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Effect of salinity stress on seedling growth of sunflower (*Helianthus annuus* L.) genotypes

A Ramaswamy, * S Seeta Ram Rao
Department of Botany, Osmania University, Hyderabad, Telangana, India

Abstract
Effects of salinity stress on seedling emergence and seedling growth characteristics were screened for four sunflower genotypes. Results revealed that there were significant differences for root length and shoot length, fresh weight and dry weight etc., under salinity. The differences among the genotypes for MDA and proline content were studied. The results suggest that, Olinda and Cleo genotypes were tolerate to salt stress and can be used for cultivation on saline soils for better yield.

Keywords: salinity, growth, sunflower, genotypes

1. Introduction

Salt- affected soils are an important ecological entity in the landscape of any arid and semi-arid region. In India nearly 9.38 million ha area is occupied by salt-affected soils out of which 5.5 million ha are saline soils (including coastal) and 3.88 million ha alkali soils (IAB 2000). These occur from Jammu & Kashmir (Ladakh region) in north to Kanyakumari in south and Andaman Nicobar Islands in the east to Gujarat in the west. Salinity poses a serious threat to our ability to increase food production to meet the expanding needs.

Soil salinity becomes a key factor in determining crop production and distribution in many areas (Gucci *et al*., 1997) [1]. High concentrations of salts in soil account for greater loss of crop yields all over the world.

Impact of salt stress on higher plants is complex and incompletely understood. The accumulation of ions in leaves under conditions of salt stress causes reduction in photosynthesis (Yeo *et al*., 1985) [2] and growth (Gadallah, 1999) [3]**.** Excesses of Na^+ and Cl^-, the predominant ions in sodic soil, create high ionic imbalances that may impair the selectivity of root membranes (Bohra and Dörffling, 1993) [4]**.** Saline conditions can induce K^+ deficiency (Botella *et al.*, 1997) [5].

In this perspective, the present study was planned to identify the sunflower (*Helianthus annuus* L.) genotypes showing tolerance to salinity levels. The selected genotypes may be recommended for use by the farmers of salt affected areas according the intensity of salinity problem.

2. Materials and Methods

Four sunflower genotypes were employed for the study. They were: Olinda, Cleo, Reyflo and Clara. Seeds were collected from Manisha Agri Biotech Hyderabad, Telangana. Healthy and uniform sized seeds for the four genotypes were sorted and were surface sterilized with 5% (w/v) thirum solution (fungicide) for 15 min followed by rinsing of the seeds with double distilled water (DDW).

The treatments employed for the study:

1. Distilled water (control).
2. 0.15M NaCl (stress treatments).

Seeds were sown at a depth of 2 cm in a plastic pot (height 85 mm, diameter 80 mm) filled with coco peat (neutral delignified coir fibres) and then added water to control and 0.15 M NaCl concentration to stress treatment up to two third of the pot. Ten seeds were sown in each pot in the upper coco peat layer at 2 cm depth which receive water/solution by capillarity. This technique simulates a semi-hydroponic system where the upper layers of coco peat medium receive water/saline solution only by capillary movement, while the roots are immersed in saturated lower coco peat medium and during capillary movement there is free flow of oxygen to constant evapotranspiration. Each of the treatments was replicated four times for all genotypes. The temperature was about 27 ^{0}C. Artificial light was provided for 12 hours for 15 days. Emergence of plumule was taken as the criteria for germination. Seedling emergence was counted on 7th day.

Percentage of seedling emergence

The percentage of seedling emergence was calculated by following formula:

% of Seed Germination = Number of seeds germinated / Total number of seeds x 100

On the 15th day, seedling growth was recorded in terms of seedling length, vigour index, shoot and root length, fresh weight and dry weight.

Seedling vigour index

Seedling vigour index was calculated by following formula: Suggested by Abdul-Baki and Anderson (1973) [6].

% seed germination x mean seedling length (root length+ shoot length)

Shoot and Root length

Shoot and root length were recorded separately in centimetres.

Fresh weight of seedlings

Fresh weights of the seedling weighted and recorded in

milligrams (mg).

Dry weight of seedlings

Seedlings were dried in oven at 110^0 C for 24 hours and their dry weight was recorded. The dry weight is expressed in terms of milligrams (mg).

