA-induced decrease in shoot dry weight was greater than that of root dry weight, when ABA was used alone but in the presence of drought conditions the root dry weight further decreased as compared to drought or when ABA treatment was made alone. This may possibly be due to the endogenous production of ABA in roots under drought stress, which have added to the effect of ABA and have inhibited the dry matter accumulation and translocation [27, 30, 31].

The observed decrease in root fresh weight as well as dry weight and shoot fresh weight and dry weight following ABA application as well as drought treatment may be a consequence of ABA inhibition of cell division, cell expansion as well as inhibition of water and nutrient uptake as has previously been reported [31-33]. In Figures 2 and 3 the fresh and dry weights of shoots and roots are plotted respectively. From these figure it can be seen that the drying process does not contribute to the shoot weight while it does for the root weight.

Addition of ABA has no significant effect on the response of salt to plants but under the combined stress of both salt and drought conditions ABA increased the root fresh weight but decreased the root dry weight. There were significant differences among the provenances as well in ABA response. There was marked reduction in shoot dry weight of provenance I plants due to ABA and ABA+ drought but the root dry weight was less affected.

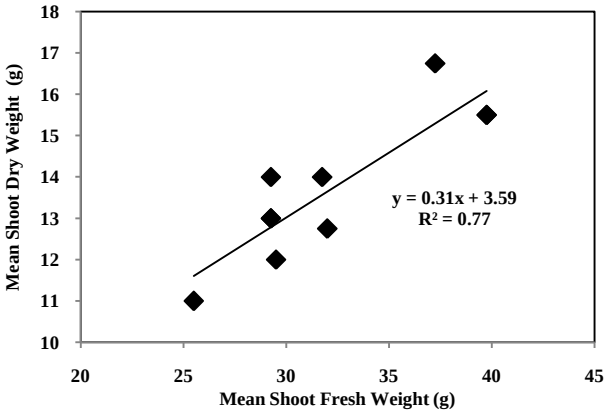

Fig. 2: Correlation between shoot fresh to dry weights

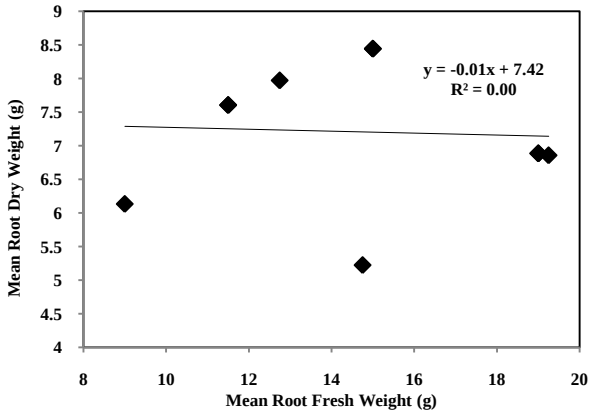

Fig. 3: Correlation between root fresh to dry weights

The provenance II plants showed marked increase both in root and shoot dry weight following different treatments whereas, provenances III and IV plants showed lower decrease in shoot dry weight but their behavior was not consistent with respect to root dry weight under drought stress inhibited dry matter accumulation [31, 34].

3.5 Transpiration (μg cm^{-2} s^{-1})

Transpiration was measured with the help of porometer (LI-1600, Steady State Porometer, LI-COR, Inc, USA) and the data obtained are given in Table 9. Transpiration was significantly influenced by NaCl, ABA and drought stresses. Maximum transpiration was recorded in the control and was lowest in T10 under the combined treatment of salt, drought and ABA. Transpiration is highest for provenance II and T5 treatment while it is lowest for provenance I and treatment T10. The differences between T1 (control) and T5 (100% FC) were not significant and all other treatments differed significantly. In T4 (ABA+ salt) and T9 (salt +ABA+ 100% FC) transpiration was reduced and provenances differed significantly.

