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NaCl Stress-Induced Reduction in Grwoth, Photosynthesis and Protein in Mustard

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Abstract

Seed germination and early seedling growth, photosynthesis and protein activity of mustard were investigated under salt stress in two experiments. The NaCl concentrations in Hoagland solution were 0 (control), 50, 100 and 150 mM. Percentage of seed germination, germination rate, length, and fresh weight of the seedlings decreased significantly under salinity. Salt concentrations significantly reduced leaf area and number of leaves while salinity showed a non significant effect on leaf water content. Chlorophyll content enhanced considerably with the increasing NaCl concentration. Furthermore, Fv/Fm ratio and electron transport rate were also appreciably reduced by salt stress. In contrast, non- photochemical quenching coefficient increased significantly with increasing NaCl concentration. Net CO_2 assimilation, stomatal conductance, transpiration rate, and intrinsic water-use efficiency decreased remarkably with increasing NaCl concentration while water use efficiency increased at 50 mM NaCl but then reduced. Correlation shows that gas exchange attributes had a significant positive relationship with chlorophyll fluorescence. There was an increase in the concentration of total protein content with the corresponding increase in NaCl level up to 100 mM. SDS-PAGE analysis showed that the intensity of band expression increased significantly as with increasing salt stress up to 100 mM but diminished at 150 mM NaCl.

Keywords: *Brassica juncea*, chlorophyll content, photosynthesis, leaf area, salt stress

1. Introduction

Salt stress has a significant impact on plant establishment and crop productivity (Munns, 2002). The crop growth is eventually declined by salt stress even though crop species vary in their resistance to saline condition (Munns & Termaat, 1986). Salt stress can significantly reduce plant growth as a result of the joint influence of high osmotic stress and explicit ion toxicity (Grieve & Suarez, 1997; Hasanuzzaman et al., 2009, 2013). Some of the harmful effects of salt stress include the reduction ingermination rate and seedling growth (Jamil et al., 2012), and the expansion in the leaf area which eventually declines photosynthetic area and biomass production (Mansour & Salma, 2004).

Photosynthesis is a major factor in the determination of plant growth. The reduction in crop production observed in various plant species exposed to salt stress is linked to the decline in photosynthesis (Long & Baker, 1986; Chaves et al., 2009). Reduction in the photosynthetic capability of various plant species by salt stress has been documented in a number of reports (Dubey, 1997; Jamil et al., 2007a; Bayuelo-Jimenez et al., 2012). The inhibition in photosynthesis under saline condition can also be explained by the decline in chlorophyll content (Delfine et al., 1999; Jamil et al., 2007). The ability of the plant to produce a stress protein is an important approach of salt stress tolerance. Most of these proteins are extremely water soluble and heat stable, related to cytoplasmic membranes and organelles and act as molecular chaperones (Wahid & Close, 2007).

Brassica is considered to be a salt resistant crop; as a result, these crops are more appropriate to evaluate the basis of salt stress tolerance in plants (Misra et al., 2001). The dominance of the Brassica amphidiploid species over the diploid species in terms of salt resistant is obvious from various documents (Ashraf & McNeilly, 1990;

Kumar, 1995; Nazir et al., 2001). It has been observed that *Brassica carinata* and *Brassica napus* were salt resistant in comparison with *Brassica campestris* and *Brassica juncea* (Ashraf & McNeilly, 1990). Thus this study was carried out to investigate to what extent salinity affects seed germination and early seedling growth, photosynthesis and protein activity of mustard and whether gas exchange related characteristics have any relation with chlorophyll fluorescence in mustard.

2. Materials and Methods

Mustard (*Brassica juncea* L. cv Gael yangjasaegas) was investigated for germination, early seedling stage, photosynthesis, and chlorophyll and protein content under salt stress in two experiments.

2.1 Germination and Early Seedling Growth

Germination and early seedling stage of mustard was observed by using Petri dishes with double filter papers saturated with 5 mL of distilled water or 50, 100 and 150 mM NaCl solution.Twenty seeds per replication with five replicate of dishes were used for each treatment. Afterwards, seeds were transferred to growth chamber at 25 ± 2ºC for germination in dark condition. Seed germination and germination rate were recorded after every 12 hours. The lengths and fresh weights of root and shoot of the plants were taken after15 days of the sowing.

2.2 Growth Condition for Greenhouse Experiment

Seedling were grown in plastic pots to investigate chlorophyll fluorescence and gas exchange attributes in sand culture experiment. The NaCl concentrations (control, 50, 100 and 150 mM) in Hoagland's solution (Hoagland and Arnon, 1950) were used to raise the plants after 15 days of sowing. All measurements were made after 28 days of salt treatments. The temperatures were 30 ºC and 20 ºC for day and night, respectively, and relative humidity ranged from 50% to 75% during the day and night. The average photosynthetic active radiation of the entire growth period was 1,095 $\mu molm^{-2}\ s^{-1}$.

2.3 PSII Photochemistry

PS II photochemistry was investigated at room temperature on upper surface (predarkened for 30 min) of leaves with a portable Mini PAM fluorometer (PAM-2000, Walz, Germany). The investigation procedure of Gentyet al. (1989) was fundamentally followed. Chlorophyll fluorescence measurements and calculations were made according to Jamil et al. (2007a).

2.4 Gas Exchange

Portable photosynthesis system (ADC 2250 Gas Analyser ADC, England) was used for measurement of photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration rate (E). All the measurements were recorded under ambient air composition (350 $\mu mol\ mol^{-1}\ CO_2$ and 210 $mmol\ mol^{-1}CO_2$). Water use efficiency (WUE) and intrinsic use efficiency (IUE) were calculated according to Ashraf (2001).

