Response of Growth, PSII Photochemistry and Chlorophyll Content to Salt Stress in Four *Brassica* Species

Muhammad Jamil[1], Shafiq ur Rehman[2], E. S. Rha[3*]

[1.] Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan
[2.] Department of Botany, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan
[3.]College of Agriculture and Life Sciences, Sunchon National University, Suncheon 540-742, Korea
euishik@sunchon.ac.kr, dr.jamil@kust.edu.pk

Abstract: Salinity is a common constrain to crop productivity around the globe. To assessed salinity effects on growth, PSII photochemistry, and Chlorophyll content of *Brassica* species differing in salt tolerance in order to clarify whether growth inhibition of *Brassica* species under saline conditions was attributable to PSII photochemistry, Seedlings of mustard (*Brassica juncea* L.), cauliflower (*Brassica oleracea botrytis* L.) canola (*Brassica napus* L.) and two cabbage (*Brassica oleracea capitata* L) varieties were grown in saline condition at salt stress of 0, 50, 100, and 150 mM NaCl. Root weight, shoot weight, and leaf area considerably reducedas the level of salt increase in saline medium while there was no change in leaf water content. Salt stress induced a significant decrease in the maximum quantum yield of PSII (Fv/Fm), electron transport rate, and photochemical quenching coefficient of chlorophyll fluorescence (q_P) in all *Brassica* species while F_o/F_m ratio increased significantly with higher salt concentration. Chlorophyll (Chl) content significantly decreased with the increase in salinity in cauliflower, whereas a significant increase was observed in other *Brassica* species. Linear regression revealed a significant negative relationship between salinity and growth, Fv/Fm, electron transport rate and q_P. There was also a positive relationship between salinity and Fo/Fm ratio. Multiple regression equations developed to predict the relationship between the growth and PSII photochemistry have shown that the dry weight of root and shoot had a significant positive correlation with Fv/Fm and electron transport rate, but a significant negative correlation with Fo/Fm ratio and photochemical quenching coefficient. It was concluded that plant growth had strong relationship with PSII photochemistry in Brassica species.

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1. Introduction

Salinity has various effects on plant physiological processes by affecting plant growth and distribution of mineral elements due to calcium dislocation by sodium (Marschner, 1986) and decreasing efficiency of photosynthesis (Hasegawa et al., 2000). Photosystem II is believed to be the major part in the response of photosynthesis to environmental challenges (Baker, 1991). The decline in plant productivity under saline condition is frequently linked with the reduction in photosynthetic capability (Long and Baker, 1986).

It has been reported that PSII Photochemistry of numerous plant species has been decreased due to Salt stress (Jamil et al., 2007; Bayuelo-Jimenez et al., 2012; Jamil and Rha, 2013). The most important component involved in defense against photo damage is perhaps the photochemical energy dissipation, which reduces the relative quantum yield of PSII in line to maintain a sufficient balance between electron transport rate and carbon metabolism (Munns, 1993). The decrease in photosynthesis under stress condition can also be due to a decline in chlorophyll (Chl) content (Heuer and Plaut, 1989). It has been earlier documented that Chl content decreased in salt sensitive plants such as tomato (Weis and Berry, 1987), pea (Delfine et al., 1999) and soybean (Lapina and Popov, 1970) but Chl content rose in salt resistant plants such as pearl millet (Hamada and El-Enany, 1994) and mustard (Seemann and Critchley, 1985; Jamil and Rha, 2013).

There are also many plant species in which no or a weak relationship between growth and photosynthetic ability is noticeable, e.g. *Triticum aestivum* (Reddy and Vora, 1986), *Hibiscus cannabinus* (Singh et al., 1990), *Trifolium repens* (Hawkins and Lewis, 1993) and mustard (Jamil and Rha, 2013). *Brassica* family is of most economic valuable vegetables interm of their utilization as a food. *Brassica* species varying in salt resistant may exhibit differing responses with respect to their PSII photochemistry and Chl content under saline conditions. Therefore, the present study was conducted to estimate salinity effects on growth, PSII photochemistry, and Chl content of *Brassica* species varying in salt resistance in order to clarify whether growth inhibition of *Brassica* species under saline conditions was attributable to PSII photochemistry.

2. Material and Methods

Plant material and salinity treatments

Seeds of commercial cultivars of mustard (*Brassica juncea* L., cv, Gael yangjasaegas) and cabbage (*Brassica oleracea capitata* L., cvs Gaeul baechu- cv$_1$ and Bom baechu cv$_2$) were obtained from Jeollabuk-do Agricultural and Extension Services, Iksan, Korea. Seeds of cauliflower (*Brassica oleracea botrytis* L., cv, Wenxing) and canola (*Brassica napus* L., cv, Si jiuhuangcaixin) were obtained from the seed section, Institute of Soils and Fertilizers, Beijing, China.