Table 9. Transpiration (μg cm^{-2} s^{-1}) of upper 3rd leaves of *E. camaldulensis*

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	5.7	4.9	5.6	7	5.77a
T2	3.5	4.2	4.4	3.9	3.99c
T3	4.4	3.7	3.7	3.7	3.88d
T4	2	3.6	3	2.2	2.68f
T5	5.7	4.9	5.6	7	5.77a
T6	4.3	4.7	4.5	3.8	4.32b
T7	1.9	3.7	3.2	2.9	2.92e
T8	1.2	3.2	3.0	1.5	2.23g
T9	2.1	3.6	2.6	2.4	2.67f
T10	1.2	2.6	2.3	1.3	1.83h
Mean	3.20d	3.92a	3.77b	3.52c	

S.E for T=0.042 and for P=. 027 Co-efficient of variation=4.03%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

3.6 Stomatal resistance of leaves (cm s^{-1})

Stomatal resistance was measured with the help of a porometer and the data obtained are given in Table 10. Leaf stomatal resistance was significantly affected by NaCl, ABA and drought. Leaf stomatal resistance increased with increase in drought and salt stress. However, application of ABA reduced the leaf stomatal resistance to some extent. But under severe condition it failed to do so. Stomatal resistance is highest for provenance I and T10 treatment while it is lowest for provenance II and treatments T1 and T6. Highest leaf stomatal resistance was observed in T10 (salt+ABA+60% FC) followed by T8 (ABA+ 60% FC) and T4 (ABA+ salt). Minimum leaf stomatal resistance was obtained in T1 (control), T9 (salt +ABA+ 100% F.C), T7 (60% FC), T3 (salt), T2 (ABA) and T6 (ABA+ 100% F.C) Comparing provenances, the lowest leaf stomatal resistance was maintained in provenance II plants while provenance I plants had maximum leaf stomatal resistance. The value for leaf stomatal resistance in all provenances differed significantly. Provenance II plants maintained the lowest leaf stomatal resistance. Measurements (3 leaf stage) were made 10 days before harvesting.

Table 10. Stomatal resistance (s cm^{-1}) of upper 3rd leaves of *E.camaldulensis*

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	5.9	6.8	6.1	4.3	5.78i
T2	10.8	7.2	8.5	9.5	8.98g
T3	6.6	11	10.8	10.0	9.59f
T4	15.8	10.6	12.2	17.8	14.10e
T5	5.9	6.8	6.4	4.2	5.79i
T6	10.7	7.6	7.3	10.2	8.75h
T7	17.1	10.5	12.4	17.3	14.32d
T8	19.4	12.2	14.6	19.1	16.33b
T9	19.3	11.8	12.1	17.3	15.11c
T10	22.4	16.2	17.5	21.5	19.41a
Mean	13.39a	9.99d	10.78c	13.10b	

S.E for T=0.065 and for P=. 041 Co-efficient of variation=1.90%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

Stomatal resistance (Table 10) increased for all 9 treatments used. It is evident from the present investigation that plants from provenances I and IV had less while provenances II and III had higher stomatal resistance. The responses of provenances I and II plants were greater with ABA and drought and showed much higher increase in stomatal resistance as compared to provenances II and III. Under drought environment the wheat variety (Sarsabz) maintained higher stomatal resistance as compared to the variety `Barani 83` and produced higher biomass [35]. However, in provenances II and III the reduction in stomatal resistance was observed under salt stress and more so when ABA used under the combined stress of salt and drought. Drought and salt were reported to decrease stomatal conductance and transpiration by many scientists under combined stress of salt and drought conditions [36, 33, 37, 38].

The higher stomatal resistance in provenances II and III plants are reflected in their lower rate of transpiration (Table 9). The interplay between hydraulic and non hydraulic signals from root to shoot has appeared in several investigations [39, 40, 41, 31]. It is noteworthy that the tolerant provenances II and III plants showed further increase in stomatal resistance in response to salt stress but decrease in transpiration in response to salt stress. The magnitude of increase in stomatal resistance and decrease in transpiration was less in response to ABA treatment in provenances II and III plants indicating their relative tolerance. Transpiration and stomatal resistance are plotted against each other in Figure 4. These parameters anti-correlate as the increase in one results in the decrease of the other.

3. 7 Water use efficiency(WUE)

Water use efficiency (WUE) was determined according to method given by Lindroth [19] and given in Table 11. WUE of the plants significantly decreased due to NaCl and ABA addition and drought conditions. WUE is highest for provenance II and T2, T6 and T8 treatments while it is lowest for the remaining provenances and treatments T3 and T4. Maximum (0.87 g kg^{-1}) WUE was observed in T2 (ABA), T6 (ABA+100%F.C) and T8 (ABA+drought). ABA treatment enhanced WUE alone and under drought condition but it failed to do so under saline condition. Minimum WUE was recorded in T4 (ABA+salt), T3 (salt) and T9 (ABA+salt+100% F.C). All the treatments varied significantly except that of T3, T4 and T9, which have similar values for WUE. On the whole provenance II plants performed better.

