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GAS EXCHANGE ATTRIBUTES CAN BE VALUABLE SELECTION CRITERIA FOR SALINITY TOLERANCE IN CANOLA CULTIVARS (*BRASSICA NAPUS* L.)

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Abstract

Present study was carried out to access inter-cultivar variation for salt tolerance in canola (*Brassica napus* L.) by using photosynthetic attributes including photosynthetic pigments as selection criteria. Four cultivars of canola viz., Oscar, Ac Excel, Cyclone and Dunkled were screened at 120 mM NaCl at vegetative stage. Salt stress reduced photosynthetic rate (*A*), transpiration rate (*E*), stomatal conductance (g_s), sub-stomatal CO_2 (*Ci*) conc. and water use efficiency (WUE) at different growth stages. Salt stress also markedly reduced chlorophyll a, chlorophyll b and total chlorophyll contents. Generally, plant biomass declined under the salt regime of all the cultivars. Nevertheless, cultivar Dunkled had higher, Oscar and Ac Excel intermediate while; Cyclone had lower shoot and root fresh weight under saline conditions. However, cultivar Cyclone was lower in chlorophyll a and chlorophyll b under the salt stress in contrast to Ac Excel. A significant variation was also shown in gas exchange attributes under the stress. Photosynthetic (*A*) and transpiration rates (*E*) was higher in Dunkled than of Oscar and Cyclone under the stress. On the basis of data, it may be concluded that fresh weight of shoots and roots had positive correlation with physiological photosynthetic rate (*A*) among all the four cultivars; thus, photosynthetic rate (*A*) can be an effectual selection criteria for salt tolerance under salt regime.

Introduction

Salt stress adversely affects growth and yield of different crop species at world level (Schwabe *et al*., 2006, Hamayun *et al*., 2010). Salt of sodium and chloride reduce seed germination, seedling stage and growth of many agricultural crops (Munns, 2005). It is reported that out of various abiotic stresses, salinity stress is the imperative one cruelly affects morphological, physiological and biochemical attributes such as gas exchange and photosynthetic pigments (Netondo *et al*., 2004; Chaum & Kirdmanee, 2009, Narusaka *et al*., 2003). It is investigated that salinity stress markedly reduced net photosynthetic rate (*A*) in oilseed crop safflower (Siddiqi *et al*., 2009), Soyabean (Hamayun *et al*., 2010a), sunflower (Noreen & Ashraf, 2008) and in *Pisium sativum* (Yildirim *et al*., 2008).

