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Assessment of Inter-Cultivar Variations for Salinity Tolerance in Winter Radish (*Raphanus sativus* L.) Using Photosynthetic Attributes as Effective Selection Criteria

[1]*Saba Munir*, [1]*Ejaz Hussain Siddiqi*, [1]*Khizar Hayat Bhatti*, [1]*Khalid Nawaz*, [1]*Khalid Hussain*, [2]*Rizwan Rashid and* [2]*Iqbal Hussain*

[1]Department of Botany, University of Gujrat, Gujrat, Pakistan
[2]GC University, Faisalabad, Pakistan

Abstract: The present study was conducted to assess the physiological and biochemical responses of winter vegetable radish (*Raphanus sativus* L.) to salinity stress. Salt stress markedly reduced biomass production of both radish varieties. Shoot fresh and dry weights were significantly reduced under salt stress, higher reduction was found at 120 m*M* NaCl as compared. Salt stress significantly reduced chlorophyll contents in both radish varieties. Higher contents of chl. a&b chl. a/b ratio was found in variety Deci White while, lower in Lal Pari under different levels of sodium chloride. Physiological parameters such as net photosynthetic rate, transpiration rate, stomatal conductance, sub-stomatal CO_2 conc. and water use efficiency was decreased in both radish varieties under salt stress. Out of various physiological and biochemical indicators, net photosynthetic rate and chlorophyll content can be used as more effective selection indicator for salinity tolerance in winter vegetable radish.

Key words: Assessment · Salinity tolerance · Radish · Photosynthetic attributes

INTRODUCTION

Accumulation of various salts in water or soil creates great dilemma to various agricultural crops worldwide [1]. Out of different soluble salts, sodium and chloride severely abridged growth and yield of various crops than those of other salts [2]. Salinity stress can detrimentally affect a variety of physiological and biochemical processes, more importantly the photosynthesis. Severely reduction in crop productivity linked due to reduction in net photosynthetic rate under salt stress [3]. Salt stress significantly reduced transpiration and net CO_2 assimilation rate in various crops such as pea [4], *Brassica* species [5], wheat [6], sorghum [7], cotton [8], sunflower [9], *Phaseolus vulgaris* [10] and *Carthamus tinctorius* [11] under salt regimes. Different selection indicator could be used to assess inter-cultivar variation for salinity tolerance in different potential crops [5]. For example, Sabar and Ashraf, 2008 evaluated 10 genotypes of pearl millet al different salinity levels using photosynthetic rate as a selection criteria for salt tolerance. Chlorophyll content was used as best selection indicator for salinity tolerance in six soybean genotypes [12]. However, for selection and breeding purpose, there is always a need to choose that selection criteria, the selection based on which must give rise plants tolerant to a stress. As is evident from the above mentioned studies, a number of physio-biochemical indicators have been recommended for screening germplasm of different crops. However, photosynthetic attributes are considered very important in view of their direct role in sustaining plant growth under saline stress [11].

In view of this, the present research work was conducted to evaluate whether photosynthetic pigments as well as net CO_2 assimilation rate could be used as effectual selection indicator for screening available radish cultivars for salinity tolerance.

MATERIAL AND METHODS

An experiment was conducted in the research area of Botany Department, University of Gujrat, Gujrat. Pakistan. Pots were filled with 10kg fresh river sand and washed two times, firstly sand was washed with tap water then

Corresponding Author: Saba Munir, Department of Botany, University of Gujrat, Gujrat, Pakistan GC University, Faisalabad, Pakistan.

with distill water. Seeds of two radish (Raphanus sativus L.) varieties were obtained from AARI, Faisalabad, Pakistan. Seeds were surface satirized with 10% sodium hypochloride (NaOCl) solution for two minutes, then washed with distill water to remove the NaOCl (Sodium hypochloride) agents. Healthy 10 seeds were directly sown in sand with equal distance and depth. Full strength Hoagland's nutrient solution was applied after 10 days of germination. NaCl treatment was applied twenty days after the start of germination. Three levels of NaCl treatment 0, 60 and 120 mM NaCl were used. The NaCl treatment was applied split doses adding 30 m*M* every day to each pot until the desired salt treatment levels were attained. After five weeks of salt treatment, data different biochemical and physiological attributes were noted.

