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POSSIBLE ASSESSMENT OF SALT TOLERANCE IN *OCIMUM BASILICUM* BY CHLOROPHYLL FLUORESCENCE

Rehana KHALIQ[1], Ovidiu TITA[1], Zafar Ullah ZAFAR[2]

[1]Lucian Blaga University of Sibiu, Faculty of Agricultural Sciences, Food Industry and Environmental Protection, 550024, Romania
[2]Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan 60800, Pakistan

Corresponding author: rehanakhaliq1@gmail.com

Abstract

The present study was carried out to evaluate the impact of NaCl on chlorophyll florescence in three ecotypes of sweet "basil" (Ocimum basilicum L.) i.e., Multan, Khanewal and Rajanpur. Four weeks old seedlings were treated by different concentrations of NaCl (0, 50, 100, 150, 200 mM). Results showed that the increasing salinity stress had a negative impact on QY (Quantum yield), NPQ (Non photochemical quenching) and OJIP in three ecotypes of Ocimum basilicum L. From the findings, it is obvious that salt stress had a slight inhibitory effect in the first week of salt treatment. Under saline conditions as compared to non saline conditions dark adopted leaves of Rajan pur ecotype have reduced fv/fm ratio in third week of salt stress but decreased with increasing levels of salt stress in other two ecotypes. Therefore, analysis of the results showed that sweet basil might tolerate a level of salt stress up to 150 mM of NaCl and chlorophyll fluorescence can be used as a criterion for assessing salt tolerance in sweet basil as well as helpful to screen which ecotype might tolerate better the salinity stress. Our findings suggested that basil can be cultivated on salt effected soils up to moderate level that can be of potential importance in Ocimum basilicum production.

Key words**:** *salt tolerance, chlorophyll fluorescence, ocimum basilicum, medicinal plant*

INTRODUCTION

High concentration of salts in soil not only affects plant growth but also photosynthesis, they are among the primary processes of plant life which are affected by salinity stress. Under saline conditions photosynthesis is particularly reduced which results in reduced growth and productivity. Salinity induces many adverse effects on the photosynthesis of plants and stomatal conductance with decrease crop productivity and ultimately death of plants [1]. Photosynthesis is an important parameter used to monitor plant response to abiotic stress [2] [3]. When plants exposed to salt stress for a long time and salt continues to accumulate in the leaves and this critical concentration of salt in the leaves affects the photosynthesis due to disturbance of structure and functions of photochemical apparatus and photochemical reactions. Reduction of Rubisco i.e. ribulose-1.5-bisphosphate carboxylase/oxygenase is closely associated with damage of photosynthetic apparatus [4] [5]. Hence, salinity and water stress negatively alter leaf water relations and osmotic balance which ultimately disturbed the photosynthesis. The analysis of chlorophyll a fluorescence (CF) is one of the widest spread method in photosynthetic research, mainly originates from PSII. It is fast, easy and noninvasive tool to measure the plant's photosynthetic performance and used by plant physiologists and ecophysiologists. Chlorophyll fluorescence is now a very powerful, unique nonintrusive mean of obtaining quickly semi quantitative information on photosynthesis, could be used for screening of salt tolerance of crops in the field and in the laboratory. The functioning of the Photo-system II is directly affected by salt stress; salt injury has been reported in various plants [6][7]. Thus chlorophyll a fluorescence kinetics is considered as informative tool in field and laboratory for studying the effects of abiotic stress on photosynthesis [8][9]. *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 production of major crops greater than 50% [10].

MATERIALS AND METHODS

An experiment was conducted in glasshouse of 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 July-september 2013. 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 were done by 5 % sodium hypochlorite solution for 5 minutes and distilled water was used to wash. Eight days after germination eight seedlings of equal size at equidistance in each pot were selected. 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. Salinity concentration was increased stepwise in aliquots of 50 mM to avoid the salt shock [11].

