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Physiological and biochemical basis of salt tolerance in *Ocimum basilicum* L.

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Globally salinity in the soil is continuously increasing affecting agricultural productivity more than other abiotic factors in arid and semi arid regions and as a result millions of hectares of usable land have now become unsuitable for cultivation. Present study was conducted to test the salt tolerance of three ecotypes of *Ocimum basilicum* L.by commonly measured physiological and biochemical parameters. Various levels of NaCl salinity stress showed inhibitory effects on roots than shoots growth, predominantly in Rajan pur ecotype. In addition to growth inhibition, NaCl reduced biomass production primarily in the roots. The adverse effect of salinity was also supported by a substantial decrease in total soluble proteins in shoots. In this study, findings indicated that ecotype of Rajan pur was more sensitive under stress than Multan and Khanewal ecotypes. It suggested that Multan ecotype as moderate tolerant could be a better choice for cultivation of salt affected soils which can be of potential importance in sweet basil production so that these plants are easily available in the traditional system of medicines.

Keyword: Aminoacids, Medicinal plant, *Ocimum basilicum*, Salt Tolerance, Proteins, Total chlorophyll.

1. Introduction

Ocimum basilicum L. commonly known as sweet basil is a popular herbaceous plant belongs to Lamiaceae family widely used for flavoring and medicinal purposes. It is an annual herb, 20-60 cm plant height with white and pink flowers and characterized by great morphological and chemical diversity. The useful parts of the plants are leaves and seeds, these highly aromatic leaves used either fresh or dried for spice. It comprises 65 species, adapted to grow in warm conditions and originally it is native to India and other countries of Asia [1].

Hot tea of basil plant leaves is good for treating nausea, dysentery and flatulence. Externally it can be used for different skin infections such as treatment of acne, snake bites and insect stings. In addition to these, basil has been used as a remedy for an enormous number of ailments, including cancer, convulsion, deafness, diarrhea, epilepsy, insanity, sore throat, toothaches, and whooping cough [2]. *Ocimum basilicum* is being utilized as a source of essential oils mainly in industries, perfumery, dental, oral products and traditional ritual. As a part of the tradition and religious rituals, basil needs more attention for the furtherance of its cultivation on a commercial scale as compared to other medicinally important plants. The aim of this study is to promote the cultivation of basil plants as well as utilization of saline lands which are unproductive for a number of field crops and reduce the average output of major crops greater than 50% [3].

Salinity not only causes great losses in crop production but also has an impact on other environmental, social and economic problems in the affected areas. It is roughly calculated that the annual global income loss of about US$ 12 billion resulted from salinization of irrigated lands impacting aggregate national incomes in countries affected by degradation of salt- affected land and saline water resources [4]. In severe cases, salt affected land causes a geographical shift of the salt affected communities. Generally, poor farming communities face economic difficulties by the negative impacts of worst salinity [5,6]. Plant growth and photosynthesis are among the primary processes of plant life which are affected by high concentration of salts in soil. During the initial exposure to salinity, appears to affect two plant processes water relations and ionic relations. When plants experience water stress, it reduces leaf expansion and in turns photosynthesis and all other process depend on photosynthesis [7]. A great deal of study and research efforts have done to the identification of medicinal and aromatic plants suitable for marginal lands but little work has been found in exploring the possibility of using saline soils for basil cultivation. By which we can generate profit and benefit to sustain cultivation on unproductive soils.

The present study on three ecotypes of basil includes effects of various levels of NaCl on plant biomass, total chlorophyll contents by SPAD, total soluble proteins and total free amino acids. The available literature might help to understand which ecotype up to what extent could tolerate salinity stress. By which we could be able to promote its cultivation on unproductive lands such as salt affected lands.

2. Material and Methods

An experiment was carried out in the glass-house of the Botanical garden of Bahauddin Zakariya University, Multan Pakistan, (30˚11N and 71˚28E). The average photoperiod 8 h and day/night temperature 24 ± 8˚C and 16± 4˚C during May to July 2012. The humidity ranged from 34.5 to 46.5 percent. Seeds of three ecotypes of *Ocimum basilicum* L. were collected from three different localities i.e. Multan, Khanewal and Rajan Pur districts of Punjab, Pakistan.