2.5 Number of Leaves, Leaf Area, Leaf Water and Chlorophyll Content

Area meter (AM-200, ADC BioScientific Ltd. England) was used for measurement of leaf area. After that, the weight of fresh leaves was taken and then were dried at 80 °C for 48 hour to find out the dry weight. The leaf water content was determined by using the procedure describe by Jamil et al. (2007a) while the portable chlorophyll content meter (CCM-200,Opti-Science USA) was used to determine the chlorophyll content.

2.6 Total Soluble Protein

Leaf protein was extracted by the procedure reported by Santoni et al. (1994) and total soluble protein was determined with Spectrophotometer according to Bradford (1976) method using bovine serum albumin as standards.

2.7 Protein Analysis

SDS-PAGE analysis of protein synthesis was performed by using the method of Laemmli (1970). Separating gel (12.5%) was made by solubilizing 20 µg protein with sample buffer (62.5 mMTris-HCl, pH 6.8, 20% (w/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.01% (w/v) bromophenol blue). Electrophoresis was performed at 80 V for 2 and half hours by using Bio-Radelectrophoresis system. Coomassie Brilliant (0.25%) Blue R-250 was used for staining for 2 hours and then destained with 50% methanol and 10% acetic acid. The molecular weight of protein was analyzed by comparing sample bands to the standard protein molecular weight marker bands (Protein Ladder 100-10KDa) in the electrophorogram.

2.8 Statistical Analysis

ANOVA was performed by using MS-Excel. The correlation was developed by using the Minitab statistical package. Mean values for above mentioned parameters were calculated by least significant difference (LSD) using a Tukey's test method (Li, 1964).

3. Results

3.1 Seed Germination and Seedling Growth

The seed germination decreased significantly with increasing NaCl concentration (Figure 1A). A significant reduction in germination was observed at 100 and 150 mM NaCl. Timing of seed germination was also affected by salinity (Figure 1B). A noticeable variation in timing was observed in the seed germination initiation and completion. In general, seed started to germinate within 36 hours and the germination was completed on the 5th day. The results showed that NaCl caused a significant diminution in seedling growth at 50 mM and at a higher salt concentration (Figure 1C and 1D). The reduction in root length with an increase in NaCl concentration was more pronounced than shoot length. Significant variations were also detected in fresh weight of root and shoot. The weight of the fresh root and shoot was declined by different NaCl solutions; while the fresh weight of shoot declined more than root fresh weight (Figure 1D).

