

## CAN LEAF WATER RELATION PARAMETERS BE USED AS SELECTION CRITERIA FOR SALT TOLERANCE IN SAFFLOWER (*CARTHAMUS TINCTORIUS* L.)

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### Abstract

An experiment was conducted to assess inter-cultivar variation for salt tolerance in safflower (*Carthamus tinctorius* L.) using water relation parameters as selection criteria. Ten available lines, Safflower-31, Safflower-32, Safflower-33, Safflower-34, Safflower-35, Safflower-36, Safflower-37, Safflower-38, Safflower-39 and Safflower-78, were screened at 150 mM of NaCl at the vegetative stage. Salt stress caused a marked reduction in shoot fresh biomass and all water relations parameters, relative water content (RWC), water potential ( $\Psi_w$ ), and osmotic potential ( $\Psi_s$ ) except leaf turgor potential ( $\Psi_p$ ). Accessions Safflower-36, Safflower-37 and Safflower-38 were higher, Safflower-39 and Safflower-78 lower, while the remaining accessions intermediate in shoot biomass production under saline conditions. Salt stress also adversely affected all water relation parameters, (RWC,  $\Psi_w$ ,  $\Psi_s$ , and  $\Psi_p$ ), however, the effect was more pronounced on leaf  $\Psi_w$ ,  $\Psi_s$ , and  $\Psi_p$ . Although a great magnitude of inter-cultivar variation for salt tolerance was observed in the set of 10 accessions of safflower with respect to shoot biomass production, no one of the water relation parameters was found helpful in discriminating among the lines for salt tolerance.

### Introduction

Of the various effects caused by salinity, reduction in osmotic potential of soil solution is the major one, which reduces the ability of plants to take up water from the growth medium (Munns, 2002). Water potential of plants growing under salt stress becomes more negative with an increase in salinity of the rooting medium (Khan, 2001; Khan *et al.*, 1999; Meloni *et al.*, 2001) which causes detrimental effects on plant growth.

Osmotic stress has the major contribution in salt-induced growth reduction at initial phase of salinity. Furthermore, Neumann (1997) while analyzing about 10 reports concluded that genotypic variation occurs in salinity-induced growth inhibition due to osmotic stress in some crops e.g., *Brassica species* (He & Cramer, 1993), maize (Cramer *et al.*, 1994), wheat (Kingsbury *et al.*, 1984), and rice (Moons *et al.*, 1995). The adverse effects of osmotic stress also depend upon severity of salinity stress.

Different plant species adopt different mechanisms to cope with these effects (Munns, 2002). Osmotic adjustment, i.e., reduction of cellular osmotic potential by the net solute accumulation, has been considered as an important mechanism of salt tolerance in plants (Ashraf & Harris, 2004). The reduction in osmotic potential in salt stressed plants mainly occurs due to the accumulation of inorganic ions ( $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{K}^+$ ) (Hasegawa *et al.*, 2000). Osmotic adjustment in all plant tissues contribute to uptake of water uptake and hence maintenance of cell turgor, thereby allowing physiological processes such as stomatal regulation, photosynthesis, and cell expansion (Serraj & Sinclair, 2002).

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Although a number of physiological and biochemical selection criteria have been recommended for screening germplasm of different crops, water relations are considered very important in view of their direct role in sustaining plant growth under saline stress (Munns, 2002; Ashraf 2004; Ashraf & Harris, 2004). In view of this, the present study was conducted to assess whether water relation parameters could be used as prospective selection criteria for screening available safflower germplasm for salt tolerance.

### Materials and Methods

Seed of 10 diverse strains of safflower (*Carthamus tinctorius* L.) was obtained from the National Agricultural Research Center, Islamabad, Pakistan. The experiment was conducted in a wirehouse in the Botanic Garden of the Department of Botany, University of Agriculture, Faisalabad (latitude 31°30' N, longitude 73°10' E and altitude 213 m) where average day/night relative humidity was 58-74% and temperature 24-8°C. Before sowing, all seed samples were surface sterilized in 5% Sodium hypochlorite solution for 10 min. Seeds were sown in plastic pots (28.5 cm diameter) each containing 10 kg of well-washed dry sand. All the pots were irrigated for 7 days with full strength Hoagland's nutrient solution. Salt (NaCl) treatments in the nutrient solution were begun 23 days after the start of the experiment. The salt treatments were 0 and 150 mol m<sup>-3</sup> in full strength Hoagland's nutrient solution.