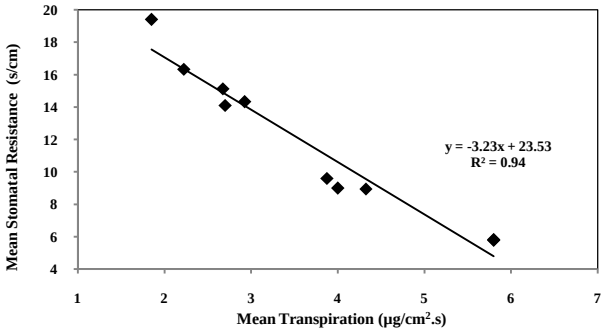

Fig. 4: Correlation between transpiration and stomatal resistance

WUE significantly increased with ABA but drought and salt treatments showed decrease in WUE in all plant provenances. However, maximum WUE was recorded in provenance II plants under ABA addition alone and in combination with drought conditions. Similar results have also appeared in woody crops [42] and in grasses [43]. The soil water and nutrients status can be sensed by roots and communicated to the shoots by changes in the concentration and flux rate of ABA in the xylem sap. Shoots may respond to the signal by a reduction in stomatal transpiration and growth rate [35, 31, 29, 33]. Results clearly indicate that ABA in combination with drought further augmented WUE but under saline conditions ABA failed to do so which may be due to the ion toxicity and osmotic imbalance created by salt stress and osmotic imbalance from ion accumulation in *Atriplex griffithii* [8].

Table 11: Water use efficiency (g kg^{-1}) of plant of *E. camaldulensis*

Treatment No.	Provenance I	Provenance II	Provenance III	Provenance IV	Mean
T1	0.65	0.67	0.62	0.65	0.65b
T2	0.86	0.91	0.86	0.86	0.87a
T3	0.19	0.19	0.17	0.17	0.18e
T4	0.19	0.17	0.15	0.16	0.17e
T5	0.65	0.67	0.67	0.67	0.67b
T6	0.86	0.91	0.86	0.86	0.87a
T7	0.53	0.60	0.57	0.51	0.55c
T8	0.83	0.88	0.87	0.84	0.86a
T9	0.19	0.18	0.16	0.16	0.17e
T10	0.37	0.41	0.38	0.35	0.37d
Mean	0.50b	0.52a	0.49b	0.49b	

S.E for T=0.074 and for P=0.005 Co-efficient of variation=4.48%

The letters in the mean column indicate order of magnitude with a being the highest and g being the lowest. Moreover mean values with same letters are not significantly different at P< 0.05

4. Conclusion:

Glycine Betaine and Proline production in *Eucalyptus* under saline and drought conditions is imperative in regulating nutrients movement. These are important organic osmolytes that can rise in *Eucalyptus* plant species as a result to NaCl and drought stresses. In this study it was found that provenances II and III plants were more successful in maintaining higher Proline and Betaine accumulation than provenances I and IV plants. It can be concluded that salt-affected soil of Pakistan (i.e. about 6 million ha^{-1}) can be utilized by planting *Eucalyptus*, which can enhance its survival more than 85% through production of these osmolytes.

References

[1] S. M. Alam, R. Ansari and M. A. Khan, "Annual Report", Nuclear Institute of Agriculture, Tando Jam, Pakistan, pp. 8-21, 2000.

[2] M.Y. Ashraf, M. Ashraf, K. Mahmood, J. Akhter, F. Hussain and M. Arshad, "Phytoremediation of saline soils for sustainable agricultural productivity", In: Plant Adaptation and Phytoremediation, M. Ashraf, M. Ozturk, and M. S. A. Ahmad, "Phytoremediation of saline soils for sustainable agricultural productivity", Eds., Springer, Berlin, Germany, p. 335–355, 2010.

[3] G.R. Sindhu and R.H. Qureshi, "Salt-affected soils of Pakistan and their utilization", Reclan. Reveg. Res. vol. 5, p. 105-113, 1986.

[4] K.M. Siddiqui, "Tree planting for sustainable use of soil water with special reference to the problem of salinity", Pak. J. Forestry, vol. 44, p. 97-102, 1994.

[5] N. E. Marcar, D.F. Crawford, P.M. Leppert, T. Jovanovic, R. Floyd and R. Farrow, "Trees for salt: A guide for selecting native species for Australia", CISRO Australia, p. 72, 1989.

[6] M.Y. Ashraf, Y. Ali and T.M. "Effect of salinity on photosynthetic efficiency yield of rice genotypes", Pak. J. Biol. Sci. vol. 1, p. 72, 1998.