Estimation of Lipid peroxidation

Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content following the method of Heath and Packer (1968) [7]. One gram seedling material was macerated in 5 ml of 0.1% (w/v) trichloroacetic acid (TCA). The homogenate was centrifuged at 10,000 x g for 5 minutes. For 1 ml of the aliquot of the supernatant, 4 ml of 20 % TCA containing 0.5% TBA was added. The mixture was heated at 95 ^{0}C for 30 minutes and cooled quickly in ice bath. The contents were centrifuged at 10,000 x g for 10 minutes and the absorbance was measured at 532 nm and the value for the non-specific absorbance at 600 nm was subtracted. The concentration of malondialdehyde (MDA) was calculated by using extinction coefficient of 155 mM^{-1} cm^{-1}. MDA content was expressed as M g^{-1} fresh weight**.**

Free Proline

The amount of proline content was estimated as described by Bates *et al*., (1973) [8]. Seedling material (0.5 g) was homogenized with 10 ml of 3 % (w/v) sulfosalicylic acid and the homogenate was filtered through whatman No. 2 filter paper. The supernatant was taken for proline estimation. The reaction mixture was composed of 2 ml of plant extract, 2 ml of acid ninhydrin reagent and 2 ml of glacial acetic acid. The test tubes containing above mixture were heated in a boiling water bath for one hour. The reaction was terminated in an ice bath followed by addition of 4 ml of toluene. The contents were shaken vigorously and then allowed to separate into phases. The chromo phase containing upper toluene phase was carefully taken out with the help of a pipette and the absorbance was taken at 520 nm. The amount of proline present was quantified with the help of proline standard graph.

3. Results

Seedling Emergence

The effect of salinity stress (0.15M NaCl) on percentage of seedling emergence of sunflower genotypes is shown in Table 1.

Salinity stress lowered the seedling emergence in case of all the four genotypes of sunflower as compared to the respective unstressed control treatments. The toxic impact of salinity stress on seedling emergence was found to be much more acute in case of Clara and Reyflo genotypes. In Clara, 83.7% reduction in seedling emergence was recorded. In case of Reyflo genotype also the toxic effect of salinity was very high (71.2% reduction over the control). In case of Olinda and Cleo genotypes, the influence of salinity on seedling emergence was found to be only marginal. Just 3.3% and 7.7% decrease in seedling emergence was found in Olinda and Cleo genotypes respectively.

Table 1: Effect of salinity stress on seedling emergence of sunflower genotypes

	Emergence (%)*	
Genotype	Control	0.15M NaCl
Olinda	99±1.0	95.7±2.4
Cleo	98±1.5	90.4±3.2
Reyflo	98±1.5	28.2±1.3
Clara	98±1.4	15.9±0.8

*Mean ± SE (N=4)

Seedling vigour index

The effect of salinity stress on the seedling vigour index of four sunflower genotypes in comparison to their respective unstressed control treatments is shown in Table 2.

Toxic impact of salinity stress was more on Reyflo and Clara genotypes. Seedling vigour index was decreased by 84.1% in Reyflo and 89.9% in Cleo in comparison to seedlings from respecive controls. Reduction of seedling vigour index was just 1.8% in Olinda and 0.6% in Clara in comparison to seedlings from control.

Table 2: Effect of salinity stress on seedling vigour index of sunflower genotypes (15days old seedlings)

	Seedling vigour index*	
Genotype	Control	0.15M NaCl
Olinda	2583.9±2.0	2536.05±2.5
Cleo	2401.0±2.5	2386.56±2.4
Reyflo	1960.2±2.5	310.02±1.5
Clara	2401.5±2.0	241.68±2.0

*Mean ± SE (N=4)

Growth Parameters

Root Growth

The effect of salinity stress on root length of seedlings of sunflower genotypes is shown in Table 3.

Salinity stress decreased the root length of Reyflo and Clara genotypes as compared to the respective unstressed control treatments. In Clara, 14.1%, reduction in seedling root length was recorded. In case of Reyflo genotype the toxic effect of salinity was very high (35. %, reduction over the control). In case of Olinda and Cleo genotypes, seedling root length was increased due to salinity stress. Root length was increased by 39.8% in Olinda, 36.6% in Cleo genotypes respectively.