It is documented by different plant physiologists that various morphological, physiological and biochemical parameters can be used as effective selection criteria for salinity tolerance for inter-cultivar variation in various crop species (Ashraf, 2004, Munns, 2007, Noreen & Ashraf, 2008, Siddiqi *et al*., 2009, Khan *et al*., 2010). For example, Yildirim (*et al*., 2008) evaluated eleven pea cultivars at 150 mM NaCl, only three were found as salt tolerant using chlorophyll a as selection criteria. Taffouo *et al*., (2009), while working with cowpea, screened 21 lines using different levels of sodium chloride. Only 8 lines of cowpea were selected as salt tolerant using chlorophyll b as selection criteria due to less reduction in chlorophyll content under salt regimes. In a similar study, three varieties of *Hordeum vulgare* were screened at 12 m*M* NaCl on the basis of photosynthetic pigments. Only one variety was selected as tolerant due to increase in photosynthetic pigments under salt stress (Demiral *et al*., 2005). Twenty five lines of wheat (*Tritcum aestivum* L.) were screened at different levels of NaCl using net CO_2 assimilation rate as selection indicator. Only five lines were ranked as tolerant due to less reduction in photosynthetic rate (*A*) under saline conditions (Ashraf & Shahbaz, 2003; Raza *et al*., 2066). While working with sunflower, Hebbara *et al*., (2003) screened 6 cultivars at various levels of sodium chloride using stomatal conductance (g_s) as selection indicator. Only one cultivar ranked as salt tolerant due to less reduction in stomatal conductance (g_s) under stress conditions. Using water use efficiency (WUE) as a physiological selection indicator, six lines of tomato (*Lycopersicum esculentum* L.) were evaluated at 150 m*M* NaCl. Only Three lines were selected as tolerant to NaCl on the basis of high value of WUE in leaves under salt regimes (Reina-Sanchez *et al*., 2005). Hebbara *et al*., (2003) evaluated 6 accessions of sunflower at 150 m*M* of sodium chloride on the basis of high value of transpiration rate (*E*). Only one accessions PAC-36 was ranked as tolerant due to high performance of transpiration rate (*E*) under saline conditions. Pakniyat & Armion (2007), working with *Beta vulgaris* assessed twenty cultivars at different levels of sodium chloride on the basis of osmotic adjustment. Only eight accessions were ranked as tolerant on the basis of higher proline accumulation in leaves as a potential biochemical indicator. Ten lines of safflower were evaluated at 150 m*M* of sodium chloride using proline as a potential biochemical indicator. Only two accessions Saff-37 and Saff-38 were ranked as salt tolerant on the basis of high accumulation of proline in leaves (Siddiqi *et al*., 2009). From the above investigations, it is evident that various potential physiological and biochemical indicators can b used for selection and breeding purpose under saline conditions. Out of various potential physiological and biochemical selection criteria, net CO_2 assimilation rate (*A*) can be used as more effective one under salt stress (Yilidirim *et al*., 2008; Taffouo *et al*., 2009; Siddiqi *et al*., 2009).

In this view, the present research work was carried out to access whether photosynthetic attributes can be used as potential physiological indicator evaluating the existing canola cultivars for salinity tolerance.

Materials and Methods

Present research work was carried to access inter-cultivar variation for salinity tolerance in a potential oilseed crop canola (*Brassica napus* L.). An experiment was conducted in the research area University of Gujrat. Seed of four canola cultivars were obtained from Ayub Agriculture Research Institute (AARI), Faisalabad, Pakistan. To avoid contamination, seeds were sterilized by using 5% sodium hypochloride solution for 10 min. After sterilization process, seeds were rinsed with double distilled water. Plastic pots (25.5 cm diameter) were filled with 10 kg river washed sand. Ten healthy seeds were sown in each plastic pot. Hoagland nutrition (full strength) was applied to each pot. After 21 days of germination, NaCl treatment was applied with Hoaglant nutrient solution. Treatment was 0 m*M* NaCl as control and 120 m*M* NaCl as salt stress. NaCl treatment was applied step wise by adding 30 m*M* NaCl daily to achieve final salinity level. 250 ml of distilled was applied to each pot daily to avoid evaporation loss. Morphological, physiological/biochemical data was recorded after the start of seven weeks of NaCl treatment.

Gas exchange attributes: Gas exchange parameters were determined using an LCA-4 ADC open system portable infrared gas analyzer (ADC, Hoddesdon, England). These parameters were determined in noon when light intensity was full (at 09 a.m. to 2.00 p.m.). A young fully expanded leaf was selected and used for the data of photosynthetic rate (*A*), transpiration rate (*E*), sub-stomatal CO_2 concentration (*Ci*) and stomatal conductance (g_s) were noted.

The conditions which were used for the equipment/leaf chamber were as follows: ambient pressure 99. 2 kPa , atmospheric CO_2 concentration (Cref) 352 μmol mol-^{1}, leaf surface area 11.35 cm^2, , PAR (Qleaf) was maximum up to 1048 μmol m^{-2} s^{-1} and the chamber water vapor pressure varied from 4.0 to 5.8 mbar (Ali *et al*., 2008).

Water use efficiency (WUE): The ratio of photosynthetic rate (*A*) divided by transpiration rate (*E*) was used as water use efficiency.