Salt treatment was started step-wise in aliquots of 40 mol m<sup>-3</sup> until the appropriate salt treatment was achieved. The salt treatment continued with the addition of 2 L of the appropriate solution to each pot once a week. To compensate evapotranspiration loss, every day 200 ml of distilled water were added to each pot. The experiment was arranged in a completely randomized design with four replicates.

After six weeks of the initiation of salt treatment, data for the following physiological parameters were recorded:

**Leaf water potential:** A fully expanded youngest leaf was excised from each plant at 08:00, and the leaf water potential measurements were made with a Scholander type pressure chamber (Arimad-2-Japan).

**Osmotic potential:** The same leaf as used for water potential measurement was also used for osmotic potential determination. The leaf material was frozen in 2.0 cm polypropylene tubes for two weeks and after which time it was thawed, and the sap was extracted by pressing it with a glass rod. The sap so extracted was used directly for osmotic potential determination in an osmometer (VAPRO vapor pressure osmometer, Model 5520, USA).

**Turgor potential:** It was calculated as the difference between water potential and osmotic potential values (Nobel, 1991).

$$\Psi_p = \Psi_w - \Psi_s$$

**Relative water content:** Leaves were excised before dawn, weighed fresh (Fw) and placed in distilled water in the dark for 24 h to re-hydrate. The following morning, leaf turgid weight (Tw) was measured and then leaves were dried at 65 °C for 48 h and dry weight (Dw) determined. RWC was calculated as:

$$RWC = [(Fw - Tw)/(Fw - Dw)] \times 100$$

After all these measurements, the plants were harvested. Plant roots were removed carefully from the sand and washed thoroughly in distilled water. Plants were separated into shoots and roots. Fresh weights of shoots of all the plants were recorded.

**Statistical analysis of data:** A completely randomized design (CRD) with four replicates was used for setting up the experiment. The COSTAT computer package (*CoHort software*, Berkeley, USA) was used for working out analyses of variance of all variables. The mean values were compared with the least significance difference test (Snedecor & Cochran, 1980).

### Results and Discussion

Salt treatment significantly reduced the shoot fresh weight of all 10 accessions of safflower (Table 1). Maximum shoot fresh weight was recorded in Safflower-36, Safflower-37 and Safflower-38 under saline conditions, while Safflower-39 and Safflower-78 had minimum shoot fresh weight under saline conditions (Fig. 1.). However, the remaining accessions were intermediate in shoot fresh biomass production under salt stress.

Salt stress significantly reduced relative water content (RWC) in all the lines (Tables 1). Although accessions differed significantly in RWC, the difference among them was not so prominent (Fig. 2.).

Relative water content has been used as one of the potential water relation parameters for assessing intra-specific variation for salt tolerance in a number of crops such as wheat (Pier and Berkowitz, 1987), maize (Premachandra *et al.*, 1990), *Vigna radiata* (Nandwal *et al.*, 1998), and sorghum (Jones *et al.* 1980). However, if parallels are drawn between the data for shoot fresh weight and relative water content of 10 diverse lines of safflower, it is evident that no clear-cut association between these two attributes exists.

Growth medium salinity significantly lowered the leaf water potential (more negative values) of all 10 lines. However, Safflower-31 followed by Safflower-32 maintained significantly higher leaf water potential values than the other accessions under saline conditions. The maximum decrease in leaf  $\Psi_w$  was observed in Safflower-36 due to salt stress.

Maintenance of high leaf  $\Psi_w$  has been related to salt tolerance in some crops, eg., *Vigna mungo* (Ashraf, 1989), and wheat (Kingsbury & Epstein, 1984). In the present study, a negative relationship was found between leaf  $\Psi_w$  values and degree of salt tolerance of all 10 safflower lines. These findings are in agreement with those of some other studies. (Blackman & Davies. 1985; Termaat *et al.*, 1985; Ashraf & Waheed, 1993) in which a negative or no association between leaf  $\Psi_w$  and degree of salt tolerance was observed.

