

# Effect of salinity stress on germination and early seedling growth of different Safflower (*Carthamus tinctorius* L.) genotypes

Naser karimi<sup>\*1</sup>, Zhaleh Soheilikhah<sup>1</sup>, Hamid Reza Ghasmpour<sup>1</sup> and Alireza Zebarjadi<sup>2</sup>

<sup>1</sup>Laboratory of plant physiology, Department of Biology, Faculty of Science, Razi University, Kermanshah, I.R. Iran.

<sup>2</sup>Department of Plant Breeding, Faculty of Agriculture, Razi University, Kermanshah, I.R. Iran.

## Abstract

Elevated soil saline levels resulting from natural geological, hydrological and pedological process, and from using salty water for irrigation may inhibit seed germination and seedling establishment of safflower, the prospective oil-seed crops. A germination study on safflower seeds and a short-term toxicity experiment with different concentrations of NaCl (control, 50, 100 and 200 mM) on safflower seedlings were conducted. Percent germination over control decreased significantly with increasing concentrations of NaCl. There were genotypic differences among the test genotypes in response to salt stress exposure. The performance of the G5 (Gilla) was the best among the genotypes. Germination of G5 was not inhibited at all up to 100 mM NaCl treatment. Root tolerance index (RTI) and relative shoot height (RSH) for safflower seedlings decreased with increasing concentrations of NaCl. In general, G5 (Gilla) has more tolerance to NaCl than the other studied genotypes.

**Keywords:** Salt stress, *Carthamus tinctorius* L., Early seedling stage, NaCl.

## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the prospective oil-seed crops which can tolerate environmental stresses including salinity and water stress [1]. It is one of the more important oil seed cultivated plant used for edible oil production in the world [2]. The importance of oil crops such as safflower has increased in recent years, especially with the interest in the vegetable oil for the human uses [3]. Generally safflower is produced on marginal lands that are relatively dry and relatively deprived in order to benefit fertilizer inputs and irrigation. Attempts to improve seed yield and quality by developing new genotypes and agronomic practices are underway throughout the world [4].

Plants exposed to stresses may undergo changes in their metabolism in order to adapt with changes in their environment. Agricultural productivity worldwide is subjected to increasing environmental constraints, particularly to salinity due to its high magnitude of impact and wide distribution [5]. The salinity problem is common in arid and semi-arid regions where rainfall is insufficient to leach salts out of the root zone. These areas often have high evaporation rates, which can encourage an increase in salt concentration at the soil surface [5]. Salt stress leads to the suppression of plant growth and development, membrane leakage, ion imbalance or disequilibrium, enhanced lipid peroxidation and increased production of reactive oxygen species which are scavenged by both enzymatic and non-enzymatic reactions [6].

In order to maintain homeostasis during stress condition, plants exhibit physiological, biochemical and molecular responses at both the cellular and whole plant levels [7, 8]. Also, the tolerance ability of some plants like rice (*Oryza sativa* L.) to salt stress depends on plant genotypes. For example, rice genotypes (Pokkali, Indica and Nonabokra) having high endogenous ABA levels during stress conditions, are classified as highly salt-tolerant ecotypes, while the majority of high yielding cultivars such as M-1-48, IR-29, IR-36 and IR72 are salt-sensitive [9, 6].

The few screens methods for salt-tolerance have been accomplished on a number of crops, using various parameters including germination and emergence, survival, growth rate, chlorosis or leaf damage, and the genotypic differences within species [10]. According to Flowers and Yeo [11] developing the procedure may itself be a difficult option, due to the complexity of environment by genotype interactions and ontogenetic drift in the response to salt, but the procedure is conceptually simple: to expose a group of genotypes to salinity, in order to choose the ones that perform the best. On the other hand, screening and selection for any character are desired at developmental stages as early as possible because plant growth is positively correlated with quantity or commercial quality of the marketed product. In this sense, a screening technique was recently developed to screen safflower genotypes for salt tolerance during germination and emergence stages [12, 13]. The objective of this research was to evaluate the effect of salinity on seed germination and early seedling growth of seven safflower genotypes which allows a quick and easy-to-measure screening tool for genotypic differences in salinity tolerance.