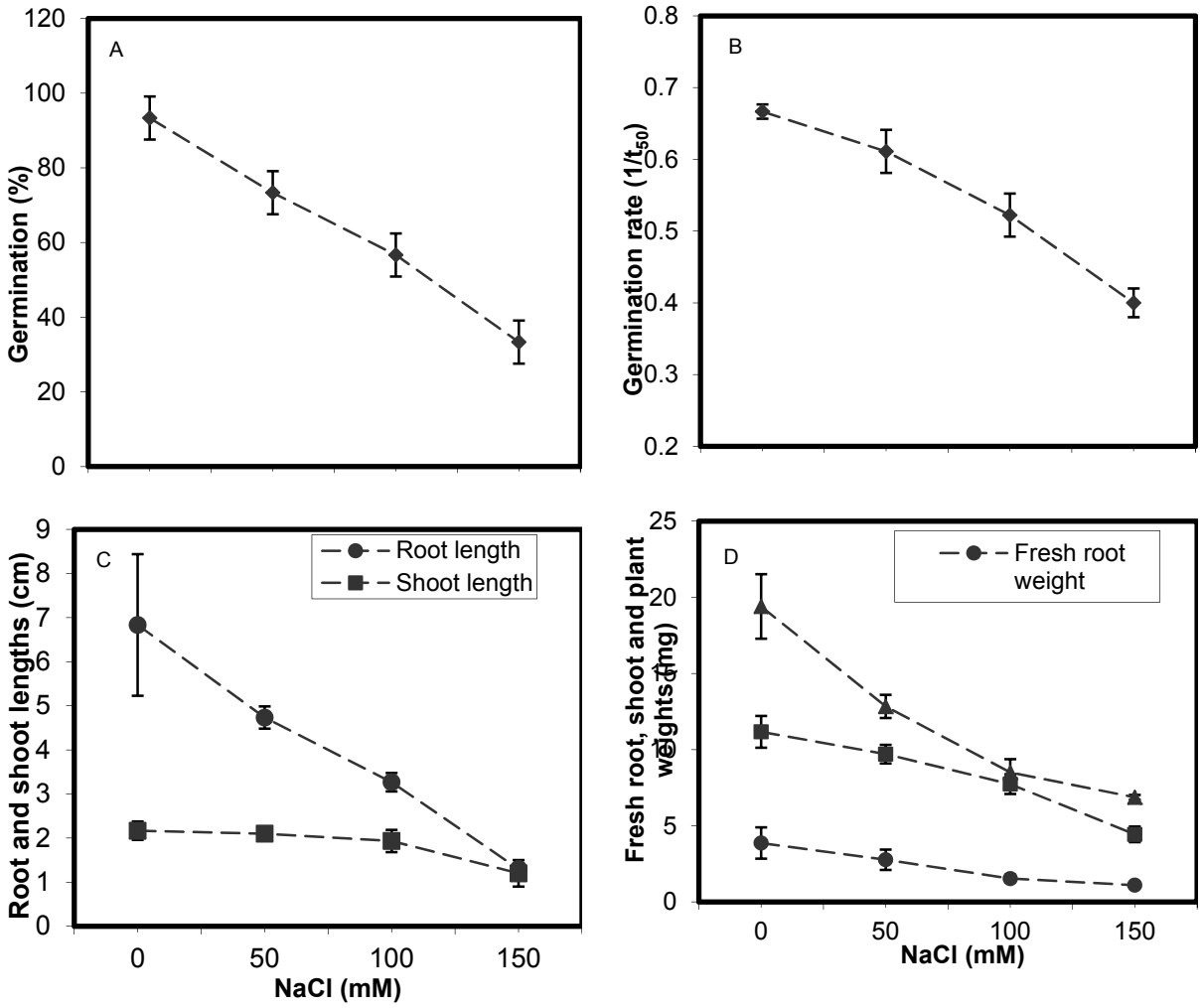

Figure 1. Germination (%) (A) and germination rate (B) root and shoot lengths (C) and fresh root, shoot and plant weights (D) of 15 days old seedling of mustard exposed to various concentrations of NaCl. Each point represents the mean of five replications ± SD

3.2 PSII Photochemistry

Fv/Fm was appreciably depressed with the rising NaCl particularly at high concentration (Figure 2A). Concurrently with this depression, the ETR also dropped constantly with the rise in the NaCl concentration (Figure 2C). This decrease was accompanied by an increase of NPQ (Figure 2B).

3.3 Gas Exchange

The net CO_2 assimilation rate decreased with increasing NaCl concentration (Figure 3A). The influence of salt stress on transpiration rate (Figure 3B) and stomatal conductance (Figure 3C) was similar to that on net CO_2 assimilation rate. Water use efficiency increased slightly at 50 mM as compared to control but then decreased at 100 and 150 mM NaCl (Figure 4A). Intrinsic water use efficiency, on the other hand decreased with the increasing external salt concentration (Figure 4B).

Correlation revealed a highly significant relationship between chlorophyll fluorescence and gas exchange attributes (Table 1). Significant positive (r^2=0.98 and 0.94 P<0.001) association was noted between g_s, Fv/Fm, and P_N. Correlation also revealed a weak (r^2=0.58, P=0.044) negative association between NPQ and WUE.

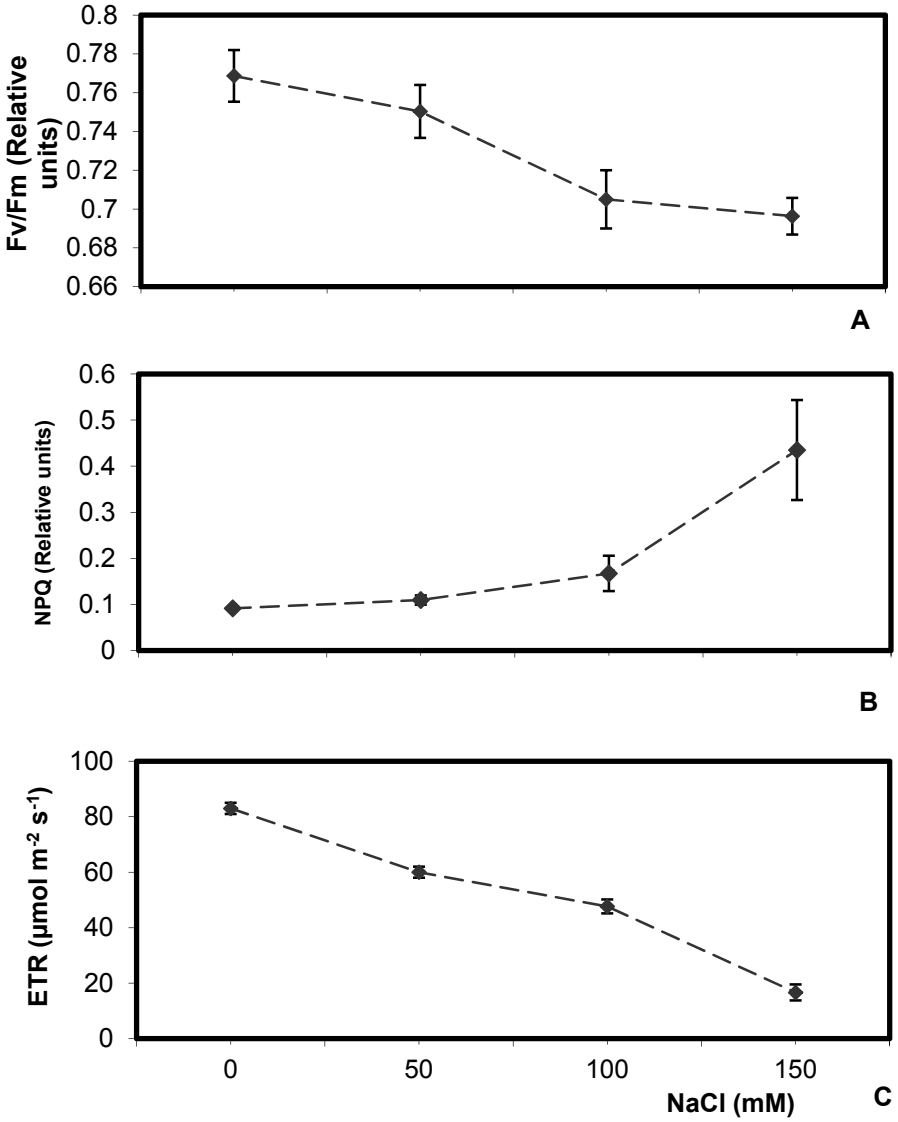

Figure 2. Effect of different concentration of NaCl on Fv/Fm ratio (A), non-photochemical quenching (B) and electron transport rate (C) of mustard. Each point represents the mean of three replications ± SD