The results of leaf osmotic potential show that salt stress significantly decreased (more negative values) the leaf osmotic potential of all lines. Of all the lines, Safflower-33, Safflower-34, Safflower-36, Safflower-37 and Safflower-38 had lower leaf osmotic potential than those of the other accessions under saline conditions (Fig. 3).

A significant reduction in leaf turgor potential was observed in all lines due to salt stress. However, the adverse effect of salt stress on leaf turgor potential was more prominent in Safflower-32 and Safflower-35 than in the other accessions. Maximum increase in leaf turgor potential was observed in Safflower-31 and Safflower-33 due to salt stress (Fig. 3).

**Table 1.** Mean squares from analysis of variance (ANOVA) for leaf water relations and shoot fresh weight of 10 safflower lines when 28 day-old plants were subjected to salt stress for 56 days.

Source of variation	df	Leaf water potential	Leaf osmotic potential	Leaf turgor potential	RWC	Shoot f.wt
<b>Main effects</b>						
Salt	1	8.160 ***	17.03 ***	1.353 ***	2041.08 ***	59900.34 ***
Cultivars (Cvs)	9	0.119 ***	0.150 ***	0.060 *	42.85 *	251.05 ns
Salt * Cvs	9	0.032 ***	0.180 ***	0.118 ***	21.26 ns	336.87 ns
<b>Error</b>	<b>60</b>	<b>0.007</b>	<b>0.033</b>	<b>0.028</b>	<b>20.038</b>	<b>433.32</b>

\*, \*\*, \*\*\* = Significant at 0.05, 0.01, and 0.001 levels, respectively.

ns = Non-significant.

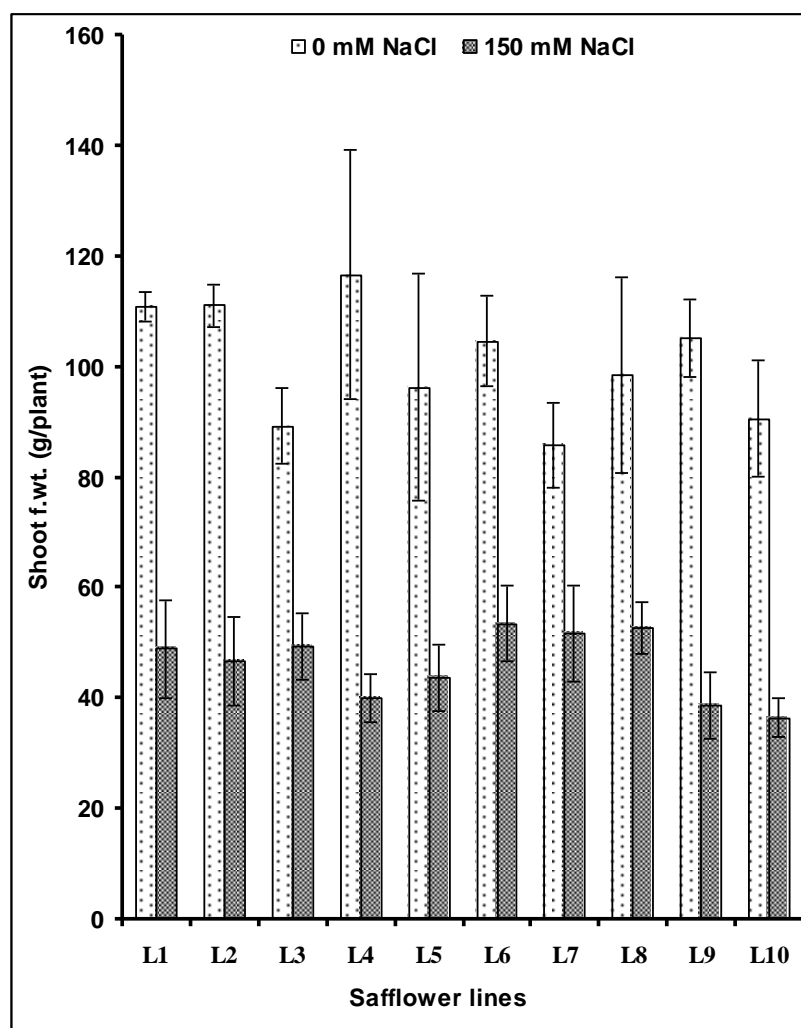


Fig. 1. Shoot fresh weight of 10 safflower lines when 28 day-old plants were subjected to salt stress for 56 days. (Mean  $\pm$  S.E.;  $n = 4$ ) (L1=Saff-31, L2=Saff-32, L3=Saff-33, L4=Saff-34, L5=Saff-35, L6=Saff-36, L7=Saff-37, L8=Saff-38, L9=Saff-39, L10=Saff-78)