Sixty pots were used in the experiment filled with river sand and thoroughly washed with tap water. There were five different regimes of salinity stress (0, 50, 100, 150 and 200 mM) of NaCl and design of experiment was randomized complete block with four replicates. Sterilization of basil seeds was done by 5 % sodium hypochlorite solution for 5 minutes and distilled water was used to wash and remove the traces of sterilizing agents before the start of experimentation. After germination, eight seedlings of equal size at equidistance in each pot were selected. To ensure a water stress was minimized it was watered all pots manually [8]. Half strength Hoagland nutrient solution was applied to all the pots for normal growth of the plants for four weeks. Treatments were started after four weeks of sowing. The treatment solution of NaCl salt was given to all pots every week and to maintain the salinity levels in the sand.

After two months growth, plants were harvested, and shoots and roots were separated and then blotted dry before measuring their fresh weights. They were dried in oven at 80^{o}C until constant dry weights and dry weights of shoots and roots were recorded. Before harvesting, total soluble proteins [9], total free amino acids [10] and total chlorophyll content by a potable Minolta chlorophyll meter SPAD-502 (Spectrum Technologies, Inc., Plainfield, IL, U.S.) were determined. The obtained data were analysed by two-way ANOVA using the statistical computer package COSTAT (Cohort Software, Berkeley, USA) and means were compared with least significant difference following Snedecor and Cochran [11].

3. Results

Various medicinal plants have different levels of tolerance to salinity stress. Salt tolerance of ecotypes of basil was investigated by subjecting under selected levels of salinity (0, 50. 100, 150 and 200 mM of NaCl). Our results showed that salinity decreased fresh and dry weights of both shoots and roots in three ecotypes but the most prominent reduction was observed in roots of Rajan pur ecotype (Figure 1-4).

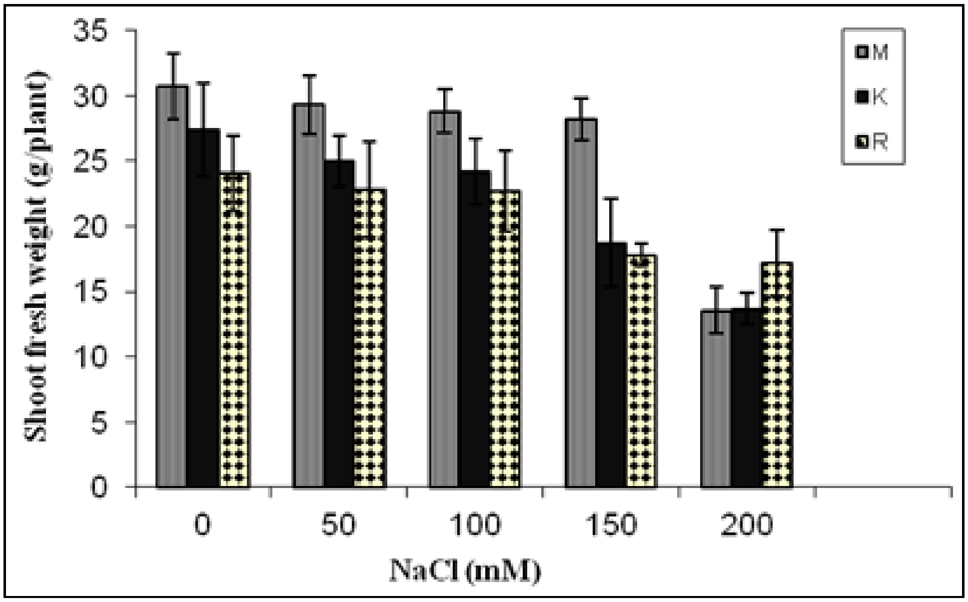

Fig 1: Shoot fresh weight (g/plant) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