The treatment solution of NaCl salt was given to all pots every week and to maintain the salinity levels in sand. After one month's growth, plants were subjected to measured chlorophyll a fluorescence in green, healthy and completely mature leaves using a small handy fluormeter (Photon System Instrument, FluorPEN FP 100, and Czech Republic). Measurements were taken on the selected leaves which were dark adapted for 20 min, a period of time sufficient for all photo-system II reaction centers to become open. Immediately after the dark adaptation, the leaves were exposed to light pulse with intensity of 2700 µmol m-2 s-1, with a wavelength of 650 nm for 5 s. The fluorescence transients were recorded from 10 µs to 5 s at 12 bit resolution [12]. The FlourPen software (PSI, CZ) was used to load the full fluorescence transients.

Statistical analysis: The collected data for chlorophyll fluorescence were analyzed by using COSTAT computer package (Cohort software, Berkeley, California) and differences between the mean values tested by following Snedecor and Cochran (1980)[13].

RESULTS AND DISCUSSIONS

From the results it was concluded that all NaCl treatments had a negative effect on the chlorophyll fluorescence such as QY (Quantum yield), NPQ (Non photochemical quenching) and OJIP in three ecotypes of *Ocimum basilicum* L. QY was decreased in plant parts such as leaves with increase salinity, whereas it was significantly ($P \leq 0.001$) reduced at high level of salt stress (200 mM NaCl) as compared to control (Table 1). Varying levels of salt application affected negatively on maximum efficiency of PSII Photochemistry (Fv/Fm) measured in the dark adapted leaves of three ecotypes under studied. Thus, in the absence of NaCl, Fv/Fm ratio was in the range of 0.730- 0.790 in all three ecotypes of sweet basil (Fig. 1-3).

Table 1. Analysis of variance of data for QY (F_V/F_M) of three ecotypes of *Ocimum basilicum* 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	Light QY after one week of salt stress	Light QY after two weeks of salt stress	Light QY after three weeks of salt stress
Ecotypes	2	0.000***	0.000***	0.0716 ^{ns}
Salt	4	0.0371*	0.000***	0.000***
Ecotypes x salt	8	0.0018**	0.0128*	0.9293 ^{ns}
Error	45	4.1393	3.8647	0.010418
Total	59			

*, **, ***, significant at 0.05, 0.01, and 0.001 probability levels respectively; ns = non-significant

Moreover, light and dark adopted QY concentrations was measured in first, second and third week of salt stress. Results showed that in first week of salt stress of NaCl there was no significant difference at all levels of NaCl salinity and no more difference between three ecotypes but in second and third week of salt stress there was significant difference between three ecotypes and all levels of salinity stress.

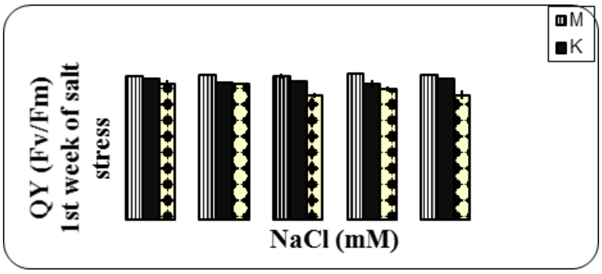

Fig. 1. QY (Fv/Fm) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further one week grown in sand culture in full strength Hoagland's nutrient solution.

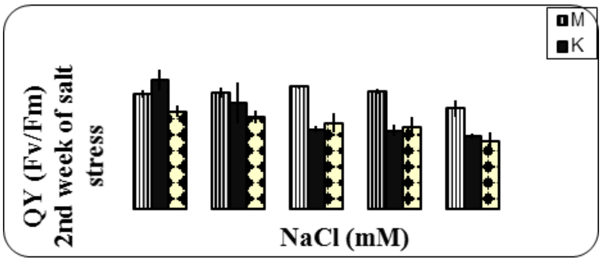

Fig. 2. QY (Fv/Fm) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further two weeks grown in sand culture in full strength Hoagland's nutrient solution.