## MATERIAL AND METHODS

### Germination assay and seedling toxicity test

The experiment was conducted at Razi University, plant physiology laboratory in summer 2009. Effect of different concentrations of NaCl on the seed germination of different safflower

Received: Oct 20, 2011; Revised: Nov 10, 2011; Accepted: Nov 23, 2011 .

\*Corresponding Author

Naser karimi

Laboratory of plant physiology, Department of Biology, Faculty of Science, Razi University, Kermanshah, I.R. Iran.

Tel: +98-831-8211869; Fax: +98-831-4274545

Email: [nkarimie@yahoo.com](mailto:nkarimie@yahoo.com), [nkarimi@razi.ac.ir](mailto:nkarimi@razi.ac.ir)

genotypes was evaluated. The concentrations of NaCl were 0, 50, 100, 150 and 200 mM and were prepared freshly. Seeds of *C. tinctorius* five genotypes (G<sub>1</sub>, LRV-51-51; G<sub>2</sub>, Lesaf; G<sub>3</sub>, Gila; G<sub>4</sub>, Kino-76; G<sub>5</sub>, Isfahan) were obtained from the Agricultural Research Institute of Razi University (Kermanshah–Iran). For the growth of seedlings in sterile conditions on square Petri dishes, seeds were surface-sterilized in 70% (v:v) ethanol for 1 min, followed by incubation in a solution of 3.5% (w:v) NaOCl and 0.05% (w:v) Tween-20 for 15 min.

Two pieces of filter paper was placed on a Petri plate and moistened with 5.0 ml aqueous solution of NaCl. Controls were set up by moistening the filter paper with 5.0 ml deionized water. Fifty seeds of each genotype were placed in each plate, covered by lid, and incubated in a growth chamber with 14/10 h light/dark cycles; temperature was kept at 25 °C during the day and 20 °C during the night. Light intensity was around 280  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . Germinated seeds were counted 7 days after initiation, and the results were expressed as percentage over control. Seeds were considered germinated when the shoot extends to half of seed length and the radical extends to the seed length. Each treatment was replicated three times. After 7 d of growth, shoot height was measured from the culm base to the tip of the longest leaf and root length was measured from the root–shoot junction to the tip of the longest root. Root length was expressed as root tolerance index (RTI) and shoot height was expressed in terms of relative shoot height (RSH); both were

calculated by dividing length of the NaCl treated with that of the control. Dry weights of seedling parts were measured after drying samples at 70°C in an oven until a constant weight is achieved.

### Data analysis

The data from the various seed treatments were pooled together and subjected to analysis of variance (ANOVA) using SAS software to determine the statistical significance difference of the mean of the treatment in the final germination percentage and growth parameters. Excel software was used to draw figures. Means were compared by applying Duncan test at 5% probability.

## RESULTS AND DISCUSSION

### Effect of NaCl on seed germination

Salinity is one of the environmental stress factors limiting the plant growth and development. Different vegetative response end points such as root length, shoot height, root biomass, shoot biomass, and total biomass (root and shoot) are generally used in studies of salt resistance to plants. In addition, germination is as the most important stage of the plant life affected seriously from salinity [14, 15]. The differences in salt tolerance of plants not only among different species, but also within certain species are observed [16].

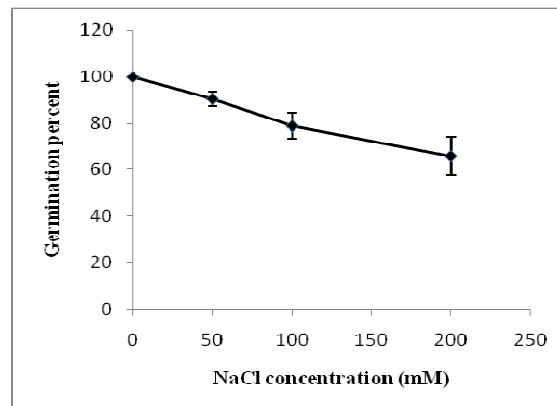


Fig 1. Seed germination percentage compared to control at different concentrations of NaCl. Each data point is the mean of seven safflower genotypes.

