

ASSESSMENT OF INTER-CULTIVAR VARIATION FOR SALT TOLERANCE IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) USING GAS EXCHANGE CHARACTERISTICS AS SELECTION CRITERIA

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Abstract

An experiment was carried out to appraise inter-accession variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using gas exchange attributes and photosynthetic pigments as useful selection criteria. Ten accessions of safflower viz., Safflower-31, Safflower-32, Safflower-33, Safflower-34, Safflower-35, Safflower-36, Safflower-37, Safflower-38, Safflower-39, Safflower-78 were screened at 150 mM NaCl at the vegetative stage. Salt stress resulted in a considerable decline in biomass (shoot and root dry mass) of all safflower lines. Safflower-36 and Safflower-38 were higher Safflower-39 lower while the remaining lines were intermediate in biomass production under saline regime. Salt stress also reduced physiological and biochemical attributes such as rate of photosynthesis, transpiration, stomatal conductance, and chlorophylls a and b in all safflower lines. A significant inter-accession variation was found in all safflower accessions with respect to difference in net CO₂ assimilation rate (*A*). Since a positive association of net photosynthetic rate (*A*) with biomass (shoot and root dry weights) was observed in the 10 diverse safflower lines under saline conditions, thus it can be used as an effectual indicator of salinity tolerance in safflower.

Introduction

High amounts of salts whether from soils or water are great problems to agriculture throughout the world (Flowers, 2004; Schwabe *et al.*, 2006). In many regions of the world, salinity stress may occur when crops are exposed to high levels of salts (Na⁺ and Cl⁻). The effects of sodium and chloride salts may be greater than those of other salts present in the soil or water (Ghassemi *et al.*, 1995; Munns, 2005). Similar to other known abiotic stresses, salinity stress can harmfully affect a variety of physiological and biochemical processes, more importantly the photosynthesis (Netondo *et al.*, 2004). The salt stress-induced reduction in crop productivity is often found to be associated with the reduction in rate of photosynthesis (Delfine *et al.*, 1999; Netondo *et al.*, 2004; Chaum & Kirdmanee, 2009). A significant reduction in photosynthetic rate (net CO₂ assimilation rate) was observed in some *Brassica* species (Nazir *et al.*, 2001; Ulfat *et al.*, 2007), rice (Tiwari *et al.*, 1997), wheat (Ashraf & Shahbaz, 2003; Raza *et al.*, 2006), sorghum (Netondo *et al.*, 2004), chickpea (Singla & Garg, 2005), cotton (Desingh & Kanagaraj, 2007), sunflower (Noreen & Ashraf, 2008), pea (Yildirim *et al.*, 2008) and *Phaseolus vulgaris* (Stoeva & Kaymakanova, 2008) under saline conditions. However, degree of salt induced reduction in CO₂ assimilation rate depends on photosynthesizing tissue, green pigments, stomatal and non-stomatal factors which eventually affect photosynthetic rate (Dubey, 2005).

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Different scientists have used different physiological/biochemical attributes as useful selection criteria to assess inter-varietal variability for salt tolerance in different crops (Cuartero *et al.*, 2006; Munns, 2007; Ulfat *et al.*, 2007). For example, Essa & Dawood (2001) screened six genotypes of soybean at different levels of NaCl using leaf chlorophyll content as a selection criterion. Thirty-four accessions of canola (*Brassica napus* L.) were screened at 150 mM NaCl using net photosynthetic rate as a physiological selection indicator of salt tolerance (Ulfat *et al.*, 2007). Pakniyat & Armion (2007) screened 20 genotypes of sugar beet (*Beta vulgaris* L.) at different levels of NaCl using leaf proline accumulation as a potential biochemical selection criterion. Similarly, 28 sugar beet (*Beta vulgaris* L.) cultivars were screened at different levels of salt (NaCl) using shoot Na⁺ as a selection criterion (Pakniyat & Armion, 2007). 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 (Ashraf, 2004; Ashraf & Harris, 2004; Yildirim *et al.*, 2008; Taffouo *et al.*, 2009).

In view of this, the present investigation was carried out to appraise whether photosynthetic parameters could be used as effective selection criteria for screening available safflower germplasm for salt tolerance.