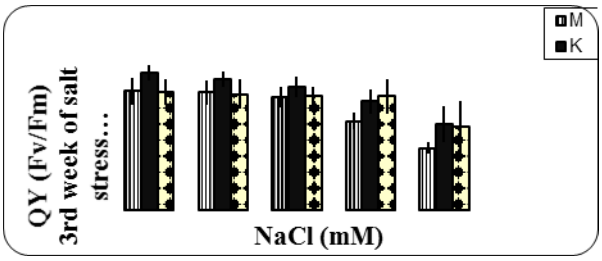

Fig. 3. QY (Fv/Fm) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further three weeks grown in sand culture in full strength Hoagland's nutrient solution.

The most pronounced effects of salt stress were observed at high level of salt stress 200mM NaCl and reduction was clearer in M. ecotype of sweet basil. However, at all levels of salt stress QY (Fv/Fm) decreased significantly (P≤ 0.001) (Table 2; Fig. 4-6).

Table 2. Analysis of variance of data for dark QY (F_V/F_M) of three ecotypes of *Ocimum basilicum* 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	Dark QY after one week of salt stress	Dark QY after two weeks of salt stress	Dark QY after three weeks of salt stress
Ecotypes	2	0.3882 ns	0.000***	0.000***
Salt	4	0.0064**	0.0076**	0.0041**
Ecotypes x salt	8	0.9832 ns	0.0986 ns	0.5365 ns
Error	45	0.01903	0.007576	0.004986
Total	59			

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

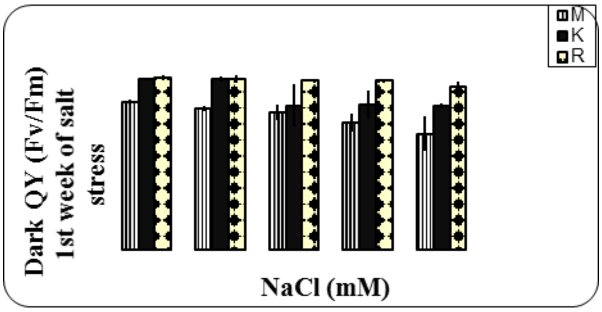

Fig. 4. Dark QY (Fv/Fm) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further one week grown in sand culture in full strength Hoagland's nutrient solution.

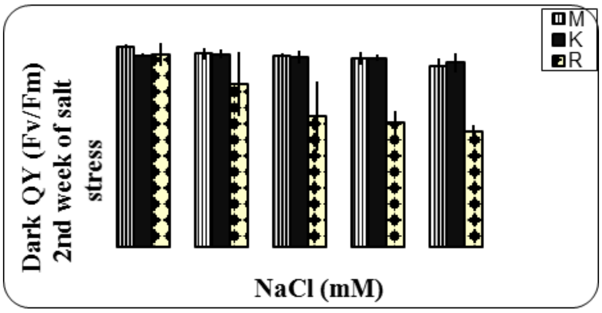

Fig. 5. Dark QY (Fv/Fm) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further two weeks grown in sand culture in full strength Hoagland's nutrient solution.

The chlorophyll fluorescence yield at three phases i.e. J, I and P were same in all levels of salt treated plants and there was no significant difference between three ecotypes of *O. basilicum.*

Absorption flux of photons per reaction center (ABS/RC) and maximum trapping rate by

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which an excitation is trapped per active PSII reaction center (TR_0 /RC) were slightly decreased in R. ecotype that treated with different levels of NaCl salinity as compared to K. and M. ecotypes.