Gas Exchange Parameters: Gas exchange parameters such as net CO_2 Assimilation (A) rate, transpiration rate (E), sub stomatal CO_2 concentration (C_i) and stomatal conductance (g_s) were noted at noon in full sun using an imported LCA-4 ADC open system infrared gas analyzer (IRGA) (Analytical Development Company, Hoddesdon, England). Youngest healthy expanded leaf was selected for the measurement of different gas exchange parameters. The given conditions for IRGA were used; leaf surface area 11.35 cm^2, ambient CO_2 conc. 352 ìmol mol^{-1}, leaf chamber flow rate (V) 251, temperature of leaf chamber varied from 31.5 to 37.8°C, ambient pressure 99.2 kpa, molar flow of air per unit leaf area (Us) 22.06 mol m^{-2} s^{-1}, chamber vapor pressure varied from 4.4 to 6.6 mbar and PAR (Qleaf) at was maximum up to 1048 ìmol m^{-2} s^{-1}.

Water Use Efficiency (WUE): Water use efficiency was measured using net photosynthetic rate over transpiration rate (A/E)

Fresh Weight of Shoot and Root (G): Fresh weight of shoots and roots of radish were measured after five weeks of harvesting plants. Each plant was washed with distilled water and separated into roots and shoots. Fresh weights of root and shoot were measured in analytical weighing balance.

Chlorophyll Contents (mg g^{-1} f. wt.): Concentration of chlorophyll contents were recorded using the method of Arnon (1949). Fresh leaf sample 0.5 g was taken and grinded with pistil and mortar using 10 ml of 80% acetone solution. The mixture was mixed with a vortex mixer and the absorbance of the leaf samples was read at 645, 652 and 663 nm with a Hitachi spectrophotometer (Hitachi, Model U2001, Tokyo, Japan). Following formulae were used to calculate the content of chlorophyll a and b.

$$\text{Chl. a (mg g}^{-1}\text{ f.wt)} = [12.7 \text{ (OD 663)} - 2.69 \text{ (OD 645)}] \times \text{V/1000} \times \text{W}$$

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

V = Volume of the Extract (ml)
W= Weight of the Fresh Leaf Tissue (g)

Experimental Design and Statistical Analysis: The design of the experiment was completely randomized (CRD) with three treatment and three replicates. The data for each attribute was subjected to statistical analysis for comparison of means using appropriate statistical packages using the MSTAT computer package (MSTAT Development Team, 1989).

RESULTS

Analysis of variance of the data showed that salt stress significantly reduced shoot fresh weight of both radish varieties. Remarkable variation was found in both varieties for this parameter. Maximum shoot fresh weight was observed in Deci White radish while lowest in Lal Pari under saline conditions. Shoot dry weight was significantly reduced in both varieties of radish under saline conditions. Both radish varieties showed a variable response under salt stress for this attribute. Shoot dry weight showed maximum weight in Deci White, while lower in Lal Pari (Fig. 1).

Chlorophyll a content of both radish varieties was markedly reduced when treated to different levels of NaCl. A remarkable variation was observed in both varieties in chlorophyll a. Highest value of chlorophyll a was found in Deci White as compared to Lal Pari under saline regimes (Fig. 1). Chlorophyll b of both varieties was significantly reduced under saline conditions. Both radish varieties showed a variable response in this parameter. Highest value of chlorophyll b was found in Deci White, while lowest in Lal Pari under salt stress. Chlorophyll a/b ratio of both radish varieties caused clear decrease under salt stress. Higher reduction in chlorophyll a/b ratio was observed at 120 m*M* NaCl level as compared to 60 m*M* NaCl level. A considerable variation was also observed in both varieties at all salt levels. Higher value of chlorophyll a/b ratio was recorded in Deci White lower in Lal Pari