Seeds of each species were grown in plastic pots (16 cm diameter, 22 cm height) in sand culture in a green house. The sand was irrigated on alternate days with a Hoagland nutrient solution for 2 weeks. Salinity treatments in Hoagland solution started after 3 weeks of the experiment. Salt treatments 50, 100, and 150 mM NaCl were added in complete Hoagland nutrient solution.The day/night temperature was 25/15 °C and photoperiod was 16 h. The average PAR during the entire growth period was 1095 µmol/(m^2 s). All the measurements were made after 30 days of sowing.

Chlorophyll fluorescence

Measurements were made with a transportable Mini PAM fluorometer (PAM-2000, Walz, Germany) from upper surface of leaves pre-darkened for at least 30 min prior to measurement. The procedure of Genty et al. (1989) was principally followed. All the fluorescence measurements and calculations were made according to Jamil et al. (2007).

Measurements of growth

The plants were harvested and then divided it into shoots (stems and leaves) and roots. Fresh weights (FW) of leaves of all the plant samples were recorded. Samples of roots, shoots and leaves were then dried at 80°C to measure the dry weights (DW). The leaf water content was calculated as (FW-DW)/FW*100 Leaf area was also measured using a leaf area Meter(AM-200, ADC Bio-Scientific Ltd., England)

Chlorophyll content

Portable chlorophyll content (CCM-200, Opti-Science, USA**)** meter was used for measurement of leaf chlorophyll contents. The measurements were made six times from leaf tip to the leaf base.

Statistical analysis

Experiment was designed by using complete randomized block design with three replication. ANOVA was done by using MS-Excel software. Mean values of all the above parameters were compared with LSD using Tukey's *t* (Li, 1964).The correlation between growth and chlorophyll fluorescence was derived by using Minitab version 14.0.

3. Results

Salinity effect on growth

Increase in salinity stress caused a significant inhibition in dry weights of roots and shoots of all *Brassica* species (Fig. 1A and B). However, the *Brassica* species varied significantly in these growth parameters in reply to salt stress conditions. Cauliflower had a considerably lower dry weight of shoots as compared to all the other species at the highest salt treatment (150 mM NaCl) while at same concentration cabbage (cv$_2$) and canola were lower in dry weights of roots.

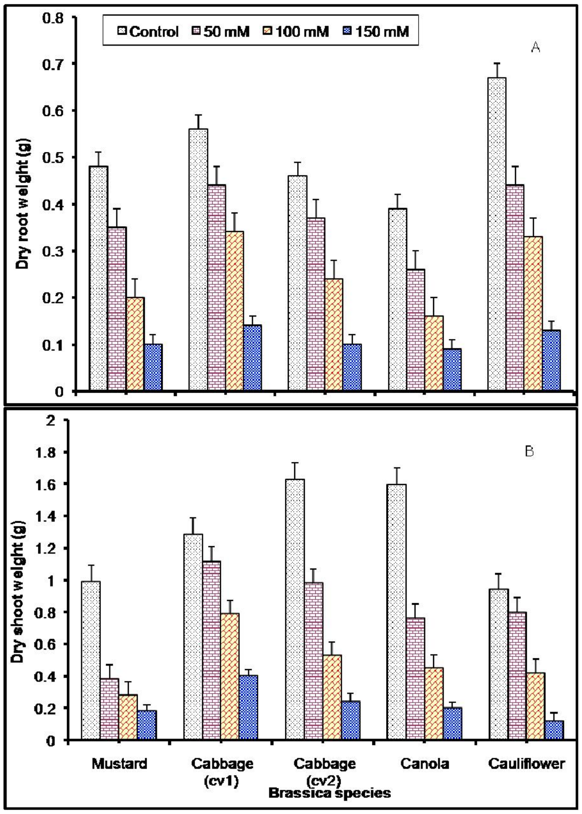


Figure 1. Root dry weight (A) and shoot dry weight (B) of four *Brassica* species exposed to various concentrations of NaCl in sand culture. Each value is the mean of three replications ± SD.