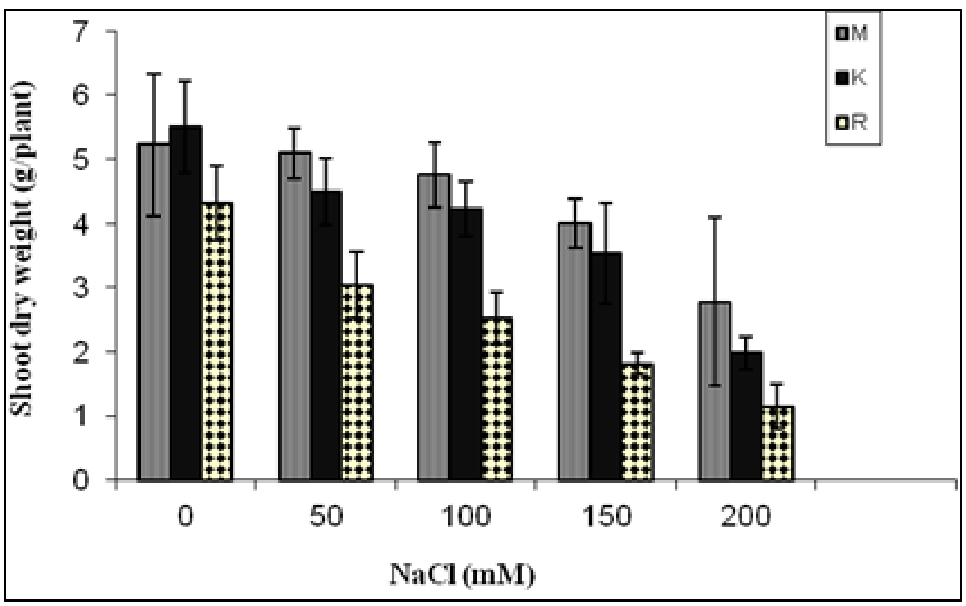

Fig 2: Shoot dry weight (g/plant) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

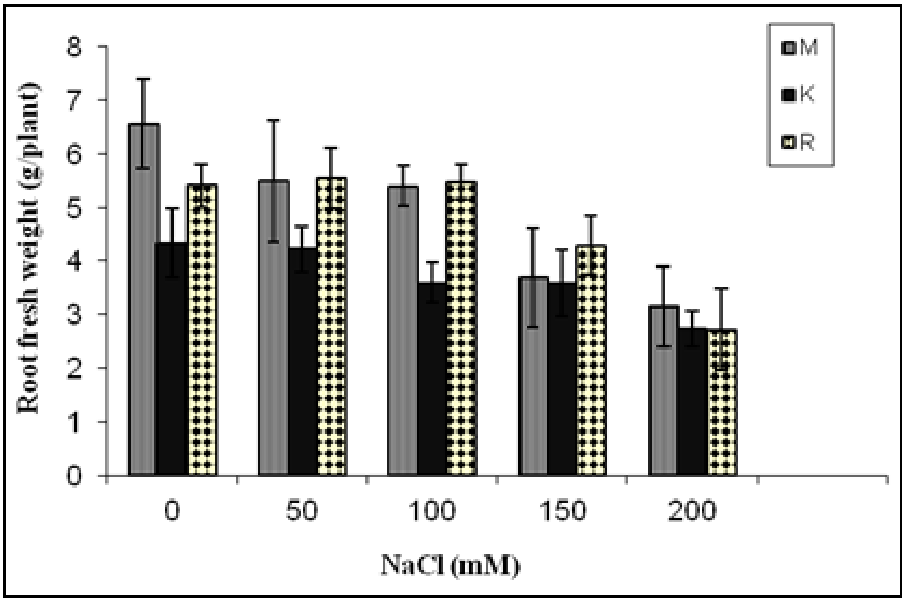

Fig 3: Root fresh weight (g/plant) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