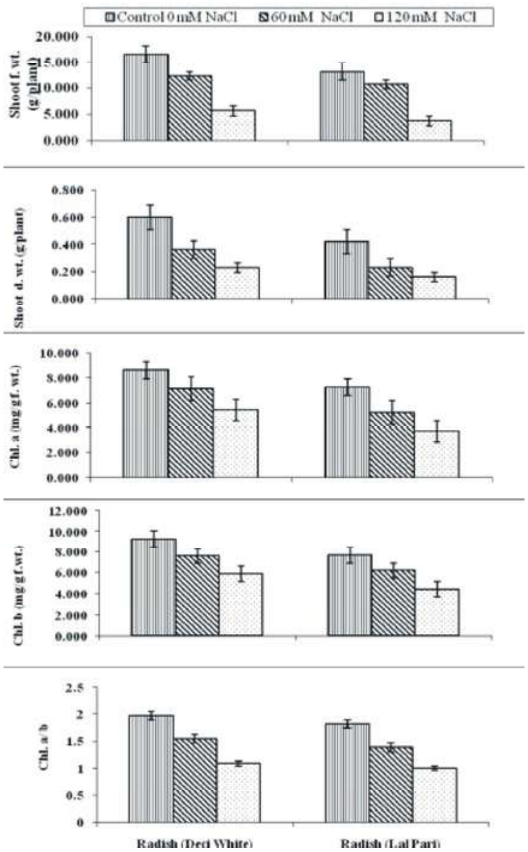

Fig. 1: Fresh and dry weight of shoot and photosynthetic pigments (Chl. a, chl. b and chl a/b ratio) of two radish varieties when 21 day-old plants were subjected to NaCl stress for 50 days. (Mean ± S.E; n= 3)

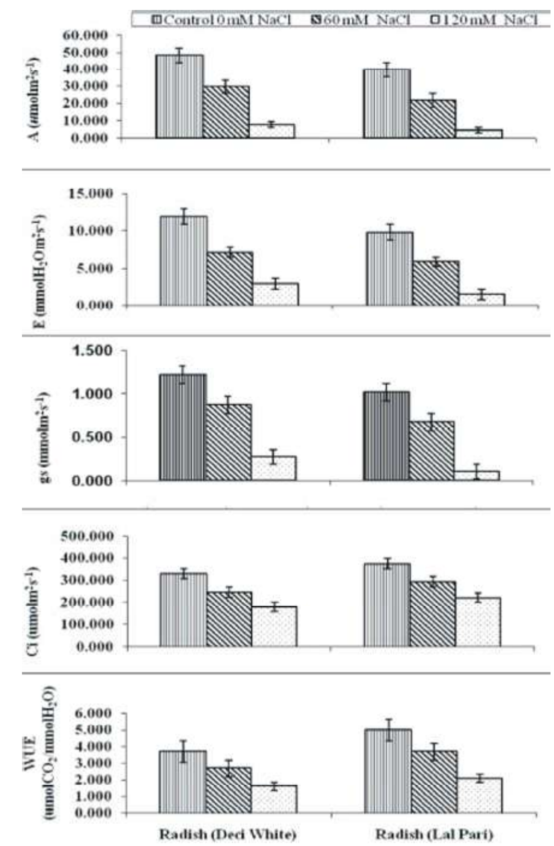

Fig. 2: Gas exchange attributes (*A, E, g_s, Ci nd WUE*) of two radish varieties, when 21 day-old plants were subjected to NaCl stress for 50 days. (Mean ± S.E; n= 3).

under NaCl stress (Fig. 1). Root zone salinity cruelly reduced net CO_2 assimilation rate in both radish varieties under salt levels. A variable response was found in both varieties in this attribute. Higher value of net photosynthetic rate was recorded in Deci White as compared to Lal Pari under salt levels (Fig. 2). Transpiration rate (*E*) was significantly reduced due to addition of sodium chloride in both radish varieties.Transpiration rate showed higher value in Deci White, while lower in Lal Pari under salt stress. Stomatal conductance (g_s) was significantly reduced in both varieties under salinity levels. A considerable variation was observed in this attribute in both varieties. Further, higher reduction in stomatal conductance (g_s) was observed in Deci White while lower in Lal Pari under salt regime (Fig. 1).