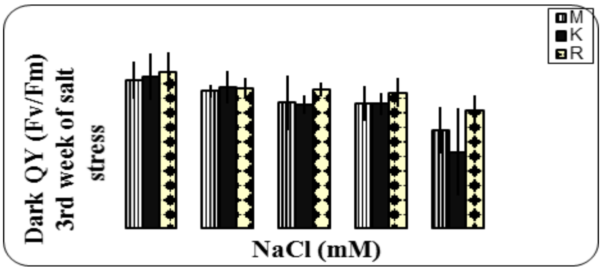

Fig. 6. Dark QY (Fv/Fm) of three ecotypes of *Ocimum basilicum* when four weeks old plants were subjected to varying levels of NaCl salinity stress for further three weeks grown in sand culture in full strength Hoagland's nutrient solution.

Table 3. Analysis of variance of data of selected parameters obtained from JIP test of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for one week

SOV	Ecotypes	Salt	Ecotypes x salt	Error	Total
Df	2	4	8	45	59
Fo	0.1095 ^{ns}	0.6323 ^{ns}	0.1044 ^{ns}	2879	
Fj	0.3718 ^{ns}	0.5281 ^{ns}	0.1301 ^{ns}	35065	
Fi	0.000***	0.3442 ^{ns}	0.0843 ^{ns}	66369	
Fp	0.000***	0.1179^{ns}	0.0756 ^{ns}	85190	
ABS/RC	0.2151 ^{ns}	0.7865 ^{ns}	0.8867 ^{ns}	0.0339	
TR0/RC	0.000***	0.0190*	0.5929 ^{ns}	0.01830	
ET0/RC	0.0767 ^{ns}	0.0032**	0.4968 ^{ns}	0.03158	
DI0/RC	0.000***	0.0436*	0.4335 ^{ns}	0.00483	
Fv/Fm	0.000***	0.004**	0.0037 **	8.5309	

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level; ns = non-significant

Therefore, ET0/RC (electron transport per active Photo-system II reaction center) concentration was decreased in R. ecotype at high level of salt stress 200 mM NaCl and DI0/RC (effective dissipated flux of untrapped excitations per active PSII RC) in K. and M ecotypes were same at all levels of NaCl but increased in R. ecotype with increasing levels of salt stress.

There was no marginal or significant decreased in quantum efficiencies of PSII like TR0/ABS (=FV/FM), ET0/ABS, ET0/TR in three ecotypes of sweet basil when compared to 0 mM NaCl salinity (Table 3,4; Fig. 7-1).

Table 4. Analysis of variance of data of selected parameters obtained from JIP test of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for three weeks

SOV	Ecotypes	Salt	Ecotypes x salt	Error	Total
Df	2	4	8	45	59
Fo	0.0000***	0.3616 ^{ns}	0.5578^{ns}	213726	
Fj	0.0000 ***	0.5376 ^{ns}	0.7043 ^{ns}	186922	
Fi	0.0000 ***	0.5648 ^{ns}	0.9383 ^{ns}	272363	
Fp	0.0000 ***	0.0506^{ns}	0.9592 ^{ns}	300797	
ABS/RC	0.0009***	0.6048 ^{ns}	0.3282 ^{ns}	0.06169	
TR0/RC	0.0062**	0.5352 ^{ns}	0.2463 ^{ns}	0.026543	
ET0/RC	0.0000***	0.1894 ^{ns}	0.1512 ^{ns}	0.080836	
DI0/RC	0.0000***	0.0065**	0.0538 ^{ns}	0.007182	
Fv/Fm	0.5852 ^{ns}	0.0252*	0.4108^{ns}	0.001355	

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level; ns = non-significant

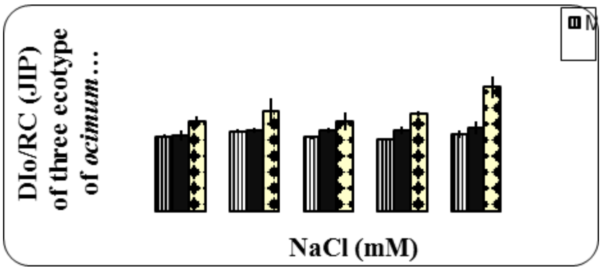

Fig. 7. DIo/RC(JIP) of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl stress for three weeks.