Salinity effect on chlorophyll fluorescence

Increase in salinity levels caused a considerable decrease in maximal quantum yield of PSII (Fv/Fm) in all species. At the highest salt treatment, cabbage (cv$_2$) and canola had lower Fv/Fm as compared to other species while mustard and cauliflower were the highest (Figure 2A). Fo/Fm (Figure 2B) of all six *Brassica* species increased significantly with an increase in salinity concentration. Mustard was notably higher in Fo/Fm values than the other species at the highest salt concentration. The cauliflower was the lowest in Fo/Fm of all the species. However, a considerable decrease in electron transport rate was observed in all the species (Figure 3A). At the highest salt treatment (150 mM NaCl), canola had a lower ETR while that of cauliflower was higher than that of the other species. Photochemical quenching coefficient (q_P) decreased with increase in salinity levels in all *Brassica* species (Figure 3B). Cabbage (cv_2) was the lowest of among all the species in photochemical quenching coefficient (q_P) at highest salt regimes. Mustard was the highest as compared to other species in photochemical quenching coefficient (q_P).

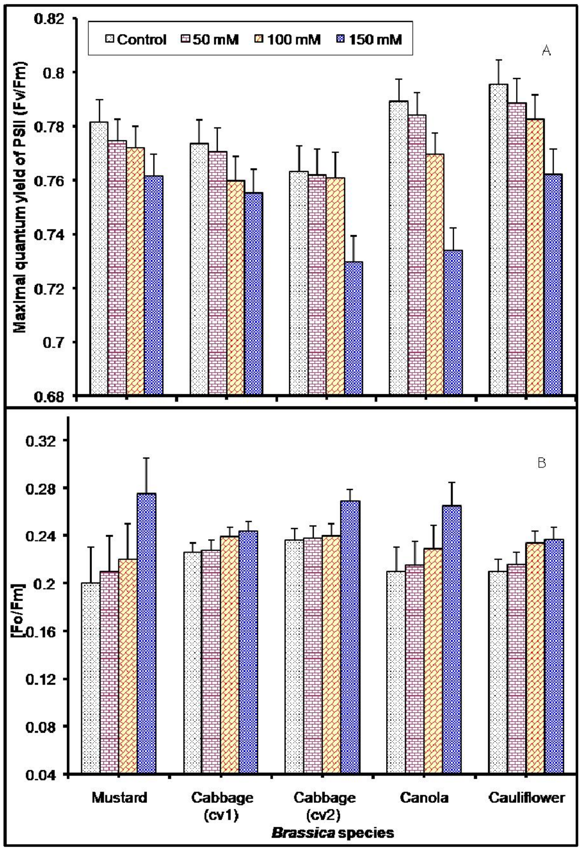


Figure 2. The maximum efficiency of PSII photochemistry (Fv/Fm) (A) and physiological state of the photosynthetic apparatus (Fo/Fm) (B) of four *Brassica* species exposed to various concentrations of NaCl in sand culture. Each value is the mean of three replications ± SD.