Table 3: Effect of salinity stress on tap root length and shoot length of sunflower genotypes (15days old seedlings)

	Root length (cm)*		Shoot length (cm)*	
Genotype	Control	0.15M NaCl	Control	0.15M NaCl
Olinda	12.3±0.1	16.2±0.2	13.8±0.4	10.3±0.2
Cleo	12.0±0.3	16.4±0.4	12.5±0.2	10.0±0.4
Reyflo	10.0±0.2	6.5±0.6	10.0±0.1	4.5±0.3
Clara	11.3±0.3	9.7±0.3	13.5±0.3	5.5±0.2

*Mean ± SE (N=4)

Shoot Growth

The effect of salinity stress on the shoot length of four sunflower genotypes in comparison to their respective unstressed control treatments is shown in Table 3.

Salinity stress caused reduction in shoot length of all four sunflower genotypes. While marginal decrease in shoot length was seen in Olinda and Cleo, drastic reduction in shoot length was observed in Reyflo and Clara genotypes. Shoot length was decreased by 25.3% in Olinda and 20.0% in Cleo in comparison to seedlings of control treatments. Reduction of shoot length in Reyflo was 55.0%, and 40.7% in Clara in comparison to seedlings from control treatments was observed.

Root fresh weight

The effect of salinity stress on the root fresh weight of four genotypes of sunflower in comparison to their respective unstressed control treatments is shown in Table 4.

Salinity stress caused substantial reduction in the root fresh weight in case of Reyflo and Clara genotypes. In case of both these genotypes, salinity resulted in 48% reduction root fresh weight. However, salinity stress caused an increase in root fresh weight in case of Olinda (25% over control) and Cleo (23% over control) genotypes.

Table 4: Effect of salinity stress on root fresh weight and shoot fresh weight of sunflower genotypes (15days old seedlings)

	Root fresh weight (mg)*		Shoot fresh weight (mg)*	
Genotype	Control	0.15M NaCl	Control	0.15M NaCl
Olinda	120.2±1.0	150.2±3.4	550.0±3.5	485.0±1.5
Cleo	132.1±2.5	162.4±2.5	570.0±2.2	515.0±2.1
Reyflo	125.6±1.5	65.5±1.2	545.0±2.3	245.0±1.2
Clara	115.4±2.1	60.0±1.1	560.0±1.4	240.0±1.5

*Mean ± SE (N=4)

Shoot fresh weight

The effect of salinity stress on the shoot fresh weight of four genotypes of sunflower in comparison to their respective unstressed control treatments is shown in Table 4.

Salinity stress lowered the seedling shoot fresh weight in case of all the four genotypes as compared to the respective unstressed control treatments. The toxic effect was more on Clara and Reyflo genotypes. In Clara 57% reduction in shoot fresh weight was recorded. In case of Reyflo, 55% reduction in shoot fresh weight was observed. The toxic influence of salinity on shoot fresh weight of Olinda and Cleo was only marginal. The decrease in shoot was fresh weight 11.8% in Olinda and 10.5% in Cleo genotypes.

Root dry weight

The effect of salinity stress on the dry weight of roots of four genotypes of sunflower in comparison to their respective unstressed control treatments is shown in Table 5.

Substantial reduction in root dry weight due to salinity was found in Reyflo and Clara genotypes. In Reyflo salinity stress accounted for 48% decrease in root dry weight as compared to unstressed control seedlings. Similarly 46.2% reduction in root dry weight as compared to the root dry weight of respective control seedlings was observed in Clara. However salinity stress resulted in increase in root dry weight in both Olinda and Cleo genotypes. The increase in root dry weight was 22.7% and 21.5% in Olinda and Cleo genotypes respectively.

Table 5: Effect of salinity stress on root dry weight and shoot dry weight of sunflower genotypes (15days old seedlings)

	Root dry weight (mg)*		Shoot dry weight (mg)*	
Genotype	Control	0.15M NaCl	Control	0.15M NaCl
Olinda	13.2±0.1	17.2±0.2	63.2±1.5	55.2±1.0
Cleo	14.0±0.3	17.1±0.5	70.5±1.7	62.4±1.6
Reyflo	11.3±0.4	5.5±0.1	65.4±1.4	30.0±1.1
Clara	12.1±0.1	6.0±0.2	61.2±1.1	28.0±1.2

*Mean ± SE (N=4)

Shoot dry weight

The effect of salinity stress on the shoot dry weight of four genotypes of sunflower in comparison to their respective unstressed control treatments is shown in Table 5.