Salt stress induced by NaCl adversely affected germination percentage of the seven safflower genotypes (Fig. 1). The mean (average of all 7 genotypes) percent germination over control decreased significantly ( $p < 0.001$ ) with increasing concentration of NaCl (Fig. 1). Germination decreased by 14, 38 and 74% at 50, 100 and 200 mM NaCl respectively. There were genotype differences ( $p < 0.001$ ) in germination with increasing concentration of NaCl. All the genotypes except G<sub>5</sub> (Gilla) showed poor germination at the highest concentration (200 mM) of NaCl (Fig. 2). It also appears from our experimental result that safflower seed can generally germinate well up to 50 mM NaCl. G<sub>5</sub> (Gilla) was the only genotype, which showed a considerable resistance to the highest NaCl concentration, with no adverse effect at 200 mM of NaCl. At the NaCl concentration

of 200 mM, germination of G<sub>5</sub> just 12% affected.

Our findings are supported by Ghorashy et al. [17], Meloni et al. [18] and Huang et al. [19] who found that higher concentrations than 120, 100 and 200 mM NaCl decreased the germination of safflower, red quebracho and saxaul, respectively. Therefore, to distinguish the differences between germination percentage of the cultivars, it is necessary to exposure the higher NaCl concentrations which cultivars show nearly maximum germination. Germination of G<sub>5</sub> at a high concentration of NaCl is not unusual as Cheesman. [20] found some salt tolerance accession of some species that originated from saline area.

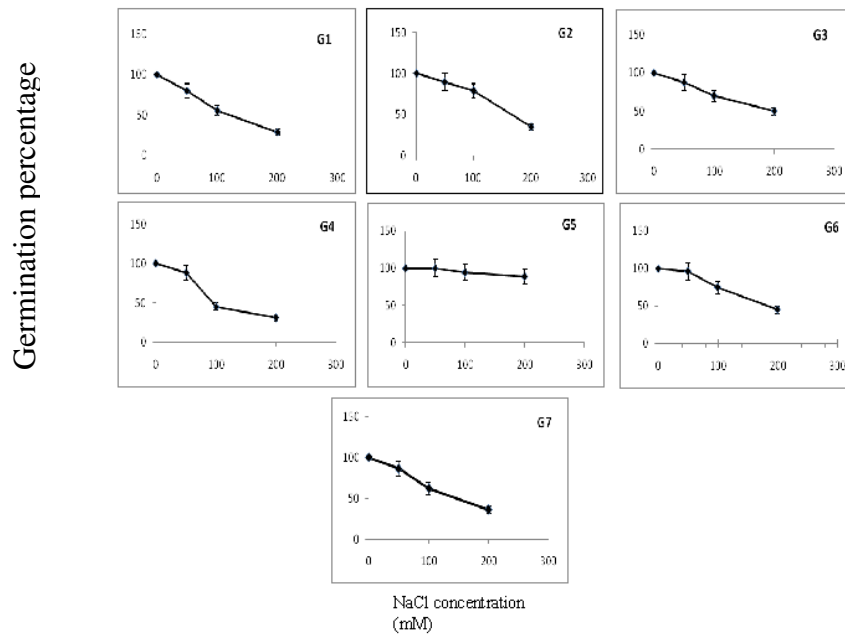


Fig 2. Effect of different concentrations of NaCl on percent seed germination compared to control of seven safflower genotypes. Each data point is the mean of 3 replicates. Error bars represent  $\pm$  SE.

**Effect of salt stress on root growth**

The effect of salinity on safflower root tolerance index (RTI) is in Fig. 3. The analysis of variance indicates that NaCl concentrations and different safflower genotypes had significant effects ( $p < 0.01$  for genotypes;  $p < 0.001$  for concentration) on RTI of safflower. Irrespective of genotypes, RTI decreased with increasing concentrations of NaCl (Figure 3). RTI reduced by 21, 50 and 71% at 50, 100, 150 and 200 mM NaCl treatments, respectively. This result indicates that NaCl caused great root length reduction at the mid

concentration range (Fig. 3 and 4). Differential root length responses to salinity were noted at different concentration levels among the safflower genotypes (Fig. 4). In general, root lengths of the G<sub>1</sub>, G<sub>3</sub> and G<sub>7</sub> were more adversely affected by NaCl than the other genotypes, especially at higher concentration range (150-200 mM). G<sub>5</sub> showed considerably enhanced resistance to NaCl applications for root growth (Fig. 4).

