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IMPROVEMENT IN GROWTH AND LEAF WATER RELATION PARAMETERS OF SUNFLOWER AND SAFFLOWER PLANTS WITH FOLIAR APPLICATION OF NUTRIENT SOLUTIONS UNDER SALT STRESS

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Abstract

Effect of nutrient solutions viz., KNO_3, H_3BO_3, Fe EDTA, and their mixture applied through foliar spray on growth and water relation was assessed in sunflower and safflower plants under salt stress. Salt stress impaired growth by reducing fresh weight of both the plants. Imposition of salt stress also had adverse effects on leaf water relation parameters, relative leaf water content (RLWC), water potential (Ψw), osmotic potential (Ψs) and turgor potential (Ψp). All the water relation parameters were improved with the foliar application of nutrient solutions. The ameliorative effect of mineral nutrition on fresh biomass of both the plants under saline conditions was due to the nutrients-induced improvement in plant water status.

Introduction

Parameters of water relation, water potential, solute potential, and turgor potential are interrelated to one another in plant cells and are markedly affected when plant grow under saline media. Reduction in solute potential of soil solution under stress reduces the ability of plants to take up water from the growing media (Munns, 2002). Under salt stress water potential becomes more negative and cause a detrimental effects on plant growth (Khan, 2001; Meloni *et al*., 2001). Neumann (1997) concluded that genotype variation occur 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). Different plant species adopt different mechanism to cope with these effects. Osmotic adjustment is an important mechanism of salt tolerance in plants (Ashraf & Harris, 2004). Osmotic adjustment involves the active accumulation of inorganic or organic solutes in a cell in response to a fall in the water potential (Ψ w) of the cell. Consequently the osmotic potential (Ψ s) of the cell decreases, which in turn attracts water into the cell and enable turgor to be maintained. Osmotic adjustment increases with the increase in salt concentration. Generally it contributes to turgor maintenance in both shoot and roots when plant experience stress condition (Sanchez *et al*., 2004). The maintenance of turgor in plant cells is the most important factor for plant biosynthesis under lower leaf water potential (Morgan, 1984; Yang *et al*., 1983; Turner & Jones, 1980). Leaf water potential, osmotic potential and relative leaf water content (RLWC) were found to be decreased with the increasing concentration of salinity and foliar application of nutrient solution reduced the decreasing tendency of these parameters (Sultana *et al*., 2001; Akram *et al*., 2009). Water relations are considered very important as they have their direct role in sustaining the plant growth under saline stress (Munns, 2002; Ashraf & Harris, 2004). In view of this the present study was conducted to investigate the ameliorative effect of foliar sprays of fertilizer solution containing K, B and Fe on growth and water relation parameters of sunflower and safflower plant.

Material and Method

Plant material and culture conditions: A moderately salt tolerant variety of sunflower (*Helianthus annuus* L. cv NuSun 636) and safflower (*Carthamus tinctorius* L. cv Spiny 321) were sown in plastic pot in Biosaline Research Field, Botany Department, University of Karachi. . These pots were 0.28m in diameter and 0.30m deep, having basal holes for leaching irrigation water, filled with 20 kg of sandy loam and cow dung manure (9:1) having pH 7.4 dS/m. The air temperature and relative humidity throughout the growing period was 25-32 ^{o}C and 60-80% respectively. NPK ratio in fertilizer was given 4:3:2 through urea, DAP and sulphates of potash (SOP) for sunflower as recommended by Nawaz *et al.,* (2003), which amounts to 0.744g Nitrogen (N), 0.558g Phosphorus (P) and 0.372g Potassium (K) per pot, and 7:15:7 for safflower as recommended by Naik *et al.,* (2007), which amounts to 0.217g Nitrogen (N), 0.465g Phosphorus (P) and 0.217g Potassium (K) per pot, given at the time of sowing and at the time of flowering. A certain amount of micronutrients were given in soil vide Hoagland solution (Hoagland & Arnon, 1938) twice along with irrigation water.

Two separate experiments were conducted for sunflower and safflower in a randomized complete block design with five replications. 90 pots, of each experiment, were divided in 6 sets comprising of 15 pots each. One set was of control (non spray) and other sets were treated with H_2O, KNO_3, H_3BO_3, Fe-EDTA and KNO_3+ H_3BO_3+Fe-EDTA mixture respectively. Out of 15 pots of each set, 5 pots of each were subjected to following different levels of saline water irrigation.

a)	Non saline water (control)	(EC 0.5 dS/m)
b)	0.3 % sea salt solution	(EC 4.8 dS/m)
c)	0.6 % sea salt solution	(EC 8.6 dS/m)

Sea salt solutions for irrigation were prepared by adding required amount of sea salt in tap water per liter. Sea salt is available in crude form in market. About 4% of its concentration was found equivalent to concentration of salts in the water of Indian Ocean (Castro & Huber, 2005).