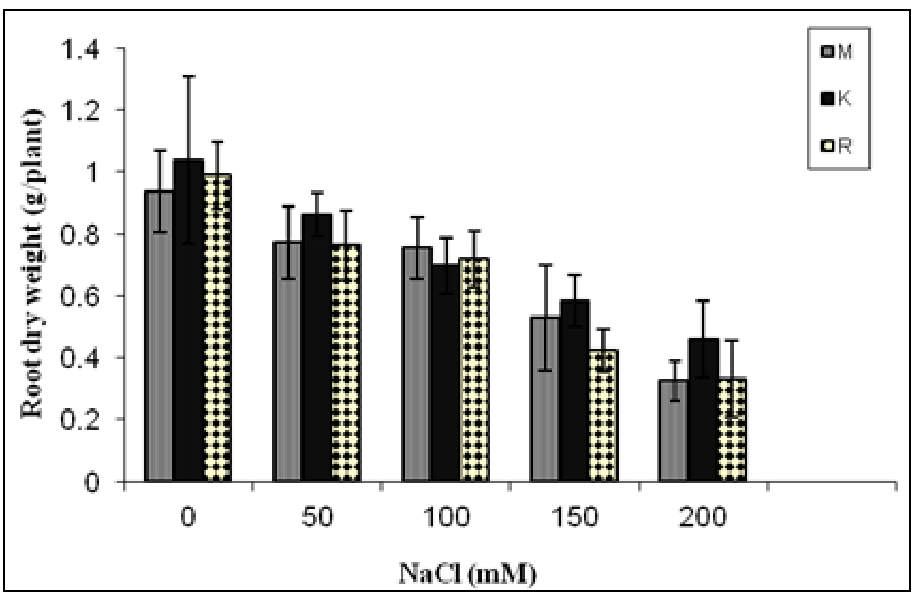

Fig 4: Root dry weight (g/plant) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

This reduction becomes more pronounced at highest salinity level. Analysis of variance of data for fresh and dry weights show that imposition of varying levels of NaCl in the growth medium had significant ($P \leq 0.001$) reducing effects on fresh and dry weights of shoots of three ecotypes of *Ocimum basilicum* (Table 1).

Table 1: Analysis of variance of data for growth attributes of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

SOV	df	Sht fwt.	Sht dwt.	Rt fwt.	Rt dwt.
Ecotypes	2	0.0009***	0.000***	0.0036**	0.4498^ns
Salt	4	0.000***	0.000***	0.000***	0.000***
Ecotypes x salt	8	0.1424 ^ns	0.9481 ^ns	0.4933 ^ns	0.9900 ^ns
Error	45	18.8371	1.2072	1.2380	0.04717
Total	59				

, *, significant at 0.01, and 0.001 probability levels respectively; ns = non-significant

Moreover, increasing levels of NaCl stress had caused a consistent decrease in fresh and dry biomass of shoots of Rajan pur ecotype. From the results, it is obvious that salt stress had slight inhibitory effect on total chlorophyll content in first week of salt stress (Figure 5).

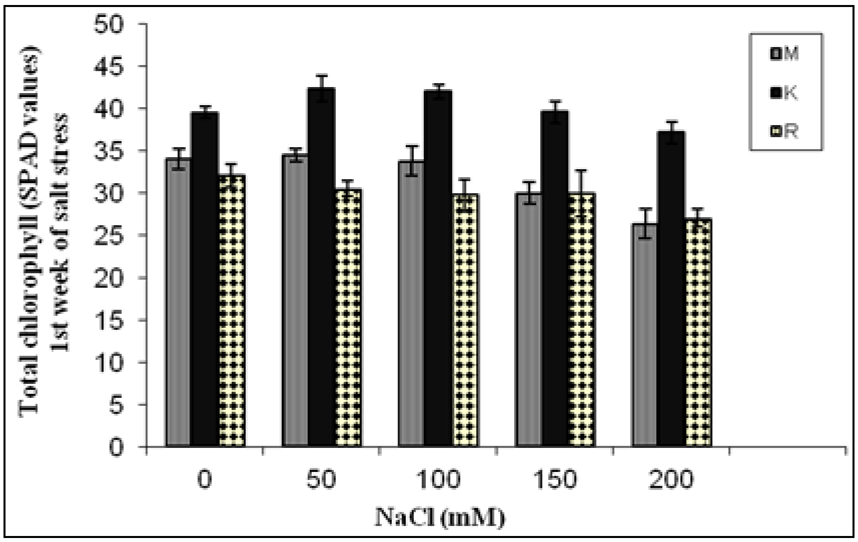

Fig 5: Total Chlorophyll content (SPAD values) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further one week grown in sand culture in full strength Hoagland's nutrient solution.

Total chlorophyll content by SPAD was significantly ($P \leq 0.001$) reduced in second and third week of salt stress in Multan ecotype (Table 2).

Table 2: Analysis of variance of data for total chlorophyll content (SPAD) of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for further one, two and three weeks grown in sand culture in full strength Hoagland's nutrient solution.

SOV	df	Total Chlorophyll after one week of salt stress	Total Chlorophyll after two weeks of salt stress	Total Chlorophyll after three weeks of salt stress
Ecotypes	2	0.000***	0.0003***	0.000***
Salt	4	0.000***	0.000***	0.0001***
Ecotypes x salt	8	0.2651 ^ns	0.6880 ^ns	0.9438 ^ns
Error	45	6.31417	27.1447	27.5645
Total	59			

***, significant at 0.001 probability level; ns = non-significant

Among the three ecotypes of sweet basil in study, increase of NaCl concentration is accompanied by a decline of chlorophyll synthesis. It clearly showed that total chlorophyll concentration was low at high levels of salt stress (200 mM) as compared to control whereas high reduction in total chlorophyll contents was observed in Multan ecotype as compared to others (Figure 6 and 7).