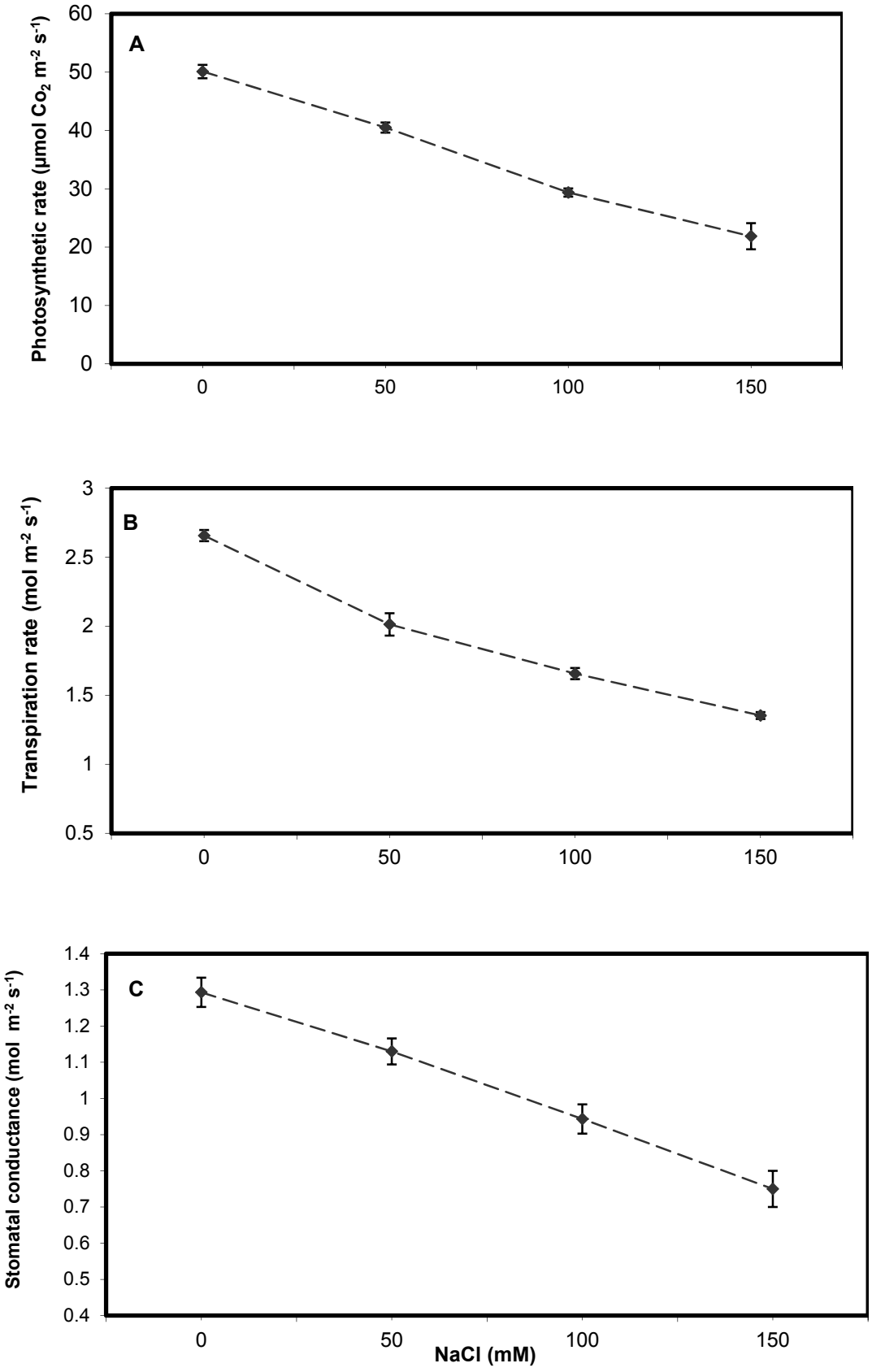

Figure 3. Effect of different concentration of NaCl on photosynthetic rate (A), transpiration rate (B) and stomatal conductance (C) of mustard. Each point represents the mean of three replications ± SD