Materials and Methods

To study the degree of inter-accession variation for salt tolerance in different accessions of safflower with diverse genetic make-up, an experiment was conducted under a net-house of the Botanical Garden of the University of Agriculture, Faisalabad (latitude 31°30' N, longitude 73°10' E and altitude 213 m) where mean day/night relative humidity was 58-74% and temperature 24-28°C. The seed of 10 safflower lines was acquired from the National Agricultural Research Center, Islamabad, Pakistan. Seeds were sterilized with 5% NaOCl solution for 5 min., after which the seeds were rinsed with distilled water to remove the sterilizing agent. Seeds were sown in plastic pots (28.5 cm diameter) each having 10 kg washed dry sand. Hoagland's nutrient solution in full strength was supplied to all pots for 7 days. Salt (NaCl) treatments in the nutrient solution were initiated 23 days after the initiation of the study. The NaCl levels were 0 and 150 mM in Hoagland's nutrient solution. The salt treatment was started step-wise by adding 40 mM every day to each pot until the requisite salt treatment achieved. Each time two liters of treatment solution were applied to each pot once a week. To compensate for evapotranspiration loss, each pot was supplied daily with 200 ml of distilled water. The design of the experiment was a completely randomized with four replicates. After six weeks of the commencement of salt treatment, data for the following physiological parameters were documented:

Gas exchange parameters: The photosynthetic efficiency and its related parameters were determined with an LCA-4 ADC open system portable infrared gas analyzer (ADC, Hoddesdon, England) in the noon when there was full light intensity (at 10 a.m. to 1.00 p.m.). A young and fully expanded leaf was used and the data for transpiration rate (E), net CO₂ assimilation rate (A), intrinsic CO₂ concentration (C_i) and stomatal conductance

(g_s) were recorded. The conditions used for the equipment/leaf chamber were as follows: atmospheric CO_2 concentration (C_{ref}) $352 \mu\text{mol mol}^{-1}$, leaf surface area 11.35 cm^2 , ambient pressure 99.2 kPa , PAR (Q_{leaf}) was maximum up to $1048 \mu\text{mol m}^{-2} \text{ s}^{-1}$ and the chamber water vapor pressure varied from 4.4 to 6.6 mbar .

Water use efficiency (WUE): The ratio of A (CO_2 assimilation rate) over E (transpiration rate) was used as (WUE).

Chlorophyll content: The concentration of photosynthetic pigments was estimated according to Arnon (1949). Fresh leaves (1 g) were triturated in a porcelain mortar by adding 2 mL 80% acetone and filtered through a filter paper (Whatman No.1). After filtration, 10 mL of 80% acetone were added and the volume of the filtrate was made up to 10 mL . The mixture was thoroughly mixed with a vortex mixer and the absorbance of the 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 chlorophylls a and b .

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

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

Where V = volume of the leaf extract (mL), W = weight of fresh leaf tissue (g)

After all these measurements, plants were uprooted from the pots and separated into shoots and roots. They were then washed with DW and surface dried with a blotting paper. Fresh masses of shoots and roots were dried in an oven at 65°C for 7 days and their dry masses recorded.

Statistical analysis of data: A completely randomized design (CRD) was employed to arrange the experimental units in four replications. The data obtained was analyzed statistically by using Analysis of Variance (ANOVA) technique COSTAT statistical package (CoHort software, Berkeley, USA). The least significance difference (LSD) values for different parameters were worked out following Snedecor & Cochran (1980) for assessing the significant differences among mean values.

Results

Shoot dry weight of all safflower lines decreased significantly due to imposition of salt to the root zone. The lines differed considerably in this attribute. Accessions Safflower-36 followed by Safflower-38 had higher shoot dry weight, while Safflower-34, Safflower-39 and Safflower-78 lower than those of the other lines under salt treatments (Fig. 1).

Root dry weight of all safflower lines decreased significantly due to addition of NaCl to the rooting medium. Higher values of root dry weight were recorded in Safflower-33 followed by Safflower-38, while the lower in Safflower-39 followed by Safflower-34 than those of the other lines in saline medium.

Salt treatment caused a marked reduction in net photosynthetic rate (A) of all safflower lines. The accessions showed a significant variation in response to salt stress in this attribute. Maximum values of net CO_2 assimilation rate were observed in Safflower-33 and Safflower-37, while minimum in Safflower-31 and Safflower-38 under salt regime. However, other accessions were almost uniformly affected due to salt stress with respect to this attribute (Fig. 1).