The seeds were sown in pots under non saline condition and saline water irrigation was started at three leaf stage after germination to get seedlings of equal size. After thinning only one seedling was kept in each pot for further work. They were irrigated with gradual increasing sea salt concentration weekly up to reaching the desired salinity levels of the experiment mentioned above. To maintain the required soil medium salt levels the EC of the soil medium was measured periodically by portable EC meter. Plants were sprayed with water and nutrient solution of KNO_3, H_3BO_3, Fe-EDTA or its mixture after 45, 75 and 95 days of planting. K was given at the rate of 250ppm and other B and Fe was given at the rate of 5ppm. The calculated amount of K, B and Fe in their respective solution, applied to the plants through foliar application was 96.5ppm, 0.85ppm and 0.76 ppm respectively. Tween-20 (0.1%) was used as a wetting agent for each treatment. A volume 300ml/plant, of the solution was sprayed on all pots with a manual sprayer. Spray was carried out between 09:00 and 11:00AM. The plants were sprayed with solutions with uniform coverage until the leaves were completely wet and the solution ran off the leaves. At the time of spray other plants were covered with plastic sheet to prevent the contamination of sprayed nutrients. Control (non saline) plants were irrigated with 3.5L of tap water and plants under saline treatments were irrigated with 3.5L of their respective sea salt solution ensuring about 40% leaching. At grand period of growth the following physiological parameters were recorded.

Leaf water potential: Youngest sunflower leaf with petiole was excised from each plant and leaf water potential was determined with a pressure chamber ARIMAD2 using N_2 gas (Hsiao, 1990). In safflower the shoot apex and part of the stem containing at least two fully expanded leaves were used for the determination of water potential as their leaves have no real petiole (Weiss, 1971).

Osmotic potential: The leaves used for water potential measurement was also used for osmotic potential determination. The leaf samples were frozen in polyvinyl bag for a week after which it was thawed, and sap was extracted by pressing it with garlic squeezer. The extracted cell sap was used directly for osmotic potential determination in an osmometer (Osmette model 5004).

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

$$\Psi p = \Psi w - \Psi s$$

Relative Leaf Water content: Leaf samples were taken from 3rd node of apex, weighed fresh (FW) and placed in distilled water for 24 h to re-hydrate. Leaf turgid weight (TW) was measured and then leaves were dried at 65 ^{0}C for 48 h to determine dry weight (DW). RLWC was calculated as RLWC = [(FW – TW) / (FW - DW)] x 100

For the fresh weight determination of shoot, plants from each pot were uprooted carefully, separated into shoots and roots. Fresh weights of shoots of all the plant were recorded.

Statistical analyses: SPSS version 13 was used for data analysis. Data sets were subjected to two-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used to measure least significant differences (LSD) between treatment methods and controls (Duncan, 1955).

Results and Discussion

Salt stress significantly reduced the growth in terms of shoot fresh weight of both sunflower and safflower plants (Table 1 a&b). Foliar sprays of nutrient solution have offset the toxic effects of salinity to various extents and improve the shoot fresh weight of both the plants irrespective to their growth under non saline or saline conditions (Fig. 1). Akram *et al*., (2009) and Jabeen & Ahmad (2011) also reported an improvement in growth of sunflower and safflower plants respectively due to foliarly applied nutrients under salt stress.

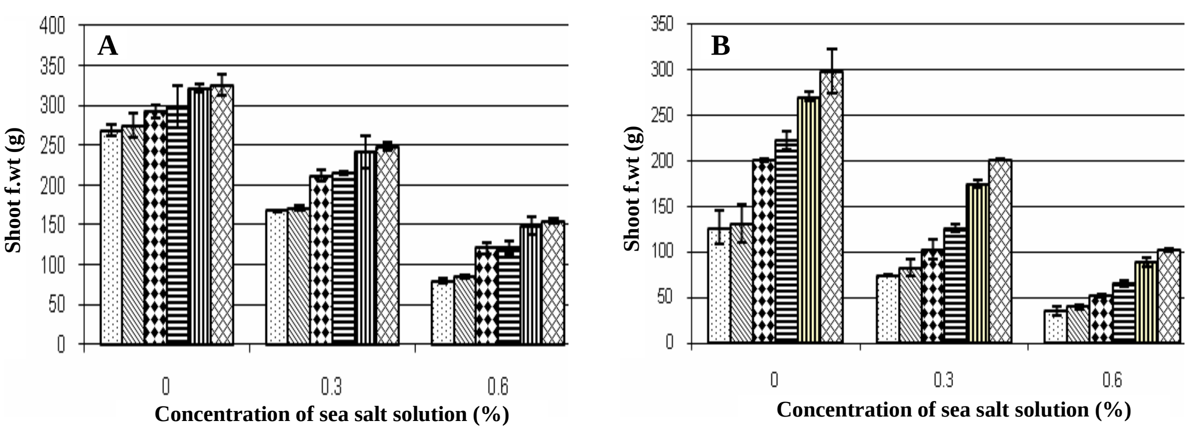

Fig. 1. Effect of salinity and foliar spray of water, potassium, iron, boron and its mixture on shoot fresh biomass of sunflower (A) and safflower (B) plant.