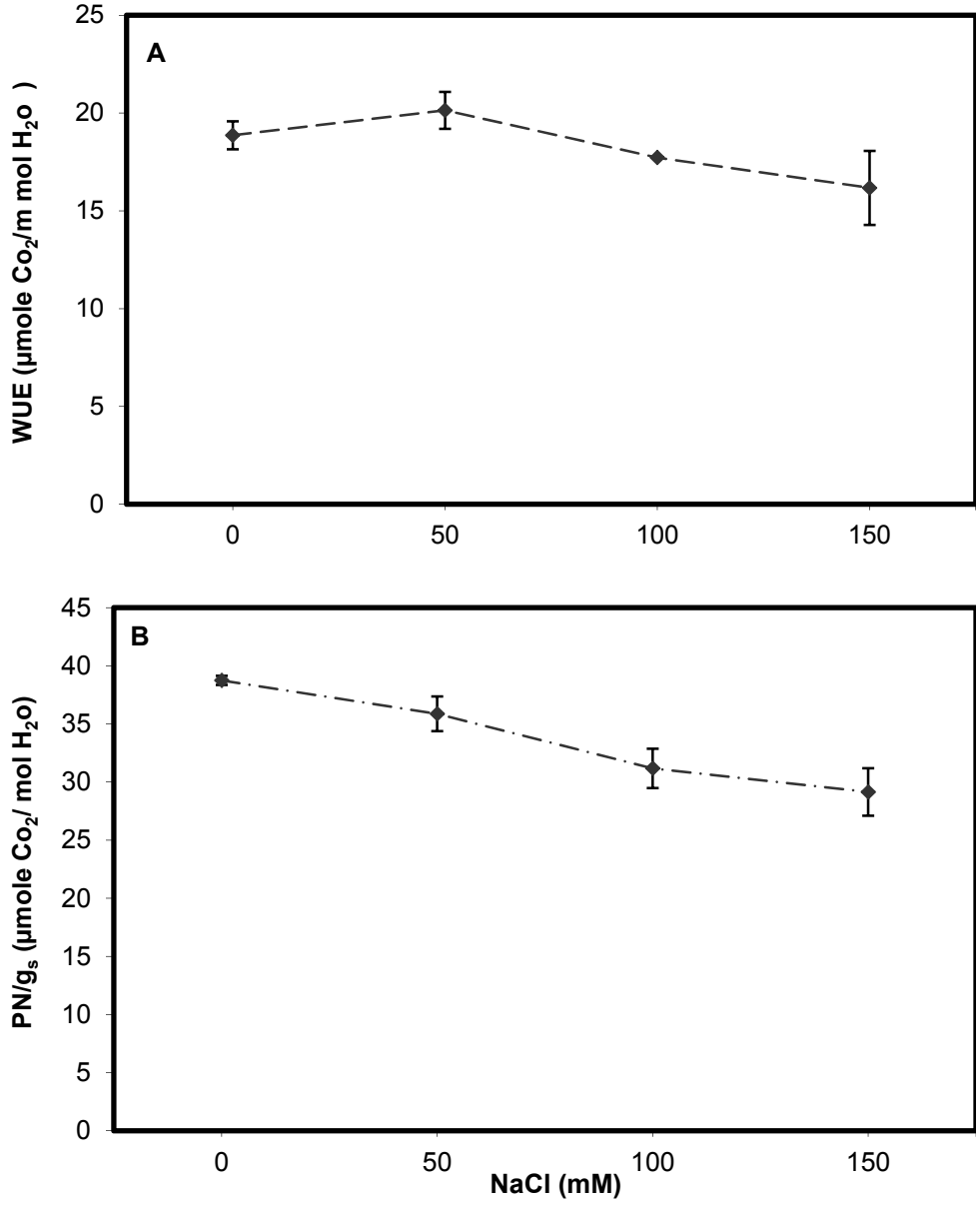

Figure 4. Effect of different concentration of NaCl on water use efficiency (WUE) (A), and intrinsic water use efficiency (P_N/g_s) (B) of mustard. Each point represents the mean of three replications ± SD

Table 1. The relationship between chlorophyll fluorescence and gaseous exchange attributes of mustard, when 20 days old seedling were exposed to different concentrations of NaClin sand culture for 28 days

	Fv/Fm	**NPQ**	**ETR**	**P_N**	**E**	**g_s**	**WUE**
NPQ	-0.672‡						
	0.017¶						
ETR	0.853	-0.890					
	0.000	0.000					
P_N	0.940	-0.775	0.961				
	0.000	0.003	0.000				
E	0.906	-0.749	0.954	0.975			
	0.000	0.005	0.000	0.000			
g_s	0.892	-0.815	0.972	0.984	0.948		
	0.000	0.001	0.000	0.000	0.000		
WUE	0.708	-0.588	0.655	0.721	0.553	0.748	
	0.010	0.044	0.021	0.008	0.062	0.005	
IUE	0.953	-0.696	0.889	0.959	0.934	0.901	0.704
	0.000	0.012	0.000	0.000	0.000	0.000	0.011

‡ = r^2, ¶ = P.

3.4 Number of Leaves, Leaf Area, Leaf Water and Chlorophyll Content

Increasing salt concentrations in the solution appreciably decreased leaf area and number of leaves (Figure 7A and 7B). However, salt stress had no significant impact on leaf water contents (Figure 7C). Salinity significantly increased leaf chlorophyll contents (Figure8) but the effect of 100 and 150 mM was far more than 50 mM NaCl (Figure 8).

3.5 Protein Content and Activity

There was an increase in the concentration of protein content with the corresponding increase in NaCl level up to 100 mM but at 150 mM, the protein content was decreased (Figure 5). SDS-PAGE analysis showed considerable variations in protein profiles. It was noted that the intensity of band expression increased significantly with increasing salt stress up to 100 mM but diminished at 150 mM (Figure 6).