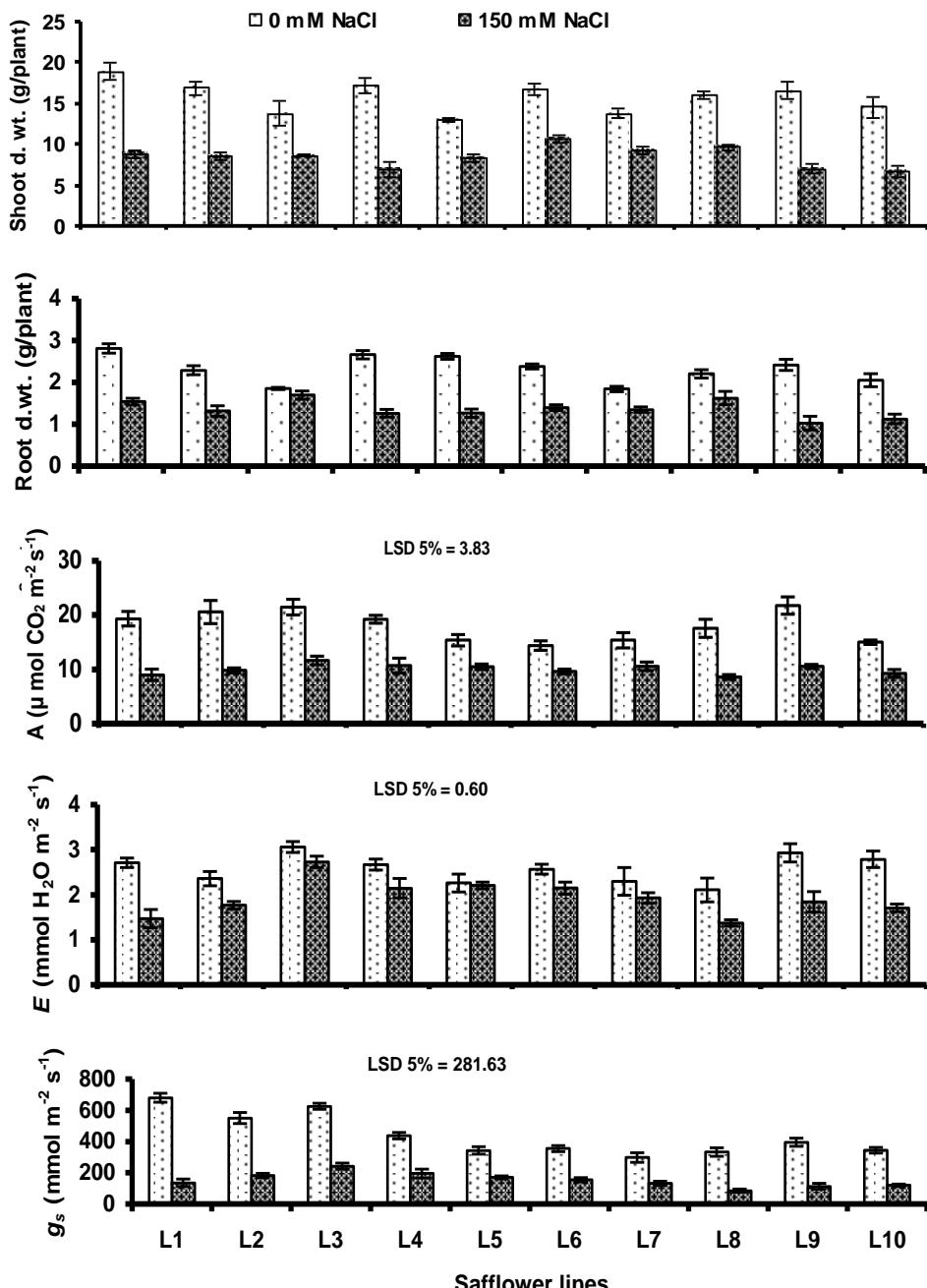


Fig. 1. Dry weights of shoot and root and gas exchange characteristics (A , E , g_s) of 10 safflower lines when 28 day-old plants were subjected to salt stress for 56 days. (Mean \pm S.E; $n=4$) (L1=Saff-31, L2=Saff-32, L3=Saff-33, L4=Saff-34, L5=Saff-35, L6=Saff-36, L7=Saff-37, L8=Saff-38, L9=Saff-39, L10=Saff-78).

Transpiration rate (E) of all safflower lines decreased significantly due to salt stress. All accessions showed a varying response to salt stress in this attribute. Highest values of E were observed in Safflower-33 followed by Safflower-34 and Safflower-36 under saline conditions. However, Safflower-31 and Safflower-38 were the lowest in E of all lines under saline regime. It was interesting to note that the value of E in Safflower-35 remained almost unaffected due to salinity.

A substantial decline in stomatal conductance (g_s) of all safflower lines occurred under saline regimes. Comparison of the lines shows that accession Safflower-33 was again the highest, while Safflower-38 the lowest in stomatal conductance under salt stress. The response of other accessions was intermediate with respect to this gas exchange parameter.