Chlorophyll content: The concentration of chlorophyll contents were determined by using the method of Arnon (1949). Fresh leaves (1g) ware obtained from each replicate, chopped it into very fine pieces, placed in test tube containing 10 ml of 80% acetone for overnight. On the coming day, chlorophyll sap was filtered using a filter paper (Whatman No.1). Absorbance of the samples was read at 645, 652 and 663 nm with a Hitachi spectrophotometer (Hitachi, Model U2001, Tokyo, Japan). Chl. a & Chl. b was calculated using following formula:

"Chl. a (mg g-^{1} f.wt.) = [12.7 (OD 663)-2.69(OD 645) ×V/1000 x W]"

"Chl. b (mg g-^{1} f.wt.) = [22.9 (OD 645)-4.68(OD 663) ×V/1000×W]"

where W = weight of fresh leaf tissue (g), V = volume of the leaf extract (ml)

After measuring all morphological/physiological parameters, plants were uprooted from each pot and separated into their roots and shoots. These roots and shoots were washed with double distilled water and dried with blotting papers. Fresh weight of roots and shoots were recorded using analytical balance.

Statistical analysis of data: A completely randomized design (CRD) was used to analyze research work in five replicates. The data obtained was analyzed statistically by using Analysis of Variance (ANOVA) technique COSTAT statistical. Package (CoHort software, Berkeley, USA).

Results

Salt stress significantly reduced shoot fresh weight of all canola cultivars. A considerable variation was found in all canola cultivars in this parameter. Cultivar Dunkled had higher value of shoot fresh weight as compared to Oscar, Cyclone and Ac Excel under saline conditions (Fig. 1.)

Root fresh weight of all canola cultivars significantly decreased due to imposition of salinity in the rooting medium. All canola cultivars showed different response in this parameter. Cultivar Dunkled and cyclone showed higher while Oscar and Ac excel lower value of root fresh weight under salt regimes (Fig. 1).

Photosynthetic rate (*A*) showed substantial reduction in all canola cultivars under saline conditions. All canola cultivars showed variable response into photosynthetic rate (*A*). Cultivar Dunkled had increased, Oscar and Cyclone decreased while Ac Excel had intermediate value under root zone salinity (Fig. 1).

Transpiration rate (*E*) significantly reduced in all canola cultivars due to addition of NaCl to rooting medium. All canola cultivars showed a considerable variation in this parameter. Higher value of transpiration (*E*) was found in Dunkled, lower in Oscar and Cyclone while intermediate in Ac Excel under salt stress (Fig. 1).

Stomatal conductance (g_s) of all canola cultivars was markedly reduced due to NaCl stress and all cultivars showed a significant variation in this attribute. Higher value of Stomatal conductance (g_s) was observed in Ac Excel and Dunkled while lower in Cyclone and Oscar due to imposition of sodium chloride in the rooting medium (Fig. 1).

Salt stress significantly reduced sub-stomatal CO_2 *(Ci)* concentrations in all canola cultivars. A considerable variation was recorded in this parameter. Higher sub-stomatal CO_2 concentrations were found in Ac Excel and Dunkled while lower in Oscar and Cyclone due to root zone salinity (Fig. 2).