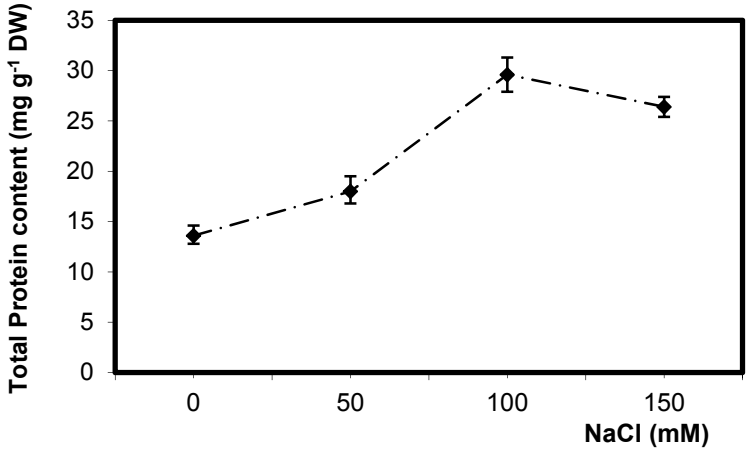

Figure 5. Effect of different concentrations of NaCl on total protein content of mustard. Each point represents the mean of three replications ± SD

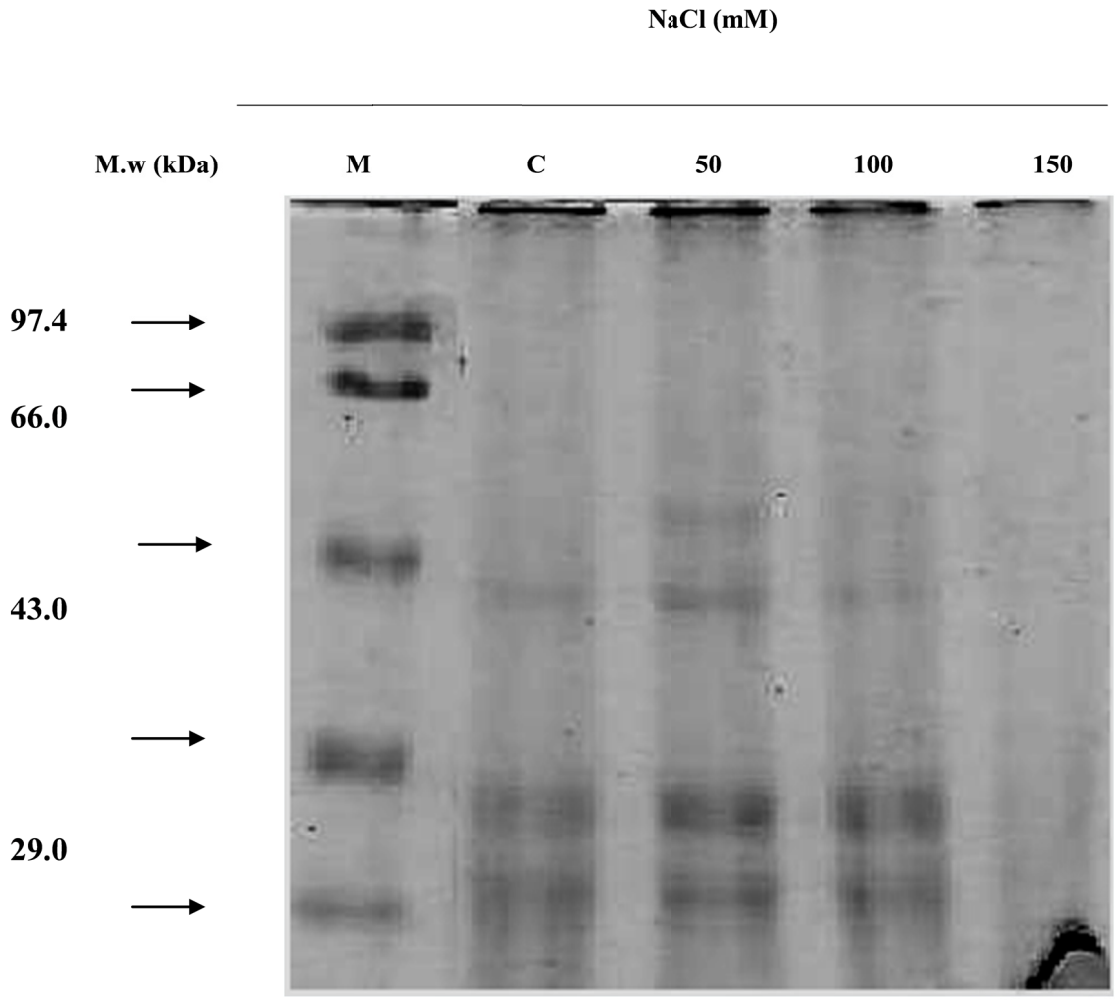

Figure 6. Effect of different concentrations of NaCl on polypeptide patterns of total leafprotein as analyzed on SDS-PAGE. Lanes C and 50, 100 and 150 represent proteins extracted from control and NaCl treated plants after 30 days of treatment and lane M represents a molecular weight marker