Salt treatment caused a marked reduction in water use efficiency (WUE) of all safflower lines except Safflower-78 in which WUE remained almost unaffected due to salt stress. Differences among the cultivars in terms of WUE were non-significant. Post hoc analysis of the data for WUE at the salt regime showed that cultivars difference was significant. Lines Safflower-31, Safflower-38 and Safflower-39 had significantly higher while Safflower-33 lower values of WUE than those of the other lines under salt stress (Fig. 2).

Salt stress markedly reduced chlorophylls a and b of all safflower lines and a significant inter-line variation was observed with respect to these photosynthetic pigments. Accessions Safflower-35, Safflower-36, Safflower-38 and Safflower-39 had higher, while Safflower-31 and Safflower-34 lower values of chlorophyll 'a' than those of the other accessions under salt stress (Fig. 2). Highest values of chlorophyll 'b' were observed in Safflower-38 followed by Safflower-37 under saline regime. However, in contrast, Safflower-31 and Safflower-34 were the lowest of lines in chlorophyll b under salt stress.

A marked reduction in chlorophyll a/b ratios of all safflower lines were observed due to root zone salinity. Comparison of the lines shows that Safflower-31, Safflower-34, Safflower-39, and Safflower-78 had higher, while Safflower-37 lower chlorophyll a/b ratio than those of the other lines under saline regime.

Discussion

Of various physiological traits, appraisal of all gas exchange attributes in a crop species is crucial so as to predict plant productivity under normal or saline conditions. Generally, salt-induced suppression in rate of photosynthesis (A) reduces biomass (shoot and root dry weights) (Athar & Ashraf, 2005). If we draw the relationship of net photosynthetic rate (A) with plant biomass of 10 safflower lines under salt stress, a positive correlation (A vs shoot dry weight, root dry weight, $r = 0.83 **$; $0.79 **$) was observed, which shows that inter-cultivar variation for salt tolerance as observed in safflower lines might have been due to differences in net CO_2 assimilation rate. Since a positive relationship between biomass and A can be observed in most of the lines, these results are analogous to earlier findings in which very strong relationship was found between these two variables in different crops e.g., canola (Ulfat *et al.*, 2007), *Brassica* spp. (Nazir *et al.*, 2001) and wheat (Arfan *et al.*, 2007). On the basis of these findings it can be concluded that that photosynthetic rate is a useful selection criterion for assessment of salinity tolerance in safflower. In the present study, saline growth medium also reduced transpiration rate (E) of all safflower lines, which is in agreement with some earlier reports on different crops, e.g., sunflower (Hebbara *et al.* 2003), wheat (Ashraf & Shahbaz, 2003) and canola (Ulfat *et al.*, 2007). A significant positive relationship of transpiration rate (E) with plant biomass (shoot dry