Salinity stress lowered the shoot dry weight in case of all the four genotypes as compared to the respective unstressed control treatments. The toxic effect was more on Clara and Reyflo genotypes. In Clara 54.2% reduction in shoot dry weight was recorded. In case of Reyflo 54% reduction in shoot dry weight was observed. The influence of salinity on shoot dry weight of Olinda and Cleo was marginal and the reduction in shoot dry weight was 12.6% in Olinda and 11.4% in Cleo genotypes respectively.

Lipid Peroxidation

Effect of salinity stress on MDA content of seedlings of sunflower genotypes is shown in Table 6.

A comparison of MDA content in sunflower seedlings under salinity stress with respective control seedlings in case of four genotypes revealed that salt stress accounted increased lipid peroxidation. The impact of salt stress was found to be much more acute in case of Reyflo and Clara genotypes. The increase in MDA content was found to be 68.5% and 64.6% in Reyflo and Clara genotypes respectively. In Olinda and Cleo genotypes, the influence salinity stress on membrane peroxidation evaluated in terms of MDA content found to be marginal. Just 8.4% and 7.3% increase in MDA content due to salinity stress was observed in Olinda and Cleo genotypes respectively.

Table 6: Effect of salinity stress on MDA content and free proline content of seedlings of sunflower genotypes

	MDA Content(mg g^- FW)*		Free proline content (mg g^- FW)*	
Genotype	Control	0.15M NaCl	Control	0.15M NaCl
Olinda	5.23±0.45	5.68±0.44	1.94±0.05	3.18±0.14
Cleo	4.94±0.37	5.39±0.56	1.74±0.07	2.89±0.16
Reyflo	4.58±0.58	7.66±0.13	1.62±0.08	1.92±0.01
Clara	5.02±0.64	8.23±0.16	1.82±0.04	2.17±0.02

*Mean ± SE (N=4)

Free Proline

The effect of salinity stress on free proline content of seedlings of sunflower genotypes and in their respective unstressed control seedlings is shown in Table 6.

Salinity stress resulted in enhancement of free proline levels in sunflower seedlings raised under salt stress. A steep rise in proline levels was observed in Olinda and Cleo genotypes. The percent increase in proline levels was observed to be 66% and 64% in Cleo and Olinda genotypes respectively. In case of Reyflo and Clara genotypes, the increase in free proline content due to salt stress was found to be marginal. Salinity stress resulted in 18.5% and 19.2% increase in proline levels in Reyflo and Clara genotypes respectively.

4. Discussion

Salt concentration caused a decrease in germination percentage. The germination percentage of genotypes in decreasing order was Olinda>Cleo>Reyflo>Clara. Seed germination was negatively affected by increased concentrations of salinity in *Atriplex patula* and *Plantago psyllium* (Ungar, 1996;) [9]. Jamil *et al.,* (2006) [10] reported that salt treatments strongly affect seed germination in sugar beat, cabbage, amaranth and pak-choi. Seed germination speed decreased when the salinity level was raised in Canola cultivars (Bybordi *et al.*, 2010) [11]. Among the four genotypes studies, Olinda and Cleo genotypes found to be salt tolerant.

Increased root length was observed in Olinda followed by Cleo and decreased root length was observed in Reyflo followed by Clara genotypes. The accumulation of Na^+ in the roots is an adaptive response used by several woody species to avoid its toxicity in the shoots (Picchioni *et al.,* 1990; Gucci and Tattini, 1997) [12, 13]. Enhancement in salinity decreased root length in *Brassica napus* cultivars and also in *Seuvium portolacastrum* (Bybordi, 2010; Slama *et al.*, 2008) [11, 14]. However, an increase in root length with increasing concentration of NaCl (0-200mM) was observed in *Kochia prostrata* (Karimi *et al.,* 2005) [15].