Control (non spray)	Foliar spray with water	ECiw: 0.5dS/m, ECe: 1.8dS/m (non saline)
Foliar spray with KNO_3	Foliar spray with Fe-EDTA	ECiw: 4.8dS/m, ECe: 6.1dS/m (0.3% sea salt solution)
Foliar spray with H_3BO_3	Foliar spray with KNO_3+ H_3BO_3 +Fe-EDTA	ECiw: 8.6dS/m, ECe: 9.9dS/m (0.6% sea salt solution)

Table 1a. Mean squares from analysis of variance (ANOVA) for leaf water relations and shoot fresh biomass of sunflower supplemented with foliar spray of nutrient solution under salt stress.

Source of variation	df	Leaf water potential	Leaf osmotic potential	Leaf turgor potential	RLWC	Shoot f. wt
			Main effects			
Salt	5	5.66 ***	20.939 ***	4.940 ***	2424.5 ***	142682.05 ***
Spray	2	0.011 ns	0.141 ***	0.075 ***	19.2 ***	7789.32 ***
Salt x spray	10	4.9 ns	0.016 ns	0.0184 ns	1.1 ns	110.34 ns
Error	34	0.011	0.023	0.012	2.46	349.95

Table 1b. Mean squares from analysis of variance (ANOVA) for leaf water relations and shoot fresh biomass of safflower supplemented with foliar spray of nutrient solution under salt stress.

Source of variation	df	Leaf water potential	Leaf osmotic potential	Leaf turgor potential	RLWC	Shoot f.wt
			Main effects			
Salt	5	4.426 ***	21.485 ***	6.517 ***	2557.16***	94374.88 ***
Spray	2	0.011 **	0.028 ns	0.004 ns	24.96 ***	21702.96 ***
Salt x spray	10	8.666 ns	0.0014 ns	2.7 ns	0.56 ns	1574.48 ***
Error	34	0.002	0.024	0.016	3.50	306.89

, * = Significant at 0.05level ns= Not significant

Osmoregulation, i.e., the maintenance of turgor is considered to be an important process for normal cellular metabolism (Taiz & Zeiger, 2002). The results for water relation parameters presented here indicate that salt stress adversely affected all these parameters (Table 2). Exogenous application of nutrient solution significantly reduced the decreasing tendency of these parameters; H_3BO_3 was found to be more effective than Fe-EDTA and KNO_3 (Table 2). While drawing a relationship among different water relation parameters and salinity levels, a negative relationship was found between plant water status and increasing salinity. During stress conditions plants alter values of internal water potential and maintained turgor and water uptake for growth (Tester & Davenport, 2003). Plants under saline condition maintained their turgor by osmotic adjustment (Hernandez & Almansa, 2002; Chaparzadeh *et al.*, 2003) which involved the net accumulation of solutes in a cell in response to salinity. It result a decrease in osmotic potential which in turn attracted water into the cell and enabled turgor to be maintained. Siddiqi & Ashraf (2008) also reported in safflower a marked reduction in all water relation parameters under saline conditions. From these results, it is obvious that plant water status decreased with increase in saline conditions, whereas foliarly applied B, Fe and K contributed to the maintenance of plant water status. These results suggest that foliar application of nutrient solution ameliorate the negative effects of sea salt by enhancing the availability of some other essential cations and phytoharmones which play an important role in regulation of stomata (Sage & Reid, 1994). Sea salt salinity increased the water deficit and decreased the nutrients by inhibiting the transportation of essential minerals from root to the shoot. However supplementation of nutrients through foliar spray can fulfill the nutrients demand of salt affected plants. The provision of above mentioned nutrients are directly or indirectly involved in cellular regulatory functions e.g. B is involved in many processes including sugar transport, Fe in metabolism of chlorophyll, K^+ in plant growth and various physiological processes such as stomatal regulation, photosynthesis, osmoregulation, protein synthesis, turgor-pressure-driven solute transport in xylem (Marschner, 1995; Ashraf, 2004). Accumulation of these and some other organic ions increased osmotic activity, causing a reduction in water potential and an inward diffusion of water from the surrounding cells which result expansion and maintenance of turgor of a cell (Akram *et al*., 2009).

In present study the application of nutrient solution improved water potential and solute potential in control and salt stressed plants. Turgor potential was not greatly changed and thus it contributed in osmoregulatory process. Similar results were also reported by Sultana *et al*., (2001), who demonstrated in seawater-stressed rice plant that turgor pressure is controlled by solute regulation within the guard cell protoplast and the relative water content of epidermal tissues.

Overall, foliar application of nutrients was found to be effective in mitigating the adverse effects of salinity on growth of both sunflower and safflower plants. Although exogenous application of nutrients caused slight changes in leaf osmotic potential, it significantly improved plant water status which might have contributed to better growth of both the plants under salt stress.

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