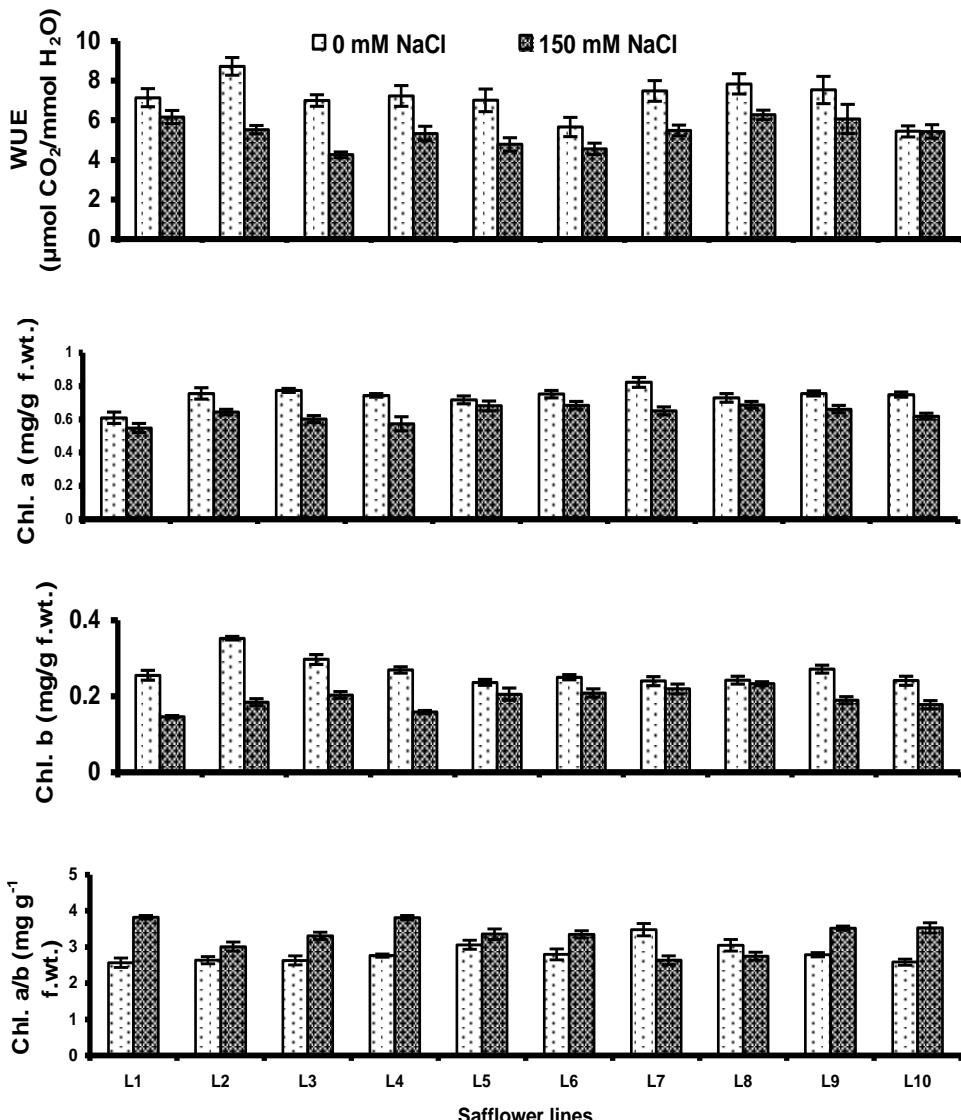


Fig. 2. Gas exchange characteristics (WUE) and photosynthetic pigments (chlorophylls a & b) of 10 safflower lines when 28 day-old plants were subjected to salt stress for 56 days. (Mean \pm S.E.; $n=4$) (L1=Saff-31, L2=Saff-32, L3=Saff-33, L4=Saff-34, L5=Saff-35, L6=Saff-36, L7=Saff-37, L8=Saff-38, L9=Saff-39, L10=Saff-78).

weight, root dry weight vs E $r = 0.66$ **; 0.60 **) was found. Such a close relationship between biomass and E has already been reported in wheat (Ashraf & Shahbaz, 2003), sunflower (Hebbara *et al.*, 2003) and canola (Ulfat *et al.*, 2007). Salt stress also resulted in a significant decline in stomatal conductance (g_s), sub-stomatal CO_2 concentration (C_i) as has been earlier reported in wheat (Arfan *et al.*, 2007). The findings of the present investigation show that root zone salinity caused a decline in g_s and C_i in all safflower lines, and they are analogous to what has been earlier reported in cotton (Meloni *et al.*, 2003), wheat (Ashraf

& Shahbaz, 2003) and canola (Ulfat *et al.*, 2007). Salt stress caused a marked reduction in WUE in all safflower lines. A positive relationship of WUE was observed with biomass production in the set of safflower lines studied.

Photosynthetic pigments such as chlorophyll 'a' and 'b' play a key role in photosynthesis (Taiz & Zieger, 2006). Reduction in photosynthesis, due to salt stress is partly ascribed to reduction in chlorophyll contents (Delfine *et al.*, 1999; Ashraf, 2004). Salt-induced suppression in chlorophyll contents can be due to deterioration of pigment protein complexes (Singh & Dubey, 1995). The findings of this investigation show that high salinity levels severely decreased leaf chlorophyll 'a' and 'b' contents in all safflower lines, which is similar to what has been earlier found in different crop species e.g., cowpea (Taffouo *et al.*, 2009), cotton (Meloni *et al.*, 2003), wheat (Raza *et al.*, 2006), and pea (Yildirim *et al.*, 2008). In the present study, a positive correlation was found between chlorophyll 'a' and 'b' and net photosynthetic rate (*A*) (chlorophyll 'a' and 'b' vs *A* $r = 0.61^{**}$; 0.81^{**}) suggesting that salt-induced reduction in net CO_2 assimilation rate might have been partly due to decrease in chlorophyll contents. Such a positive association between *A* and chlorophyll pigments has been earlier observed in different crops e.g., sunflower (Ashraf & Sultana, 2000), wheat (Arfan *et al.*, 2007) and pea (Yildirim *et al.*, 2008). In conclusion, of various physio-biochemical parameters examined in the present investigation, only net CO_2 assimilation rate (*A*) was the most effective indicator of salt tolerance in the set of 10 safflower lines. Thus, screening and selection of safflower germplasm using photosynthetic rate may provide salt tolerant lines/accessions of safflower.

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