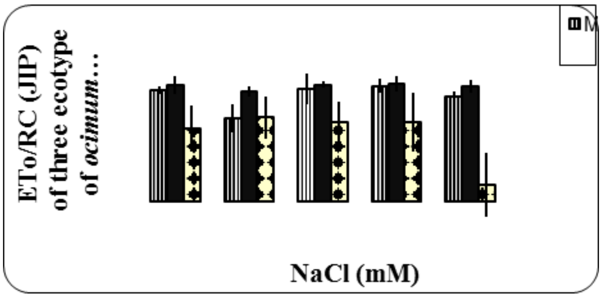

Fig. 8. ETo/RC(JIP) of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl stress for three weeks.

The chlorophyll fluorescence was used to detect changes induced by salinity in the maximum quantum yield of PSII and can estimate photosynthetic efficiency of leaf under various conditions of stress. It has been shown that these are to be reliable indicators of stress.

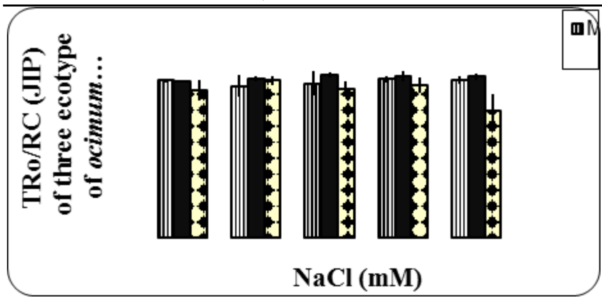

Fig. 9. TRo/RC(JIP) of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl stress for three weeks.

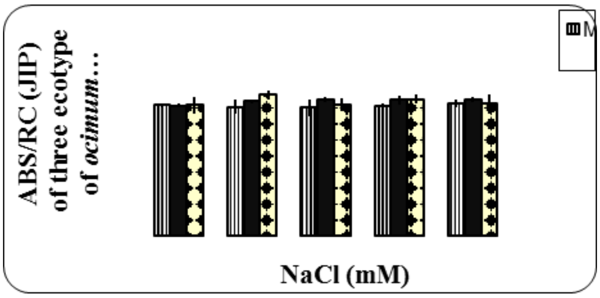

Fig. 10. ABS/RC(JIP) of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl stress for one week.

Non photochemical quenching coefficient was reduced as salt levels increased in all three ecotypes of basil plants (Table. 5, 6; Fig. 11).

Table 5. Analysis of variance of data of selected parameters obtained from NPQ test of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for three weeks

SOV	Ecotypes	Salt	Ecotypes x salt	Error	Total
Df	2	4	8	45	59
NPQ_L1	0.2941 ^{ns}	0.1984 ^{ns}	0.0293*	0.03349	
NPQ_L2	0.9954 ^{ns}	0.5023 ^{ns}	0.1254 ^{ns}	0.046615	
NPQ_L3	0.6989 ^{ns}	0.3978 ^{ns}	0.2479 ^{ns}	0.06633	
NPQ_L4	0.3454 ^{ns}	0.3290 ^{ns}	0.2145^{ns}	0.08225	
NPQ_Lss	0.1881 ^{ns}	0.3143 ^{ns}	0.2555^{ns}	0.086541	
NPQ_D1	0.1243 ^{ns}	0.7481 ^{ns}	0.4687 ^{ns}	0.00827	
NPQ_D2	0.0012**	0.0869 ^{ns}	0.6174 ^{ns}	0.004331	
NPQ_D3	0.0418*	0.1993 ^{ns}	0.5198 ^{ns}	0.004240	

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level; ns = non-significant

The photochemical quenching energy dissipation is the main process which protects the photosynthetic machinery from photo damage. Energy dissipation by photo damage due to salinity causes reduction in the relative quantum yield of PSII creates and maintains balance between and carbon metabolism and photosynthetic electron transport [14].