Turgor potential of a cell plays an important role for the normal functioning of metabolic phenomena under adverse environmental conditions (Taiz & Zeiger, 2002). Maintenance of high turgor potential of plant cells under saline conditions was thought to be one of the vital water relation attributes for sustaining growth under salt stress (Hsiao, 1973; Greenway & Munns, 1980). However, in view of the results for leaf  $\Psi_p$  presented in the present study do not show a positive association with the degree of salt tolerance of the diverse safflower lines examined here, because most of the low biomass producing lines (salt sensitive) had higher values of leaf turgor potential than those of the high biomass producing lines (salt tolerant). These results support some earlier studies in which salt sensitive lines of different species maintained higher leaf turgor than their salt tolerant relatives e.g. *Beta vulgaris* (Heuer & Plaut, 1989), *Sorghum* spp., (Yang *et al.*, 1990), *Triticum aestivum* (Kingsbury & Epstein, 1984), and *Citrus sinensis* (Walker *et al.*, 1983).

Taken overall, no one of the water relation parameters (RWC,  $\Psi_w$ ,  $\Psi_p$ ) was found useful in discriminating the set of safflower lines for salt tolerance.

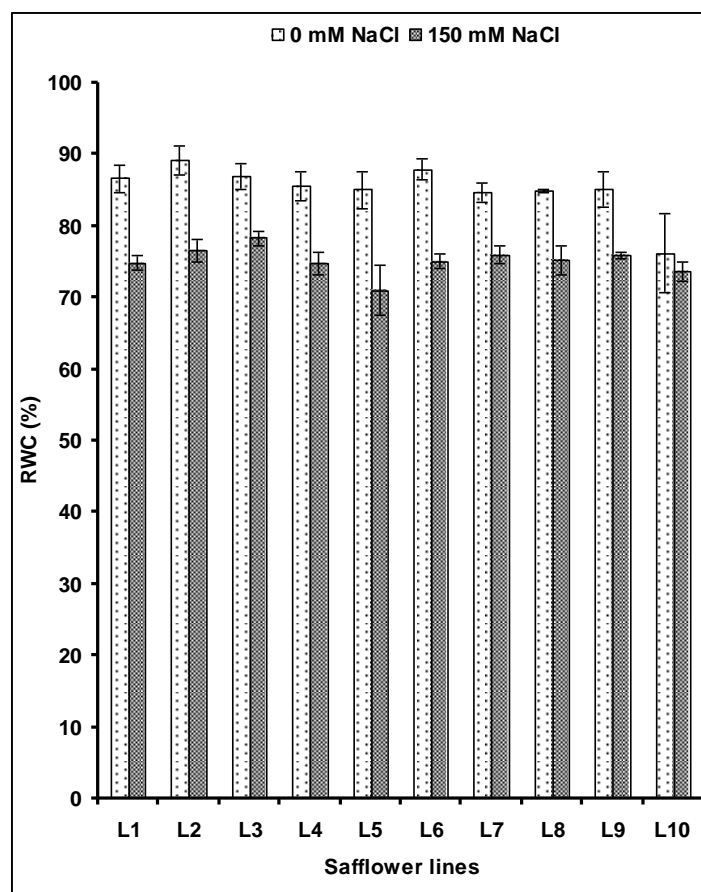


Fig. 2. Relative water content of 10 safflower lines when 28 day-old plants were subjected to salt stress for 56 days. (Mean  $\pm$  S.E.;  $n = 4$ ) (L1=Saff-31, L2=Saff-32, L3=Saff-33, L4=Saff-34, L5=Saff-35, L6=Saff-36, L7=Saff-37, L8=Saff-38, L9=Saff-39, L10=Saff-78)