Salinity stress severely reduced water use efficiency (WUE) of two radish variety, when subjected to different NaCl levels. Higher reduction in this attribute was observed at 120 m*M* NaCl as compared to 60 m*M* NaCl. A significant variation was recorded in both varieties in the attribute. Variety Lal Pari showed less reduction in WUE at both level as compared to the Deci White under saline regimes (Fig. 1).

DISCUSSION

Salinity either in water or soil causes unfavorable consequence on various agricultural crops. Soil salinity reduces growth and yield of different agricultural crop species. Biomass production (shoot fresh and dry weight) is an important factor to increase growth and yield of agricultural crops. Our findings are accord with the results which has been reported in okra, canola, wheat and safflower cultivars [11].

Growth and yield of potential agriculture crops frequently depends on the capably of photosynthetic pigments which are present in thylakoid membrane of chloroplast, capture light and perform photosynthetic process. Salt stress ruptures thylakoid membrane, solute leakage out and as a result, reduces photosynthetic efficiently [11]. Chlorophyll a content was decreased in both radish varieties by increasing level of NaCl. Our results showed similarity with other researcher works in which reduction of photosynthetic pigments by the increase level of NaCl [13]. Chlorophyll b content was decreased by the increasing root zone salinity in both radish varieties. Our findings are similar with the results of [14], while working with rice, rice, maize, wheat, sugarcane and alfalfa where salt stress significantly reduced chlorophyll b and a/b ratio.

Gas exchange attribute is an important selection criterion for salt tolerance in different agricultural crops. Photosynthetic pigments, chlorophyll a & b etc. play a key role to enhance gas exchange characters [15]. Under saline condition, salt of Na & Cl enter into thylakoid membrane, rupture it and overall reduces the gas exchange parameter [16,17]. Net carbon dioxide assimilation rate (*A*) of both radish varieties was markedly reduced with the addition of NaCl. Less reduction in photosynthetic rate (*A*) was observed in variety Deci White as compare to Lal Pari. Reduction in photosynthetic rate (*A*) is due to reduction chlorophyll contents in various crops [17]. Photosynthetic rate (*A*) are adversely affected due to high concentration of salinity and maximum reduction in net carbon dioxide assimilation rate (*A*) was observed at higher level of salinity [18]. Our results are in agreements with the findings of Wahid *et al*. 2001, where salt stress reduced net photosynthetic rate in wheat, rice, sugarcane. Reduction in transpiration rate in both radish varieties under salt stress was in agreements with the results of Stepien and Klobus, [19] while working with wheat, rice, maize and sunflower. Sub stomatal carbon dioxide concentration (C_i) was decreased by the increasing level of sodium chloride in both radish varieties. Our results are in agreement with the findings of [18], where sub stomatal carbon dioxide concentration decreased with increasing salt level in wheat crop. Water use efficiency (WUE) of two radish varieties, significantly reduced by increasing the level of NaCl. Results of present study correlate with earlier findings e.g. wheat, rice, brassica, carrot, radish, safflower [11].

In the view of our present results and with the findings of research workers, it is concluded that salt stress significantly reduced growth biomass, photosynthetic pigments and photosynthetic processes in various agriculture crops. Of various physiological and biochemical attributes, net photosynthetic rate and chlorophyll could be used as efficient selection criteria for salinity tolerance in radish vegetables.

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