[7] V.P.G. Moezel, L.E. Watson, G.V.N. Pearce-Pinto and D.T. Bell, "The response of six *Eucalyptus* species *Casuarina obesa* to the combined effect of salinity water-logging", Aust. J. Plant. Physiol. vol. 15, p. 465-474, 1991.

[8] M. A. Khan and I.A. Ungar, "The response of six *Eucalyptus* species *Casuarina obesa* to the combined effect of salinity water-logging", Annals of Bot. vol. 85, p. 225-332, 2000.

[9] I. M. Zeid and N.A. El-Semary, "Response of two differentially drought tolerant varieties of maize to drought stress", Pak .J. Biol. Sci. vol. 4, p. 779-784, 2001.

[10] C. F. De Lacerda, J. Cambraia, M.A. Oliva and H. R. Ruiz, "Changes in growth in solute concentrations in sorghum leaves roots during salt stress recovery", Environ. Expt. Bot. vol. 54, p. 69-76, 2005.

[11] N. Misra and A.K. Gupta, "Effect of salt stress on praline metabolism in two high yielding genotypes of green gram", Plant Sci. vol. 196, p. 331-339, 2005.

[12] M. Nasim, R.H. Qureshi, T. Aziz, M. Saqib, S. Nawaz, S.T. Sahi and S. Pervaiz, "Growth ionic composition of salt stressed *Eucalyptus camaldulensisa* and *Eucalyptus tereticornis*", Pak. J. Bot., vol. 40, p.799-805, 2008.

[13] M.A. Khan, M.U. Sherazi, M.A. Khan, S.M. Mujtaba, E. Islam, S.A. Mumtaz. A. Shereen, R.U. Ansari and M.Y. Ashraf, "Role of Proline", Pak. J. Bot. vol. 41, p. 633-638, 2009.

[14] H. Sixto, J.M. Grau, N. Alba and R. Alia, "Response to sodium chloride in different species clones of genus populus" Forest vol. 78, p. 93-104, 2005.

[15] H. Fuji and J.K. Zhu, "Arabidopsis mutant deficient in 3 abscisic acid-activated protein kinases reveals critical roles in growth, reproduction and stress", PNAS vol. 106, p. 8380-8385, 2009.

[16] U. S. Salinity Laboratory Staff, "Diagnosis and Improvement of Saline and Alkali Soils. U. S. Department of Agriculture", Handbook p. 60, 1954.

[17] L. S. Bates, R.P.Waldern and I.D.Teare, "Rapid determination of free Proline for water stress studies", Plant and Soil, vol. 39, p. 205-207, 1973.

[18] C. M. Grieve and S.R. Gratan, "Rapid assay for determination of water soluble, quaternary ammonium compounds", Plant and Soil, vol. 70, p 303-307, 1983.

[19] A. Lindroth and E. Cienciala, "Water use efficiency of short rotation salix viminalis at leaf, tree and stand scales", Tree Physiology, vol. 16, p. 257-262, 1996.

[20] R.G.D. Steel and J.H. Torrie, "Principles Procedures of Statistics" McGraw Hill Book Co. Inc. New York, 1980.

[21] T. M. Qureshi, M.Y. Ashraf, A. Bano, F. Hussain and M.H. Naqvi, "Physiological responses of *Eucalyptus* under saline environment", In: Abstracts: 9th International Cong. of Soil Science, Nuclear Institute for Agriculture and Biology Faisalabad Pakistan, p. 45-46, 2002.

[22] S. S. M. Naqvi, S. Mumtaz, S. A. Ali, A. Shereen, A.H. Khan, M.Y. Ashraf and A. Khan, "Proline accumulation under salinity stress. Is abscisic acid involved", Acta Physiol. Plant vol. 16, p. 117-122, 1994.

[23] R. H. Qureshi. "Alternative strategies for tackling the soil salinity problem", Dept. of Soil Sci. Univ. of Agric Faisalabad Pakistan, p. 120, 1993.

[24] K. Akilan, R.C.C. Forrell, D.T. Bell and JK.Marshal, "Response of clonal river and gum (*Eucalyptus camaldulensis*) to water logging

by fresh and salt water", Aust. J. Ext Agri. vol. 37, p. 243-248, 1997.

[25] M. Qasim, M. Ashraf, M.Y. Ashraf and S.U. Rehman, "Salt induced changes in two canola cultivars differing in salt tolerance," Biol. Plant, vol.46, p. 629-632, 2003.