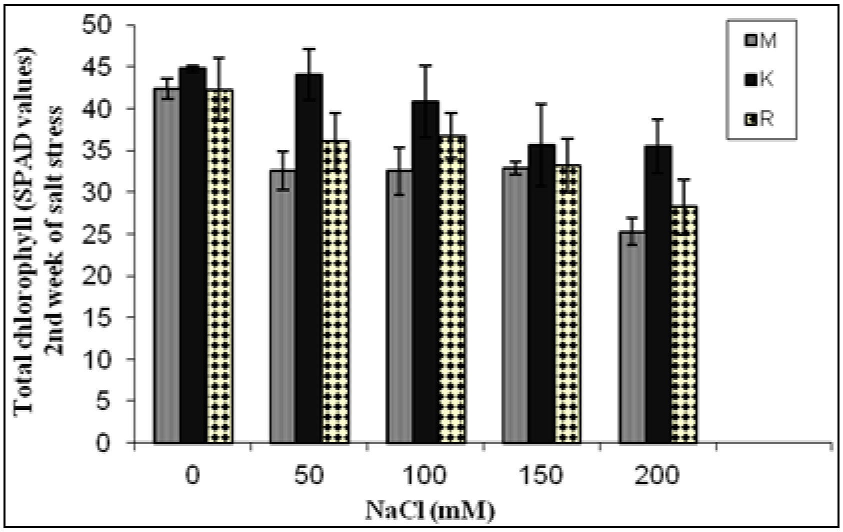

Fig 6: Total Chlorophyll content (SPAD values) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further two weeks grown in sand culture in full strength Hoagland's nutrient solution.

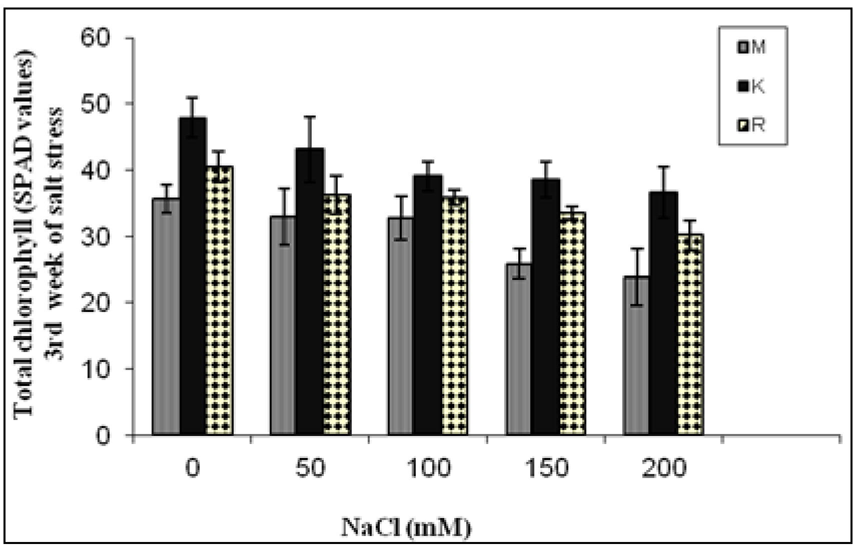

Fig 7: Total Chlorophyll content (SPAD values) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further three weeks grown in sand culture in full strength Hoagland's nutrient solution.

Total soluble proteins were reduced by increasing levels of salt stress in three ecotypes of basil but more apparently in Rajan pur ecotype. Total soluble proteins of *O. basilicum* were slightly reduced by increasing levels of salt stress (Figure 8). At control and low salinity stresss there was no prominent difference between total soluble proteins, whereas at the higher salt stress it decreased in three ecotypes but more prominent reduction was observed in Rajan pur ecotype. Increasing salinity resulted in lower concentration of total soluble proteins (Table 3). Statistically it showed that there were significant reduction at all salt treatments.