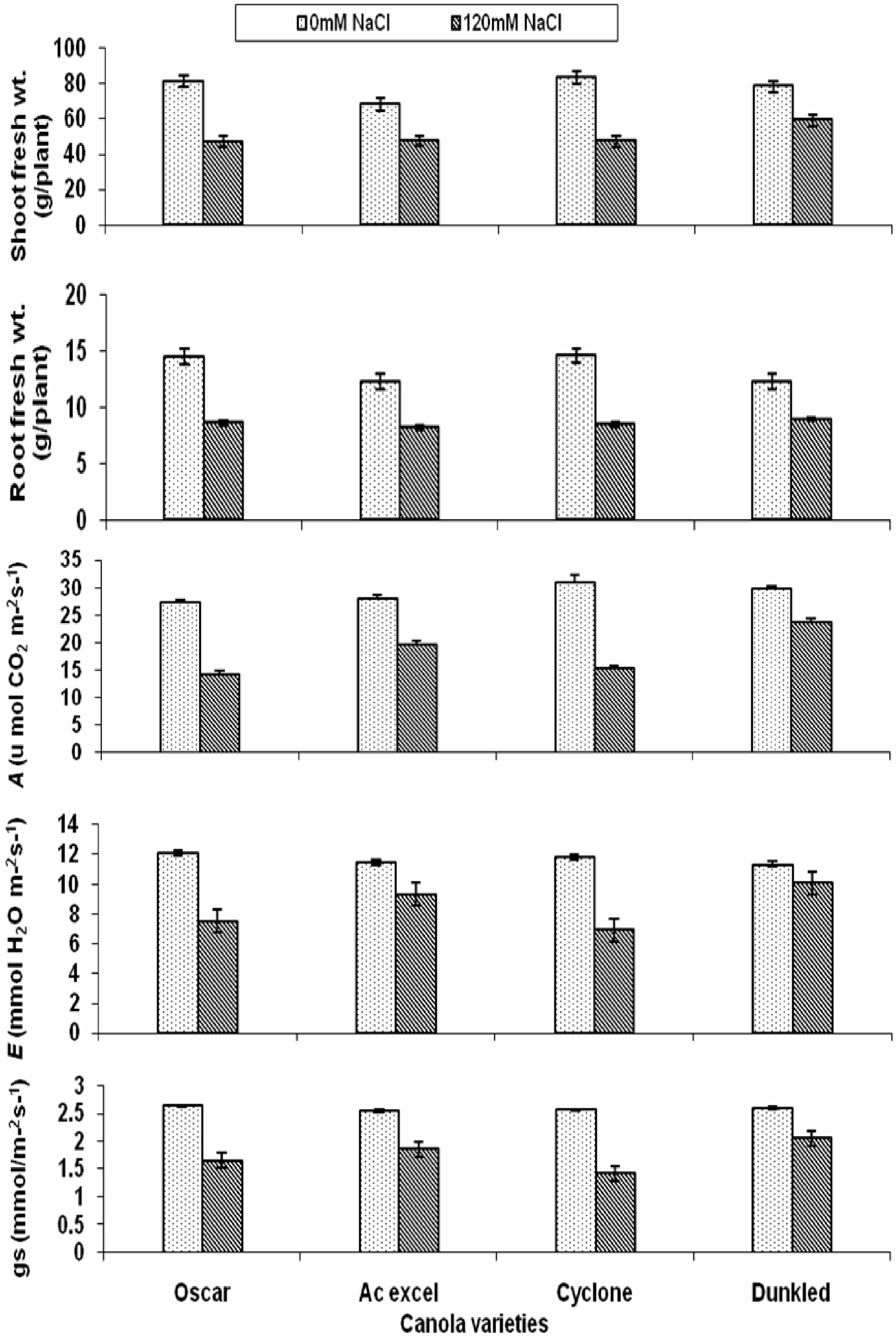

Fig. 1. Fresh weight of shoots and roots and gas exchange parameters (A, E, g_s) of four canola cultivars when 21 day old plant subjected to NaCl stress for 48 days. (Mean ± S.E; n= 5).