Table 6. Analysis of variance of data of selected parameters obtained from NPQ_Fm test of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl salinity stress for three weeks

SOV	Ecotypes	Salt	Ecotypes x salt	Error	Total
Df	2	4	8	45	59
Fm_L1	0.0005***	0.0039**	0.4763 ^{ns}	196231	
Fm_L2	0.0006 ***	0.0057 **	0.2806 ^{ns}	783427	
Fm_L3	0.0004***	0.0120*	0.2457^{ns}	488245	
Fm_L4	0.0002***	0.0123*	0.2201^{ns}	377020	
Fm_Lss	0.0000 ***	0.0065 **	0.2159^{ns}	301186	
Fm_D1	0.0009***	0.0045 **	0.2553 ^{ns}	868836	
Fm_D2	0.0055***	0.0139 *	0.2671 ^{ns}	118243	
Fm_D3	0.0017**	0.0109 *	0.3442^{ns}	145854	

*, **, ***, significant at 0.05, 0.01, and 0.001 probability level; ns = non-significant

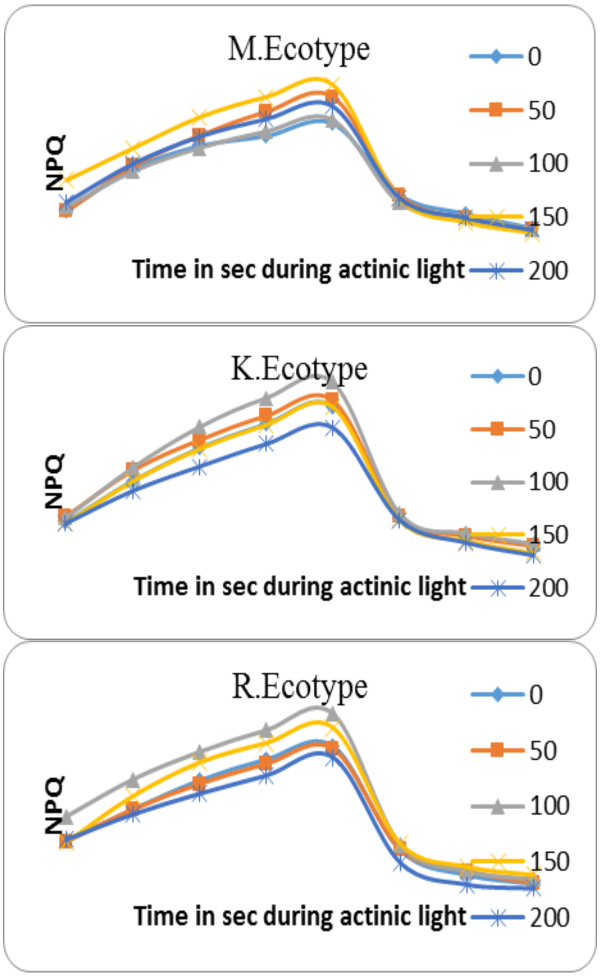

Fig. 11. NPQ Curves of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl stress for three weeks.

From the results it was concluded that second and third week of salt stress had significant effect on three ecotypes of sweet basil at all levels of salt stress as compared to control. It has been concluded from the studies that salinity alone had no significant on PS II photochemistry at relatively low light but effects of both salt stress and high light induced photo damage to PSII (Fig. 12).