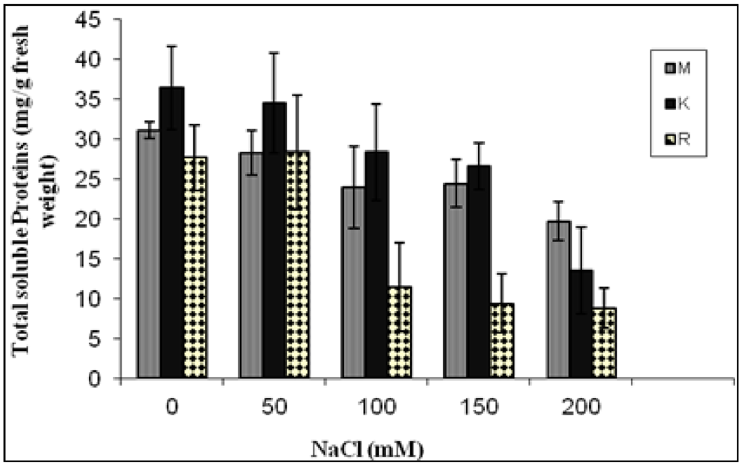

Fig 8: Total soluble proteins (mg/g fresh weight) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

Table 3: Analysis of variance of data for total soluble proteins and total free amino acids of three ecotypes of *ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

SOV	df	Total Soluble Proteins	Total Free Amino Acids
Ecotypes	2	0.0002***	0.0008***
Salt	4	0.000***	0.4605 ns
Ecotypes x salt	8	0.3870 ns	0.775 ns
Error	45	62.1379	0.2992
Total	59		

***, significant at 0.001 probability levels respectively; ns = non-significant

Total free amino acids were higher under saline conditions as compared to the non saline conditions in Rajan pur ecotype but decreased with increasing levels of salt stress in Multan ecotype. Thus, analysis of these results showed that Multan ecotype of sweet basil might tolerate high level of salt stress up to 150 mM of NaCl and can be cultivated on salt affected soils. Amino acids were higher under salt stress conditions as compared to the non saline conditions in Rajan pur ecotype but decreased with increasing levels of salt stress in Multan ecotype (Figure 9).

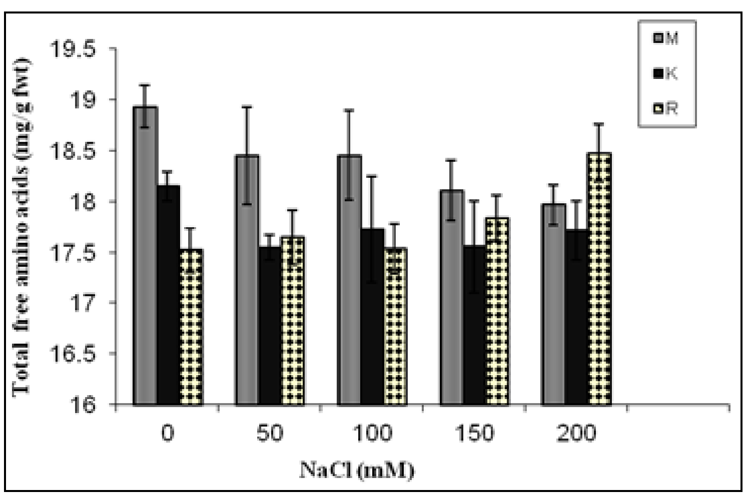

Fig 9: Total free amino acids (mg/g fresh weight) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further four weeks grown in sand culture in full strength Hoagland's nutrient solution.

ANOVA revealed that total free amino acids significantly ($P \leq 0.001$) increased with increasing levels of NaCl salinity in Rajan pur ecotype (Table 3). Moreover, total free amino acids were increased at high salinity level (200 mM NaCl) and reduced with decreasing the level of salt stress. It was concluded that all three ecotypes were different in total free amino acids concentration.

4. Discussion

According to the results under varying levels of salt stress in the study, fresh and dry biomass of both shoots and roots decreased as compared to control**,** this is the symptoms of salinity stress. In this present study reduction in growth of plants may be due to low water content in the soil which increased osmotic pressure, this is usually occurred in salt stress conditions. Therefore, different levels of NaCl salt stress affects the growth of basil plants and non-saline basil plants had greater fresh weights as compared to treated plants. Mainly there are two reasons for suppression of plant growth in salt affected soils. Firstly, salinity in soil reduce the ability of plants to take up from soil and results in reduced growth. This osmotic effect results from salt stress. Secondly, when critical amount of salts accumulate in photosynthetic leaves will damage cells in leaves this may affects photosynthesis and growth of plants [12].