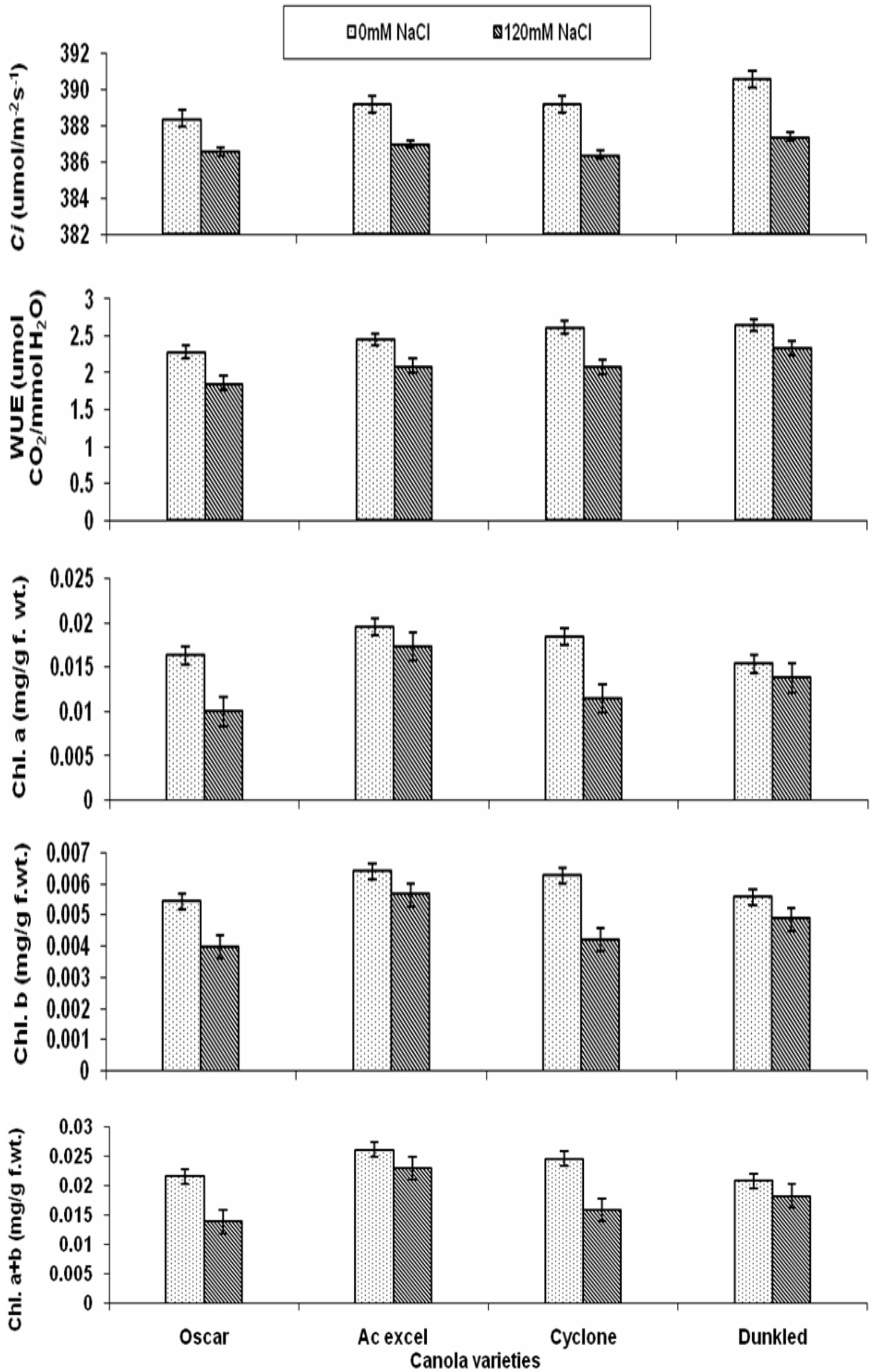

Fig. 2. Gas exchange parameters (Ci, WUE) and photosynthetic pigments (Chl a, b. a+b) of four canola cultivars when 21 day old plant subjected to NaCl stress for 48 days. (Mean ± S.E; n= 5).

Reduction in water use efficiency (WUE) was found in all canola cultivars due to addition of sodium chloride in the rooting medium but there is a little variation in canola cultivars in this parameter (Fig. 2).