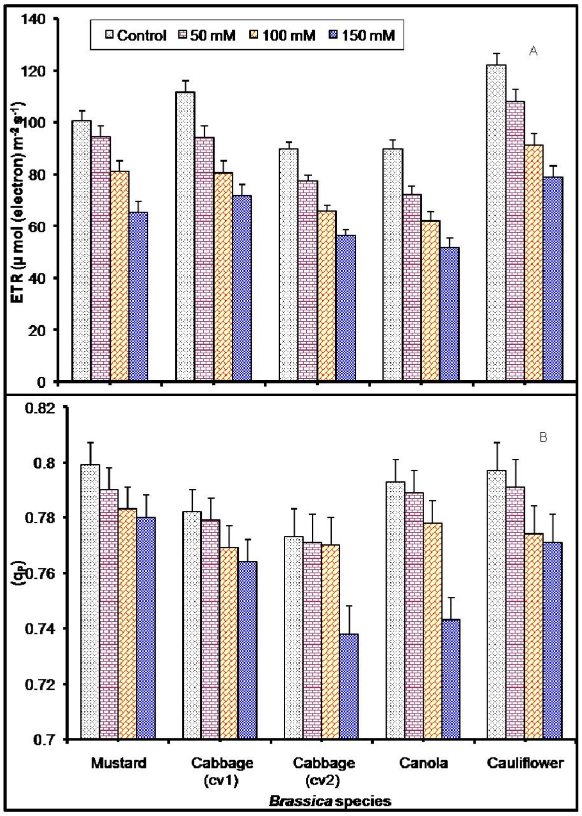
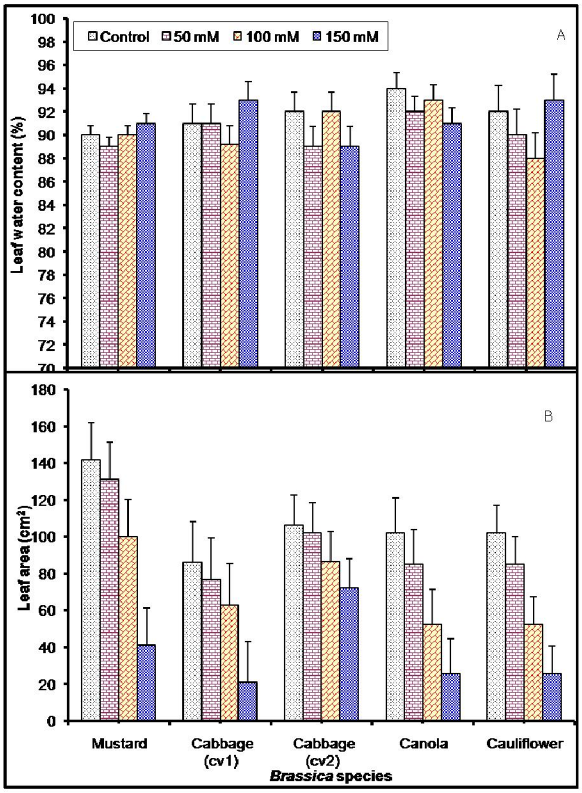
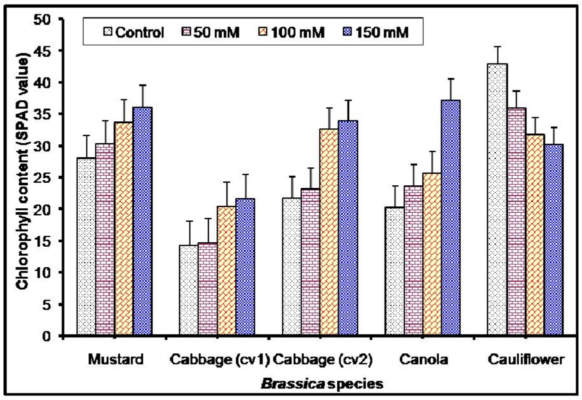


Figure 3. The electron transport rate (ETR) (A) and photochemical quenching coefficient (q_P) (B) of four *Brassica* species exposed to various concentrations of NaCl in sand culture. Each value is the mean of three replications ± SD.

Salinity effect on leaf area, leaf water and chlorophyll contents

Leaf area significantly decreased in all species with an increase in salt concentration (Figure 4B). However, salt treatments had a non significant effect on leaf water contents (Figure 4A). Cabbage (cv_2) had a maximum leaf area at the highest salt level. At the same salt concentrations, cabbage (cv_1) had minimum leaf area among all species. The leaf chlorophyll content increased as the peripheral salt concentration increased in all species except cauliflower in which it decreased with increase in salinity stress (Figure 5). At 150 mM NaCl, canola had maximum leaf chlorophyll content among all the species while cabbage (cv_1) was the lowest at the same concentration (Figure 5).





salinity and index characterizing the physiological

Parameters	Linear regression equation [Salinity (dS m^{-1}) =]	Regression coefficient (R^2)	Probability (*P*)
DRW	1.03 - 1.87x¶	0.82	*P*< 0.001
DSW	0.91 - 0.65x	0.76	*P*< 0.001
Fv/Fm	11.46 - 14.3x	0.49	0.001
Fo/Fm	-2.55 + 12.94x	0.59	*P* < 0.000
ETR	1.107 - 0.04x	0.34	0.007
Qp	12.4 - 15.38x	0.50	*P* < 0.001

¶x denotes the parameters in linear equation

Parameters	Multiple regression equation	Regression coefficient (R^2)
(1) DRW = 1.17 + 2.58 Fv/Fm - 2.06 Fo/Fm + 0.016 ETR - 0.36 q_p		0.66
(2) DSW = - 0.10 + 4.20 Fv/Fm - 7.49 Fo/Fm + 0.012 ETR - 1.10 q_p		0.26

4. Discussions

Growth reduction under saline conditions has been well documented in various plants by many researchers (Alpaslan and Gunes, 2001; Greenway and Munns, 1980). Salinity imposes a considerable decrease in dry weights of roots and shoots and leaf area but it had no significant effect on leaf water contents (Figures 1 and 4). The plant growth is eventually decreased by salt stress, even though plant species differ in their tolerance to salt stress (Munns and Termaat, 1986). Salt stress reduces the leaf growth rate by restricting the length of the leaf elongating zone and reducing the growth capacity (Bernstein et al., 1993). Greenway and Munns (1980) investigated that the influence of salt stress on leaf area was higher than on dry weight, as salt addition in the old leaves occurs through shoot transpiration streams, which killing them.