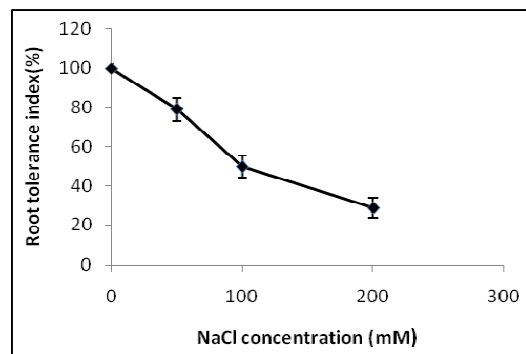


Fig 3. Effect of different concentrations of NaCl on safflower root tolerance index. Each data point is the mean of 7 safflower genotypes. Error bars represent  $\pm$  SE.

Reduction of both root and shoot length is a typical of response to salinity [15]. Reduced root length growth in response to salt exposure has been reported by a number of investigators (Chandru et al. [21] in the sunflower, Ozdemir and Engin [22], Rao et al. [23] in the chickpea and Ghorashy et al. [17]. Significant reduction of root length growth with increasing concentration of NaCl is due to

the fact that plant roots were the first point of contact for these toxic NaCl species in the nutrient media.

Our result of RTI shows that the G<sub>5</sub> (Gilla) genotypes have a significantly higher tolerance to salinity, although the actual differences in tolerance of other genotypes are not large. This tolerance to salinity could be related to some physiological and

biochemical adaptation strategies, which have been described to be heritable, often as a result of multiple or single gene differences, and genetic modifiers [24]. Salt-tolerance mechanisms are complex and include osmotic adjustment through the accumulation of compatible

solutes, lowering the concentrations of toxic ions in the cytoplasm by restriction the influx of Na<sup>+</sup>, its sequestration in the vacuole, and/or Na<sup>+</sup> ion extrusion [25], and scavenging of reactive oxygen species (ROS) [26].

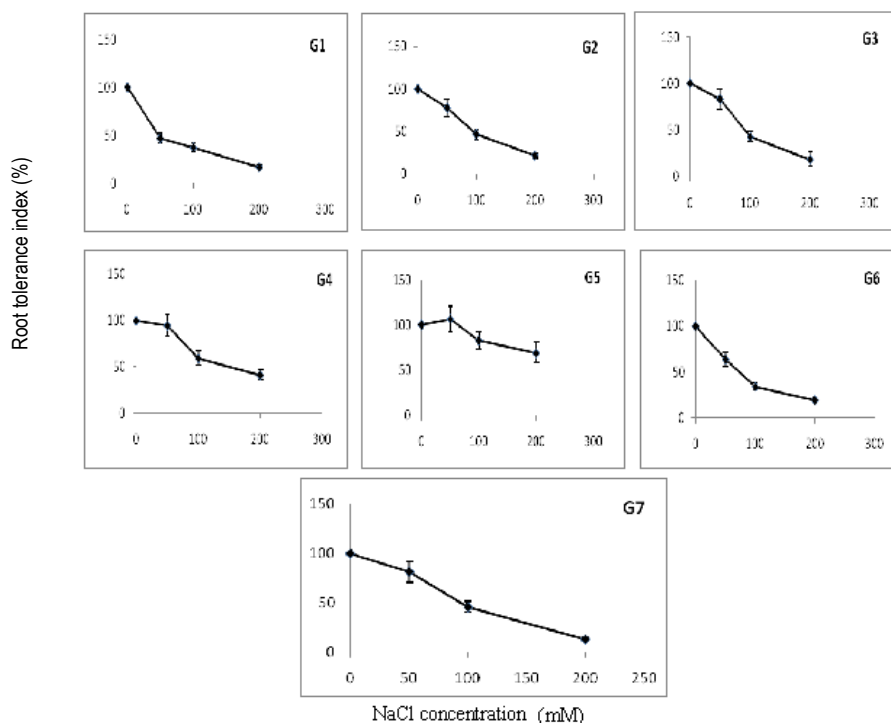


Fig 4. Effect of different concentration of NaCl on root tolerance index of 7 safflower varieties. Each data point is the mean of 3 replicates. Error bars represents ± SE.

**Effect of salinity on shoot height**

Significant reduction ( $p < 0.001$ ) of relative shoot height (RSH)

was observed due to increase in NaCl concentrations. Mean relative shoot height reduced by 16, 31 and 62% at 50, 100 and 200 mM NaCl (Fig. 5).