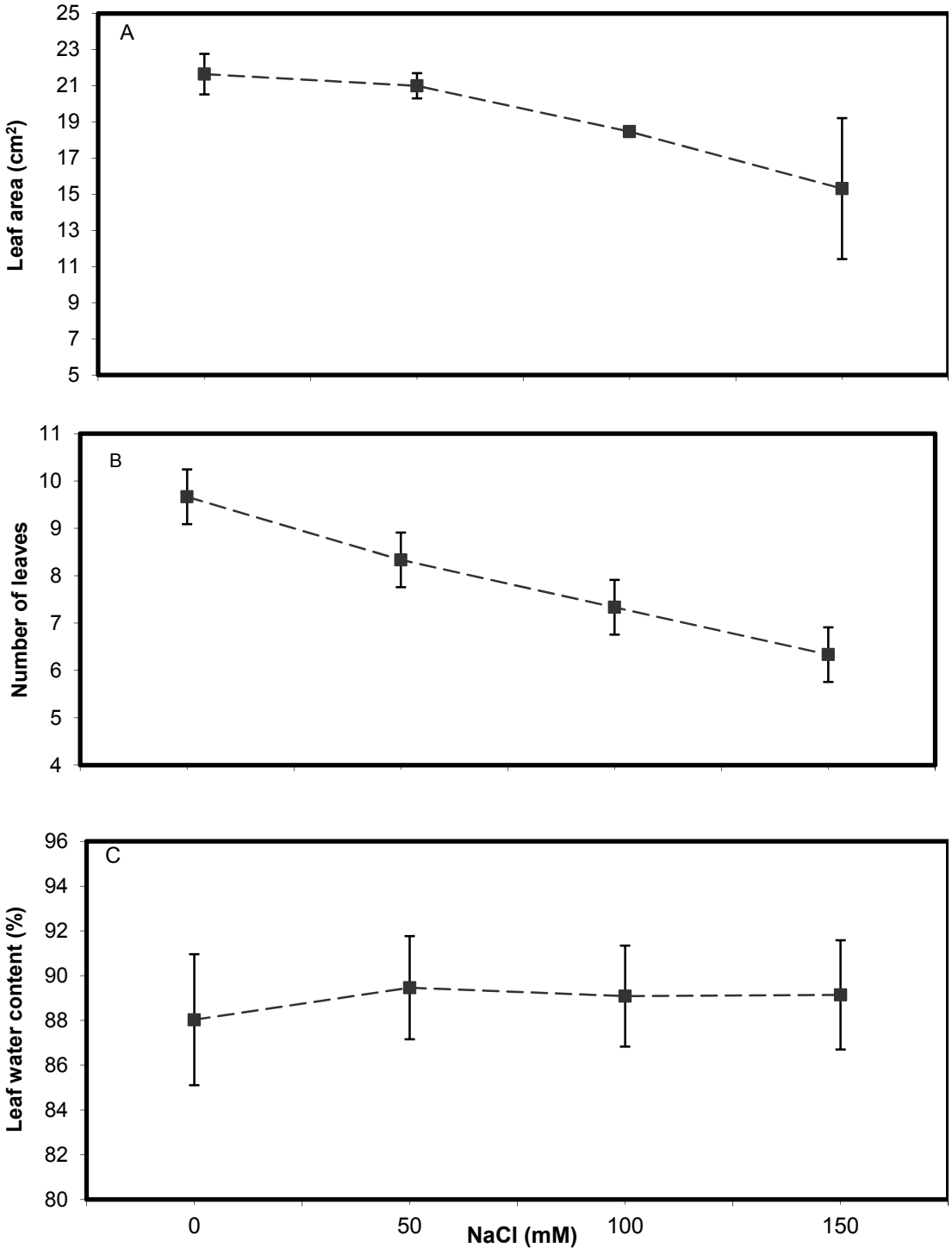

Figure 7. Effect of different concentration of NaCl on leaf area (A), number of leaves (B) and leaf water content (C) of mustard. Each point represents the mean of three replications ± SD

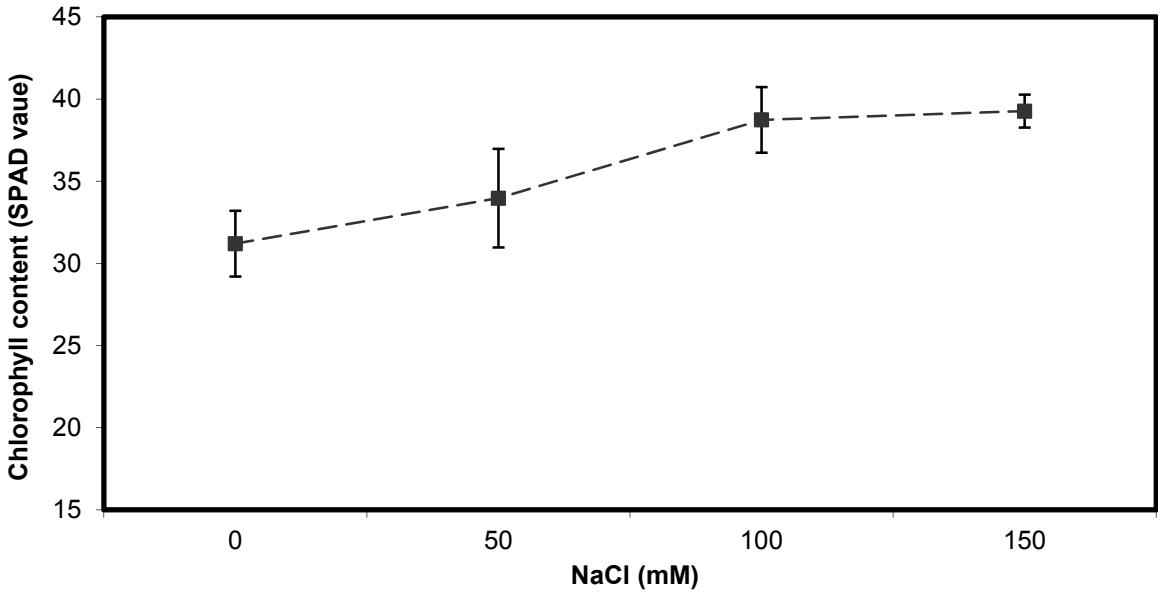

Figure 8. Effect of different concentrations of NaCl on chlorophyll content (SPAD value) of mustard. Each point represents the mean of three replications ± SD