Salt stress significantly decreased chlorophyll a in all canola cultivars. A significant variations were observed in all canola cultivars in chlorophyll a. Cultivar Ac excel had higher, Oscar had lower, while other two cultivars had intermediate value of chlorophyll a under saline conditions (Fig. 2).

Chlorophyll b showed marked reduction in all canola cultivars under salt stress. All canola cultivars showed clear variations in this attribute. Chlorophyll b in Ac Excel had highest value as compared to Oscar, Cyclone and Dunkled under NaCl stress.

Root zone salinity significantly reduced total chlorophyll (Chl. a+b) content in all canola cultivars. Ac Excel had higher value of total chlorophyll content than those of Cyclone, Dunkled and Oscar under sodium chloride stress (Fig. 2).

Discussion

Of various physiological and biochemical attributes, assessment of all gas exchange and photosynthetic pigments parameters in various crop species is essential one so as to enhanced plant productivity in salt affected areas. It is investigated that reduction in photosynthetic parameters under salt stress, reduces shoots and roots fresh weight and ultimately adversely affects crop growth and yield (Ashraf, 2004; Athar & Ashraf, 2005; Ulfat *et al*., 2007; Sidiqi *et al*., 2009).

In the present study, salt stress significantly net CO_2 assimilation rate (*A*), this ultimately reduces biomass production. These findings are closely related to earlier studies in various crop species e.g. safflower (Siddiqi *et al*., 2009), wheat (Arfan *et al*., 2007). From these observations, it is clear that inter-accession variation in canola cultivars for salt tolerance were only due to genetic variation in photosynthetic rate (*A*) which could be used as effective selection criteria for salt tolerance in different crops.

Addition of NaCl to rooting medium also caused a marked reduction in transpiration rate (*E*) in all canola cultivars, this result correlates with earlier findings on different crops e.g. Sunflower (Noreen & Ashraf, 2008), safflower (Siddiqi *et al*., 2009), wheat (Shahbaz & Ashraf, 2003; Raza *et al*., 2007). Salt stress significantly reduced stomatal conductance (g_s) in all canola cultivars and also showed inter-cultivar variations, these result are analogues to earlier findings in different crops (Shahbaz & Arhraf., 2003; Arfan *et al*., 2007; Raza *et al*., 2006). Sub-stomatal CO_2 concentrations (*Ci*) and water use efficiency (WUE) were significantly reduced in all canola cultivars due to addition of sodium chloride to rooting variations in these traits which analogues to earlier findings in various crop species e. g. sunflower (Hebbara *et al*., 2003), wheat (Ashraf & Shahbaz, 2003; Raza *et al*., 2006; Arfan *et al*., 2007), safflower (Siddiqi *et al*., 2009) and pea (Yildirim *et al*., 2008).

Chlorophyll contents (chl.a and chl. b) play a vital role in photosynthetic process which ultimately increases crop growth and yield (Taiz & Zieger, 2006). Reduction in chlorophyll contents further reduces photosynthetic process under saline conditions (Delfine *et al*., 1999; Ashraf, 2004, Hamayun *et al*., 2010b). In the present study, salt stress markedly reduced photosynthetic pigments such as chlorophyll a, b and total chlorophyll (Chlorophyll a+b) in all canola cultivars. Reduction in chlorophyll contents reduces biomass production and ultimately decline yield of canola cultivars. These results are analogues to earlier findings in different crops e.g., *Helianthu annuus* (Ashraf & Sultana, 2000), cotton (Meloni *et al*., 2003, soybean (Essa & Dawood, 2001), wheat (Raza *et al*., 2006), *Pisum sativum* (Yildirim *et al*., 2008), cowpea (Taffouo *et al*., 2009) and safflower (Siddiqi *et al*., 2009).

From the present research work, it is concluded that inter-accessions variations was found in all canola cultivars under saline conditions. Various morphological, physiological and biochemical traits were observed, only photosynthetic rate (*A*) can b used as an effective selection indicator for salt tolerance in four canola cultivars.

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