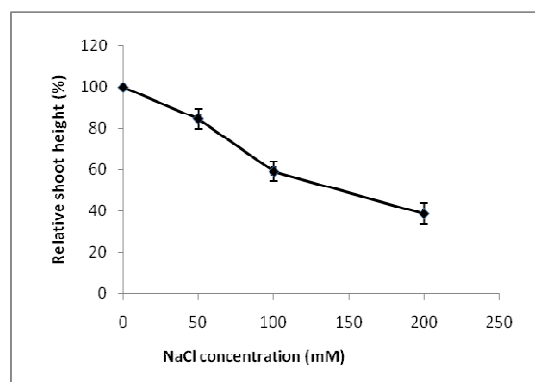


Fig 5. Effect of different concentrations of NaCl on relative shoot height of safflower. Each data point is the mean of 7 safflower varieties. Error bars represent ± SE.

Analysis of variance for RSH shows significant statistical ( $p > 0.05$ ) difference between different NaCl concentration and genotypes ( $p < 0.001$ ). Highest mean RSH of 95.63 (%) was noted in G<sub>5</sub> followed by G<sub>4</sub> (74.77%) and G<sub>2</sub> (56.83%) in 100 mM NaCl treatment. The lowest RSH was shown by G<sub>6</sub> (37.56%) (Fig. 6). A strong relationship ( $r^2 = 0.68$ ) exists between RTI and RSH when safflower plants are exposed to different concentrations of NaCl (Fig. 7). RSH increased with increasing RTI. This result indicates that reduction of shoot height growth is positively dependent on the reduction of root length growth.

**Effect of salinity on shoot and root biomass**

Significant reductions in safflower shoot length with increasing NaCl concentration suggest that safflower shoot length can also be used as a good indicator for NaCl toxicity. Reduced shoot height due to application of NaCl in this study also corroborates with the result of Ghorashy *et al.* [17], who found significant reduction of safflower shoot height when NaCl was applied at a relatively lower dose of 75 mM NaCl.

The reduction of shoot height due to NaCl exposure can be

an important consideration for safflower cultivation as reduced shoot height will decrease safflower leaf area, net photosynthesis, and ultimately safflower yield. Comparing the inhibition of root length growth with the shoot height growth, it seems that inhibition of root growth is more prominent than shoot growth especially at the higher

NaCl concentrations (100-200 mM of NaCl) (Fig. 3–6). This result indicates that at a concentration at which shoot length growth was inhibited to some extent, root length growth was completely halted.

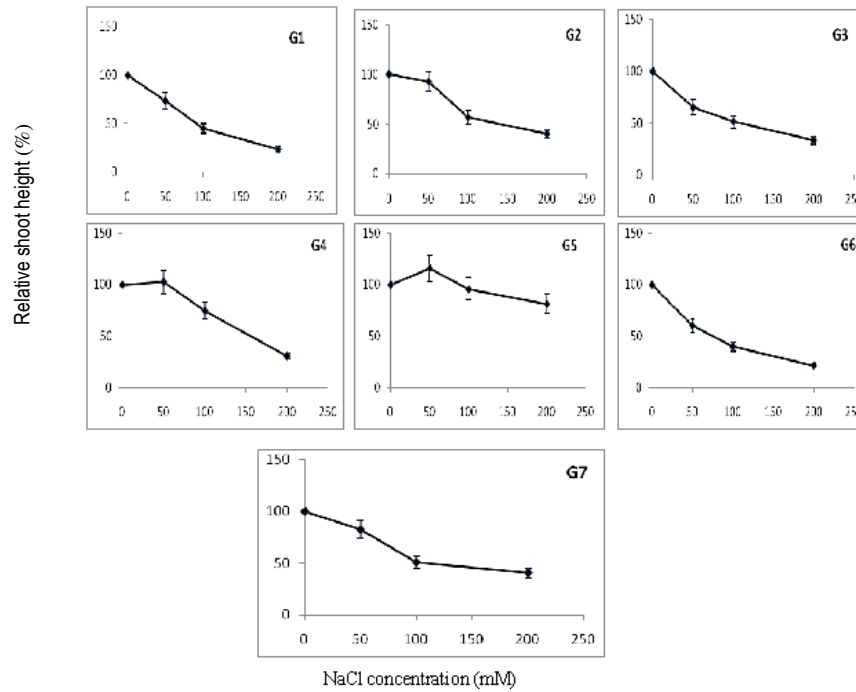


Fig 6. Effect of different concentrations of NaCl on relative shoot height of safflower. Each data point is the mean of 3 replicates. Error bars represents  $\pm$  SE.