The Fv/Fm is a sign of possible yield of photochemical reaction of PSII (Weis and Berry, 1987). A significant decrease was observed in Fv/Fm, ETR and q_P of all the *Brassica* species (Figures 2A, 3A and 3B). The increase of Fo/Fm in all species grown at a various salt concentration (Figure 2B) may be due to structural changes of PS II, in which Fo reflect the fluorescence emitted by PS II and where Qa is oxidized. The increase of Fo/Fm also depends on the loss of excitation energy while it migrates along the pigment matrix. The q_P energy indulgence reduces the Fv/Fm in turn to maintain an sufficient balance between ETR and carbon metabolism (Weis and Berry, 1987). The decrease of photosynthesis is often linked with the increase in Na^+ deposition (Dionisio-Sese and Tobita, 2000). The decline of q_P by salt stress may be a result of salt stress induction of stomata closure, caused by the decrease in osmotic potential (Weis and Berry, 1987). As salinity increases, the deposition of NaCl in chloroplasts of higher plants increases, it influence growth rate, and it is often linked with decrease in ETR activities in photosynthesis (Kirst 1989). Similar kind of results has been documented for rice, mungbean and *Brassica* seedlings (Lutts, 1996; Dionisio-Sese and Tobita, 2000; Misra et al., 2001). They observed that the effects of salt on the potential photochemical efficiency of PSII may be species specific. Salt decreased the efficiency of photosynthesis has also been reported by Hasegawa et al. (2000).

The chlorophyll content increased as the external salt concentration increased in all species except with cauliflower (Figure 5). In this study, we had observed that cauliflower was salt sensitive as compared to other *Brassica* species. It has been observed previously that chlorophyll content decreases in salt sensitive plants such as tomato (Lapina and Popov, 1970) pea (Hamada and El-Enany 1994) and soybean (Seemann and Critchley, 1985). The decrease in chlorophyll content in cauliflower could be attributed to increased activity of Chlorophyll degrading enzyme cholorophyllase (Rao and Rao, 1981) or breakdown of chloroplast structure and instability of pigment protein complexes (Singh and Dubey 1995). An increase in chlorophyll content in other *Brassica* species may be due to the deposition of NaCl in the chloroplast. The increase in chlorophyll contents were also observed in salt resistant plants such as pearl millet (Reddy and Vora, 1986) and mustard (Singh et al., 1990; Jamil and Rha, 2013).

Table 1 revealed a significant negative relationship between salinity and dry root weight, dry shoot weight, Fv/Fm, ETR and q_P. There was also a positive relationship between salinity and Fo/Fm. In this study, multiple regression equations (Table 2) were developed to predict the relationship between growth and PSII photochemistry. These equations show that DRW and DSW had a significant positive relationship with Fv/Fm and ETR but a significant negative relationship excited with Fo/Fm and q_P. The objective of these predictions was to fine the relationship between growth and PSII photochemistry and whether growth of *Brassica* species under salt stresses conditions was attributable to their PSII photochemistry. In the present study, it was observed that growth and PSII photochemistry has close association with each other in all *Brassica* species. The results for growth and PSII photochemistry of all *Brassica* species conflicting in salt tolerance can be simply related to some previous reports in which a close association has been found between growth and photosynthetic capacity, in many crop species such as *Triticum aestivum* (Hawkins and Lewis, 1993) *Hibiscus cannabinus* (Curtis and Lauchli, 1986) and *Trifolium repens* (Rogers and Noble, 1992). There are many reports in which inhibition of the growth and photosynthetic capability of various plant species by salt stress has been documented (Munns and Termaat, 1986; Hasegawa et al., 2000; Jamil et al. 2007; Jamil and Rha, 2013).

It was concluded that salinity effect growth and PSII photochemistry and close relationship was observed between growth and PSII photochemistry in all *Brassica* species. All the *Brassica* species were varying in their ability to salt tolerance but cauliflower was more sensitive than other *Brassica* species.

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Corresponding Authors:
Dr. Eui Shik Rha
Department of Well-being Resources
College of Agriculture and Life Sciences, Sunchon National University, Suncheon 540-742, Korea.
E-mail: euishik@sunchon.ac.kr
Dr. Muhammad Jamil
Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Kohat 26000, Pakistan.
E-mail: dr.jamil@kust.edu.pk

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