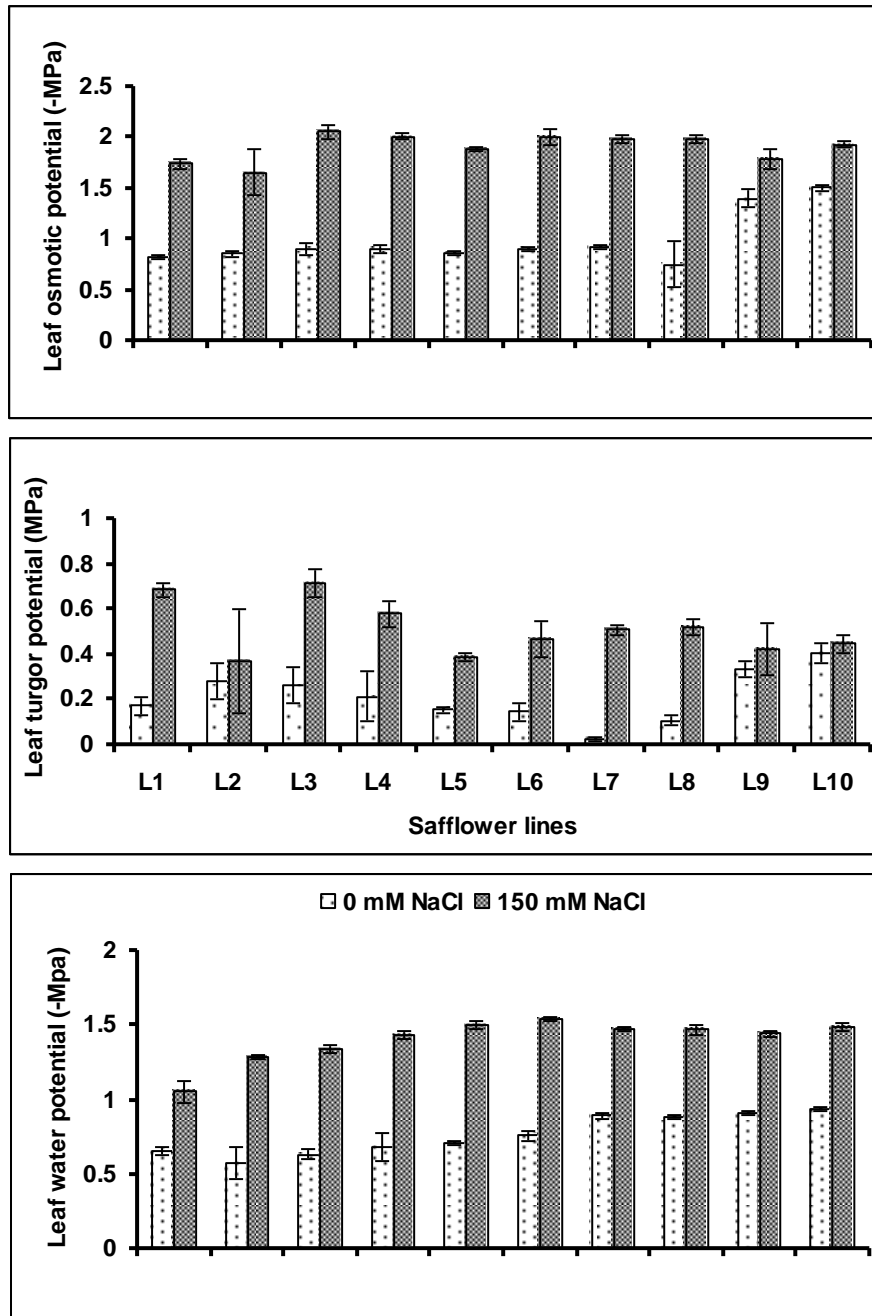


Fig. 3. Leaf water relations of 10 safflower lines when 28 day-old plants were subjected to salt stress for 56 days. (Mean  $\pm$  S.E.;  $n = 4$ ) (L1=Saff-31, L2=Saff-32, L3=Saff-33, L4=Saff-34, L5=Saff-35, L6=Saff-36, L7=Saff-37, L8=Saff-38, L9=Saff-39, L10=Saff-78)

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