4. Discussion

Seed germination and seedling growth are the most important stages of plant growth and development. In the present study, salt stress markedly reduced germination percentage and delayed germination rate (Figure 1A and B) which might be due to high osmotic potential or particular ion toxicity (Huangand, 1995). It was also reported that germination rate and the germination percentage declined with the reduction of the water passage into the seeds during imbibitions (Hadas, 1977; Kaya et al., 2006). It has been documented in various studies that seed germination significantly reduced with the increasing salinity in the growth medium (Gulzaret al., 2001; Jamil et al., 2007a, 2012). Salinity reduced seedling growth with the increasing NaCl concentration (Figure 1C and D).The effects of the salinity proved more discernible on the root length than shoot (Figure 1C) while a shoot fresh weight was affected more than root fresh weight (Figure 1D). The decrease in lengths of root and shoot may be due to slowing down the water absorption by the plant (Werner & Finkelstein 1995). Demir and Arif (2003) also obtained similar results. They have found that the root growth was more badly influenced by salinity than shooting growth. It was observed that fresh mass of root and shoot of cultured plants was considerably abridged by increasing the salt concentration (Jeannette et al., 2002).

A decline in quantum yield of PSII was observed in our experiment with increasing salinity (Figure 2A), may result from the closure of stomata induced by osmotic stress and the accumulation of salt (Dionisio-Sese & Tobita, 2000). Similar results have been reported by Misra et al. (2001). They investigated that salinity affected the process of photosynthesis in mung bean and Brassica seedlings. A change in PS II, is recommended by the boost of NPQ in plants developed under salt condition (Figure 2B). The change in NPQ may also imitate the fact that decreased CO_2 assimilation reduces the requirement for production of electron transport, and therefore increases thermal dissipation of light energy. A significant relationship was observed between Fv/Fm, ETR, NPQ and CO_2 assimilation (Table 1) as has already been observed in sorghum (Cornic & Briantais, 1991). There was a reduction in ETR between control and 150 mM NaCl (Figure 2C). Salinity increases the deposition of NaCl in chloroplasts influence growth rate, and is frequently linked with a decline in photosynthetic electron transport activities in photosynthesis (Kirst, 1989). P_N was markedly decreased by salt stress (Figure 3A) and this was accompanied by a decrease in g_s (Figure 3C). Transpiration rate also decreased considerably with increasing salt concentration (Figure 3B). A positive significant (r^2=0.98 and P<0.001) correlation between P_N and g_s (Table 1) may suggest that the reductions in P_N were largely associated with stomatal closure, and therefore stomatal effects could be the most important to justify photosynthesis depression. WUE increased to some extent at 50 mM NaCl but then decreased (Figure 4A) while IUE decreased with the increasing external salt concentration (Figure 4B). A positive correlation was also observed between WUI, IUE, and photosynthesis (Table 1) as which had already been observed in Brassica species (Ashraf, 2001; Nazir et al., 2001).

It has been reported since 1940 that variations in chlorophyll fluorescence are the regular signs of variations in photosynthetic activity (McAlister & Myers, 1940; Kautsky & Zedlitch, 1941). In recent times, it was observed that chlorophyll fluorescence could be used to estimate the quantum effectiveness of electron transport through PSII in leaves (Genty et al., 1989) and this PSII is associated with CO_2 assimilation (Genty et al., 1989; Genty & Harbinson, 1990). Similar kind of association was observed between Fv/Fm, NPQ, ETR and P_N,g_s, and E in our experiment (Table 1).The increasing salinity of the growth solution caused a considerable reduction in leaf area and the number of leaves (Figure7A and 7B) while non significant differences was observed in water leaf content (Figure 7C). Inhibition in leaf growth of salt stress may be probably take place through an effecton this region (Lazof & Bernstein, 1998).It has been reported that different concentration of NaCl had significantly decreased the number of leaves per plant (Seema et al., 2003). Leaf area reduced consistently andsignificantly per plant as NaCl concentration increased from low to high salt concentration (Grieve et al., 1999). Chlorophyll content increased significantly (Figure 8) may be due to the deposition of NaCl in the chloroplast. It has been documented that the chlorophyll content decreased in plants such as tomato (Lapina & Popov, 1970), radish (Jamil et al., 2007a), rice (Jamil et al., 2012) and pea (Hamada & El-Enany, 1994), but has been increased in some plants such as pearl millet (Reddy & Vora, 1986), mustard (Sing et al., 1990) sugar beet and cabbage (Jamil et al., 2007b) under saline condition.

Proteins generally deposit in plants grown under stress condition and they may supply a storage form of nitrogen which play a vital role in osmotic adjustment, which is utilized after stress (Singh et al., 1978), SDS-PAGE revealed that the intensity of band expression increased significantly as with increasing salt stress up to 100 mM but diminished at 150 mM (Figure 5). Similar kind of trend was also observed in protein content, which increases up to 100 mM but at 150 mM it was decreased (Figure 6). Agastianet al. (2000) investigated that soluble protein increases at low salt concentration and decreases at elevated levels in mulberry. Similarly, Bozarth et al. (1987) noted the deposition of membrane protein in the cell wall portion of the soybean stem when seedlings were treated with abiotic stress.

In conclusion, salinity strongly affects seed germination and early seedling growth, photosynthesis and protein synthesis. The gas exchange attributes had a strong association with PS II photochemistry of mustard under different concentration of salt stress.

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