The maximum average shoot length was recorded for Olinda which was followed by Cleo, Clara and Reyflo. Reduced shoot length with high concentration of salt level suggested by Khan *et al.,* (1994) [16] Ibrar *et al.,* (2003) [17] and Jabeen *et al.*, (2003) [18]. Length of shoot decreased in response to salinity in two wild species of potato *i.e.*, *Solanum stoloniferum* and *Solanum bulbosus* (Daneshmand *et al.*, 2010) [19]. *Pisticia vera* underwent a significant decrease at 60 and 80mM NaCl in terms of stem length (Chelli-chaabouni *et al.,* 2010) [20]. The toxic impact of salt stress was found to be minimal in case of Olinda and Cleo, where the shoot length under salinity stress was almost to unstressed control treatments.

The decrease in shoot fresh weight was more pronounced in Reyflo and Clara as compared to the sunflower genotypes Olinda and Cleo. The maximum mean shoot fresh weight was noted in Cleo. Similarly considerable variation in salt tolerance among sunflower genotypes (FS1, FS2 and FS5) using 40, 80 and 120 mM NaCl has been reported by Wahid *et al*., (1999) [21].

The results of this experiment showed that the magnitude of reduction in shoot dry weight was more in Reyflo and Clara genotypes than in the Olinda and Cleo genotypes. The differential salt tolerance in alfalfa genotype was linked to of their differential selectivity for K^+ over Na^+ (Ashraf, 2002) [22]. Salt tolerance in Olinda and Cleo might be due to osmo-regulation and potassium selectivity. Kingsbury and Epstein (1986) [23], Weimberg (1987) [24], Yeo *et al.,* (1990) [25] and Barrett-Lennard *et al.,* (1999) [26] suggested that osmotic adjustment, selectivity of potassium, Na^+ compartmentation or its exclusion and production of organic solutes can be linked to the salt tolerance of cultivars of different species.

An increased root fresh and dry weight was observed in Olinda and Cleo genotypes over the control, whereas decreased root fresh and dry weight was observed in Reyflo and Clara genotypes. Similar results were obtained in spider plants (Mwai, 2001) [27], and in white seed coat bambara (Tafouo *et al.*, 2008) [28]. The reduction of the plant dry weight due to increased salinity may be a result of a combination of osmotic and specific ion effects of Cl^- and Na^+ (*Turan et al.*, 2007; Tafouo *et al.,* 2010) [29, 30].

In our study, salt stress resulted that in increased lipid peroxidation levels. Under salt stress the lipid peroxidation levels were more in Reyflo and Clara compared to Olinda and Cleo genotypes. Oxidative stress is induced by salt stress results in the oxidative damage of lipids and Cell membrane proteins (Mano 2002) [31]. Olinda and Cleo genotypes exhibited relatively less membrane injury and lipid peroxidation levels compared to other genotypes. These results confirms the observations made by Azevedo-Neto *et al.,* (2006) [32] in case of salt-tolerant and salt-sensitive maize genotypes.

In the present study, it was observed that proline accumulation was more in Olinda and Cleo and less in Reyflo and Clara genotypes. It has been found that salt tolerant alfalfa plants showed high proline content in the roots; whereas salt sensitive plants the increase was low (Petrusa and Winicov, 1997) [33]. Proline is known to act as a mediator of osmotic adjustment (Handa *et al*., 1986) [34], stabilizer of subcellular structures (Smirnoff and Cumbes 1989) [35], free radical scavenger (lyer and Caplan 1998; Smirnoff and Cumbes, 1989) [36,35], sink for energy (Saradhi and Saradhi 1991) [37] and stress-related signal molecule (Werner and Finkelstein, 1995) [38]. Sivakumar *et al.,* (2000) [39] reported that proline alleviates NaCl stress-induced enhancement in oxygenase activity of Rubisco. Proline could act as a free radical scavenger in

sunflower genotypes under salt-stressed conditions. High accumulation of proline in Olinda and Cleo compared to other genotypes suggests that Olinda and Cleo possesses a better potential to maintain osmotic balance compared to other genotypes under salt stress.

5. Conclusion

The effect of salinity on the growth parameters in sunflower genotypes showed that all of the considered parameters were affected by salinity with a varietals difference. The studies revealed that the toxic effect of salt stress was more in case of Reyflo and Clara genotypes and least in case of Olinda and Cleo genotypes. From the studies it can be concluded that Olinda and Cleo genotypes are salt tolerant and Reyflo and Clara genotype are salt susceptible.

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