[26] A. J. Thompson, J. Andrews, B.J. Mulholland, M.T.J. McKee, H.W. Hilton, J.S. Horridge, G.D. Farquhar, R.C. Smeeton, I.R.A. Smillie, C.R. Black and I.B.Taylor, "Overproduction of abscisic acid in tomato increases transpiration efficiency and root hydraulic conductivity and influences leaf expansion", Plant Physiology vol. 143, p. 1905-1917, 2007.

[27] A. Bano, K. Dorffling, D. Bettin and H. Hahn, "Abscisic Acid and Cytokinins as Possible Root to Shoot Signals in Xylem Sap of Rice Plants and drying Soil", Aust J Plant Physiol. vol. 20, p. 109-115, 1993.

[28] M. A. A. Gadallah, "Absisic acid, temperature and salinity interactions on growth and some mineral elements in *Carthmus* plants," J. Plant Growth Regulation vol. 20, p. 225-236, 1996.

[29] M. Q. Andrea, J.B. Federico, M. Daniela, J.B. Cavagnaro and B. Rubén, "Abscisic acid sprays significantly increase yield per plant in vineyard-grown wine grape (*Vitis vinifera* l.) cv. cabernet sauvignon through increased berry set with no negative effects on anthocyanin content and total polyphenol index of both juice and wine", J. Plant Growth Regulation, vol. 28, p.28, 2009.

[30] N. Chakrabarti and S. Mukherji, "Alleviation of NaCl stress by pretreatment with phytohormones in *Vigna radiata*", Biol. Plant, vol. 46, p. 589-594, 2003.

[31] T. S David, M.I. Ferreira, J. David and S. Pereira, "Transpiration from a mature *Eucalyptus globulus* plantation in Portugal during a spring- summer period of progressively higher water deficit," Oecologia, vol. 110, p. 153-159, 1997.

[32] A. Rohde, S. Kurup and M. Holdsworht, "ABA-GE emerges from the seed," Trends Plant Sci., vol. 5, p. 418-419, 2000.

[33] M. Abou, and A.A.W. Hartung, "Tissue culture of the desert plant, *Anastatica hierochuntica*," Plant Cell Rep. vol. 14, p. 657-661, 1995.

[34] W. J. Davies, S. Wilkinson and B. Loveys, "Stomatal control by chemical signaling the exploitation of this mechanism to increase water use efficiency in agriculture," New Phytol. vol. 153, p. 449-460, 2002.

[35] M.Y. Ashraf and G. Sarwar, "Salt tolerance potential in some members of brassicacea physiological studies on water relation and mineral contents", In: Prospects for Saline Agriculture (eds: Ahmed R and K A Malik) Kluwer Academic Publishers The Netherlands, p. 237-245, 2002.

[36] J.T. Baker and L. H. Allen Jr., "Contrasting crop species responses to CO_2 and temperature: rice, soybean and citrus", Vegetation vol. 104, p. 239-260, 1993.

[37] A. Arshi, M.Z. Abdin and M. Iqbal. "Growth and metabolism of Senna as affected by salt stress", Biol. Plant, vol.45, p. 295-298, 2002.

[38] J. Pospisilova. "Participation of Phytohormones in the stomatal regulation of gas exchange during water stress", Biol. Plant, vol. 46, p. 491-506, 2003.

[39] M. A. Else, K.C. Hall, G.M. Arnold, W.J. Davies and M.B. Jakson, "Export of abscisic acid. 1-amino-1-cabo acid, phosphate, and nitrate from root to shoots of flooded tomato plants- accounting for effects of xylem sap flow-rate on concentration and delivery", J. Plant Physiol., vol 107, p. 377-384, 1995.

[40] W. Tsuji, M. E. K. Ali, S. Inanaga and Y. Sugimoto, "Growth and gas exchange of three sorghum cultivars under drought stress," Biol. Plant, vol.46, p. 583- 587, 2003.

[41] E. V. Mass, "Salinity Citriculture", J. Tree Physiol. vol. 12, p. 195-216, 1993.

[42] R. J. Beale, "Energy crop water nutrient use efficiency", Int Energy Agency Bioenergy Task 17 Short Rotation Crops Research Centre Foulum Tjele Denmark, p. 9-10, 1999

[43] F. Caravaca, E. Diaz, J.M. Barea, "Photosynthetic and transpiration rates of *Olea europea* and *Rhamnus lycioides* as affected by water deficit and mycorrhiza", Biol Plant vol.46, p. 637-639, 2003