This reduction of photosynthesis due to salinity may be decrease in conductance of CO_2 in mesophyll and stomatal cells. In this investigation when NaCl concentration increased morphological parameters in three ecotypes of *Ocimum basilicum* L. were changed. For instance, fresh and dried biomass of both shoots and roots in three ecotypes were decreased by increasing salinity. In our results, depression of growth in three ecotypes of sweet basil may be due to decrease in water uptake from saline growth medium [13], reduced photosynthetic capacity, lower chlorophyll contents [14, 15] and can be explained in view of Hussain and Ilahi, (1992) [16] and Anwar *et al*. [17]. However, salinity-induced growth reduction was also observed in many plant species e.g., canola [18] and Lucerne [19] . Furthermore, growth reduction under the saline conditions in other medicinal plants that also strengthens our results finding [20]. Our findings are in agreement with those of Farhoudi and Tafti (2011) [21] in soybean and Keshavarzi (2011) [22] on savory found also that under salinity stress plant growth is inhibited due to the low water potential, ion toxicity and imbalance excreted by salinity [23] .

From the present study, our results showed that chlorophyll contents decreased with increasing levels of salts stress in three ecotypes of sweet basil. The decrease in chlorophyll contents under saline conditions is also reported by Ashraf *et al*. [24]. But in first week of salt stress there was no significant difference among three ecotypes of sweet basil. It had been indicated that salt stress in wheat significantly reduced chlorophyll contents. Moreover, it has been reported by Lapina and Popov, (1970) [25] that chlorophyll contents also decrease in tomato plants and pea due to salt stress [26]. Decreasing chlorophyll contents of three ecotypes of sweet basil with increasing salinity could be related to degrading chlorophyllase enzyme [27] and destruction and instability of chloroplast and complexes of pigment proteins respectively [28].

It has been indicated in this study that total soluble proteins were reducing in three ecotypes by applying the salt stress. Reduction of total soluble proteins was found more pronounced in R. ecotype of sweet basil. Similar results were also found in given literature of Demiral and Turkan (2006) [29]. Results were found in barley, sunflower, and rice plants, Parvaiz and Satyavati, (2008) [30] is also strengthening our findings of present study. It was observed that proteins content in *Catharanthus roseus* had been significantly decreased along with increasing NaCl concentrations [31]. Furthermore, it was reported that level of soluble proteins decreased in salinity stressed Chamomile and sweet marjoram. Decreased total soluble proteins in R.ecotype as compared to other ecotypes at 100, 150 and 200 mM level of salt stress showed that there was some proteolytic activity occurred in this stress condition for the synthesis of osmoprotectant it might be one possibility in this regard. Other possibility is that if accumulation of proteins appeared in plants under salinity stress then it may provide nitrogen in storage form that is again utilized for biosynthesis of chlorophyll which is directly correlated with photosynthesis and helps in osmotic adjustment [32].

From the results, it was concluded that three ecotypes of basil plants differed in amino acids at all levels of salt stress, slight reduction of amino acids was appeared in M. ecotype of basil plant with increasing level of salt stress but increased with increasing levels of salinity stress in R.ecotype. Increased total free amino acids would have more advantages than the other plants which significantly decreased with increasing levels of salt stress. By accumulation of total free amino acids in two ecotypes of sweet basil clearly indicated that proteins hydrolysis releases and also accumulate free amino acids in plants tissues and helps in the osmotic balance of plants which was supported by Morgan, (1992) [33]. According to literature, amino acids were increased in plants of *Catharanthus roseus* and *Matricaria chamomilla* subjected to salt stress conditions [34].

5. Conclusion

Our results showed that biomass, chlorophyll and total soluble proteins of basil ecotypes were inhibited by increasing salt concentration. Salt stress also affected amino acids in ecotypes of basil. From this study it is also assumed that Rajan pur and Multan ecotype can tolerate moderate level of salt stress might be suitable for cultivation on marginal lands. In addition, essentially little information is available concerning salt tolerance of basil plants. Because of high potential value of basil plants there appears to be a need for much cultivation on a commercial scale.

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