Increasing salinity levels caused remarkably decreases in shoots and root dry weight (Table 1). Although our genotypes showed different responses to each salinity level, the highest values in all salinity levels were usually obtained from G<sub>5</sub> (Gilla). As

expected, salt levels had a significant effect on the shoot dry weight of all genotypes. Salt concentrations resulted in a decline of both root and shoot dry weight.

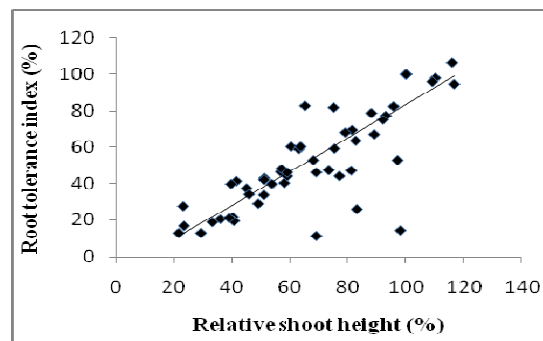


Fig 7. Relationship between relative shoot height and root tolerance index as affected by different concentration of NaCl.  $r^2 = 0.687$ .

Decreasing shoot dry weight resulted in the decline of shoot length in all genotypes, but the reduction which occurred in G<sub>5</sub> and G<sub>2</sub> was lower than that in G<sub>7</sub> genotype. This gave a lower shoot length and this was attributed to higher shoot dry weight at higher salinity levels, meaning that genotype G<sub>5</sub> could accumulate more dry

matter in high soil salinity levels. The dry weight of the roots of the genotypes declined as root length fell, but the decline of root dry weight was lower than that of root length. Among the genotypes, G<sub>5</sub> gave a higher root/shoot dry weight ratio at all salinity levels.

Table1. Relative toxicity of NaCl on germination and early seedling growth of safflower. Each data point is the mean of 7 safflower varieties.

NaCl concentration (mM)	0	50	100	200
Germination percent (%)	100.00	86.40	62.25	36.15
Relative root length (%)	100.00	79.12	49.68	28.53
Relative shoot height (%)	100.00	84.63	59.13	38.71
Relative root weight (%)	100.00	87.03	57.40	40.74
Relative shoot weight (%)	100.00	50.00	38.46	27.30

Sodium chloride solution treatments reduced the growth of the plumule and radicle. There is a direct relation between salt concentration and reduction in growth (Plumule and radicle), because as the NaCl concentration level increased, the plumule and radicle weight decreased (Table 1). It has been reported that during imbibitions, the effect of salt is merely osmotic until a hydration threshold is surpassed [27]. The combined toxic and osmotic effects could be lethal at high NaCl concentration levels (100-200 mM) leading to lack of germination at these levels. Some plants are sensitive to salinity at the seedling stage, because the mechanism of the tolerance to salinity is not yet fully developed [27]. Some other plants might also show tolerance to salinity at the seedling stage [28].

## CONCLUSION

Based on these results, the studied safflower genotypes (except G5) are sensitive to salinity and therefore cannot tolerate high salinity levels of 100–200 mM. The G<sub>5</sub> (Gilla) genotype showed tolerance up to 200 mM NaCl. Salt stress was found to be more toxic to seedling growth than to germination. Root length and shoot height was significantly reduced with increasing NaCl concentration in growing media. There was considerable variation in toxicity among the genotypes. The G<sub>5</sub> (Gilla) showed higher tolerance to NaCl than the other studied genotypes. The tolerance characteristic could be an important criterion to select a genotype to grow in soils with elevated NaCl concentration or with contaminated water. Although a considerable magnitude of variation for salt tolerance was observed in a set of 7 genotypes of safflower while screening them at both germination and seedling stages, but a further study needs to be carried out to assess whether the lines marked as salt tolerant at the initial growth stages, maintain their degree of salt tolerance when tested as adult.

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