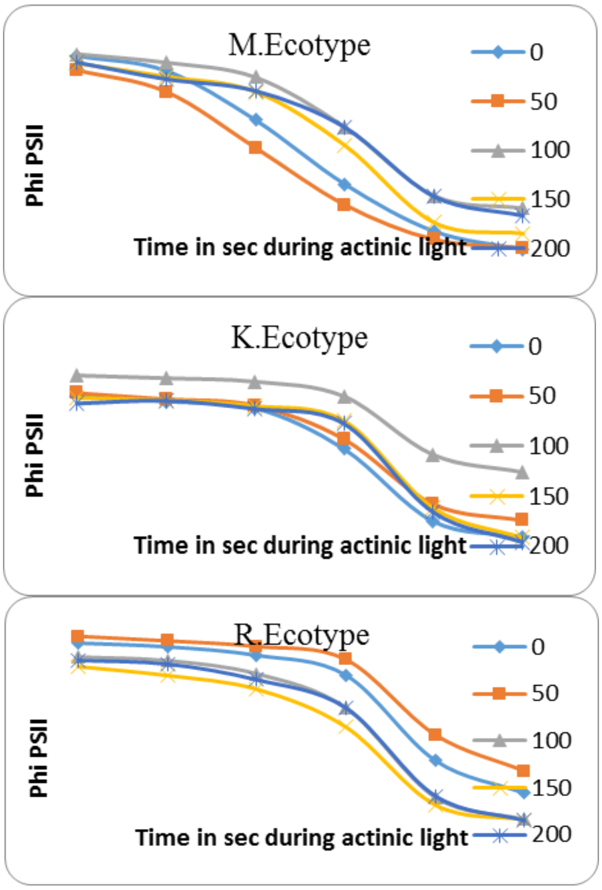

Fig. 12. Phi PSII of three ecotypes of *Ocimum basilicum* L. when four weeks old plants were subjected to varying levels of NaCl stress for three weeks.

When leaves of three ecotypes of basil plants were exposed to different levels of salinity stress, it had no effects on quantum yield (Fv/Fm) of dark adopted leaves. Different plant species have almost constant Fv/Fm ratio ($0.80 \leq Fv/Fm \leq 0.86$) measured under non stressed conditions ([15]). But Fv/Fm ratio in different plant species decrease up to these values 0.588±0.019 under severe salinity stress [16]. Furthermore, slight change in Fv/Fm ratio at high salt levels suggesting that only small reductions in photosynthesis occurred at a high level of salinity stress and causes inhibition in the ability of PSII to reduce Q_A which is primary acceptor of PSII. On the other hand, it is observed that lower Fv/Fm in salt stress conditions as compared to control, will regenerate RUBP and this regeneration need some electron translocation from PSII to electron acceptor, may be disturbed by salt stress [17].

In our findings, slight change in Fv/Fm was appeared in first week of salt stress and at high level of NaCl salinity in three ecotypes of basil plants. There might be two reasons for this small change one is related to damage of chlorophyll and other is ionic imbalance under high salinity ([18][19]). It was reported in other study that negative effects on growth related to decrease in PSII activity and chlorophyll contents [20].

Quantum yield of photosystem II (ΦPSII) decreased by increasing salt concentration and ΦPSII reflected electron transport rate was highest at low salt level of NaCl Therefore, Quantum yield of photo-system II was markedly reduced in R. ecotype as compared to other two ecotypes at high level of salinity stress. The selected JIP-test parameters were listed in Tables 3 and 4. The results of these parameters have contributed to a better understanding of the responses of basil plants to salinity stress. It was concluded that salinity had inhibitory effects on the photochemical activity of plants which results in increase number of closed PSII reaction centers and hinder the participation of large number of closed PSII reaction centers in electron transport [21]. Present results in chlorophyll fluorescence confirmed that photosynthetic activity was more inhibited under high salinity than that under low and moderate salt stress.

CONCLUSIONS

The above mentioned parameters of chlorophyll fluorescence are appropriate criteria for the diagnosis of salt stress in plants. Quantum yield of photo-system II and Fv/Fm ratio (maximum photochemical efficiency of PS II) of three ecotypes of *Ocimum basilicum* L. was reduced at high level of salt stress and this could be a reliable indicator of salt stress in all plant species.

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