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THE EFFECT OF WATER STRESS AND SALINITY ON GROWTH AND PHYSIOLOGY OF TOMATO *(LYCOPERSICON ESCULENTUM* MILL.*)*

ANASTASIA E. GIANNAKOULA and I. F. ILIAS

Department of Crop Production, Alexander Technological Educational Institute of Thessaloniki, Sindos 54700, Greece

Abstract - Carotenoids like lycopene are important pigments found in photosynthetic pigment-protein complexes in plants. They are responsible for the bright colors of fruits and vegetables and perform various functions in photosynthesis. Our research has shown that the application of moderate salt stress on tomato plants can enhance lycopene and potentially other antioxidant concentrations in fruits. The increase in lycopene in response to salt stress in the tomato fruits varied from 20% to 80%. Although the specific biological mechanisms involved in increasing fruit lycopene deposition has not been clearly elucidated, evidence suggests that increasing antioxidant concentrations is a primary physiological response of the plant to salt stress. Additionally drought stress during cultivation increased the antioxidant capacity of tomato fruit while maintaining the lycopene concentration. In addition, the effects of silicium were investigated, added to the nutrient solution either at low concentration or at an increased concentration. The present study clearly indicates that an enhanced silicium supply to tomato increases markedly the lycopene contents, irrespective of the salinity status in the tomato fruit.

Key words: Chlorophyll fluorescence, gas exchange, drought stress, lycopene, salinity treatment, *Lycopersicon esculentum* L, peroxidation, total antioxidant capacity gas exchange parameters, NaCl, photosystem II, silicium (Si)

INTRODUCTION

The physiology of plant responses to salinity and their relation to salinity resistance have been much researched and frequently reviewed in recent years (Neumann 1997; Lu et al., 2003). Many crop species, including bean, tomato, onion, pepper, corn, potato etc., are sensitive to salinity, resulting in reduction in crop productivity (Ashraf, 2004). The decline in growth observed in many plants subjected to salinity stress is often associated with a decrease in their photosynthetic capacity. Although much effort has been invested into investigating the cause of decreased photosynthetic capacity, the underlying mechanisms are still unclear.

Tomato is an important agricultural commodity worldwide. More than 80% of tomatoes are consumed in the form of processed products such as tomato juice, paste, puree, ketchup, sauce, and salsa. Lycopene is responsible for the characteristic deep-red color of ripe tomato fruits and tomato products. (Helyes et al., 2009). It is the major carotenoid in tomato representing 80-90% of the total pigments in it. It also participates in photoprotection and protects photosynthetic organisms from excessive light damage. Lycopene is a key intermediate in the biosynthesis of many important carotenoids, such as beta-carotene, and xanthophylls. Plants produce various phytochemicals that are of nutritional and medicinal value to humans (Clinton, 1998). Phytochemicals having antioxidant capacity are drawing increased interest from consumers. Many studies have consistently demonstrated inadequate consumption of fruit and vegetables. Improving the intake of fruit and vegetables has been a major public health effort for many

years with minimal success. One plausible approach is the development of fresh produce containing a greater concentration of phytochemicals known to improve health. Controlled environments provide a unique opportunity to modify the concentrations of selected phytochemicals in fruit and vegetables, yet practical information is limited regarding methods effective in optimizing antioxidant capacity. Lycopene is correlated with reduced incidence of some cancer types. It is an acyclic, biologically active carotenoid found in foods, and its preventive role in several cancerous diseases has been proved by epidemiological and experimental data (Lugasi et al., 2004).

Although silicium (Si) is not considered an essential element for plant nutrition, many authors have reported on beneficial effects when its supply to various cultivated plants is enhanced. In most cases, the favorable effects of Si on crop plants seem to originate from reinforcement of the cell walls due to deposition of Si in the form of amorphous silica (SiO_2 nH_2O) and opal phytoliths (Inanaga and Okasaka, 1995; Epstein, 1999). In other reports, Si was implicated to ameliorate the adverse effects of aluminum toxicity (Hammond et al., 1995), manganese toxicity (Iwasaki et al., 2002), and salinity (Bradbury and Ahmad, 1990; Liang et al., 1996).

The main objective of the present study was to evaluate the effects of salt and drought stress, alone and in combination, on growth, photosynthesis, biochemistry and quality in tomato plants and their fruits. In addition, it is not clear how Si can increase the carotenoid content of the tomato fruit. A more detailed study in this area is needed including examination of the accumulation of antioxidant phytochemicals after exposure to drought stress conditions during the cultivation and application of Si.

MATERIALS AND METHODS

Plant material and culture

Local varieties of tomato seeds were sown in plates with perlite in a greenhouse and five weeks later the seedlings were transplanted in the ground. Experiments were conducted at the Technological Educational Institute of Thessaloniki (northern Greece) (22°55′E, 40°38′N) to determine the effect of preharvest application of salt on plant growth, photosynthesis, biochemistry and quality. Plants were sown individually and randomly inside the greenhouse, in experimental plots. Experiments were established on a sandy loam soil whose physicochemical characteristics were silt 18%, clay 5.6% sand 70.4%, organic matter 0.88%, $CaCO_3$ 0.9%, electrical conductivity 1.5μS cm^{-1}, and pH (1:2 H_2O) 7.4. The region is characterized by continental climatic conditions. Each plant was watered as required and fertilized weekly at each irrigation with 300 cm^3 of nutrient solution containing 60.0 mg N, 26.2 mg K and 49.8 mg P or P_2O_5 (water-soluble fertilizer 20-20-20, *F-TOP Ledra*, Thessaloniki, Greece). The photosynthetically active radiation (PAR) at plant height in the greenhouse was of 500-700 μmol m^{-2} s^{-1} (measured by a *Li-6200* portable photosynthesis meter, *LiCor*, Lincoln, NE, USA). Plants were maintained in the greenhouse under natural sunlight; average day/night temperatures were 30±2/26±2°C.

Pigment estimation

Chlorophylls (*a*+b) of the youngest fully expanded leaf were quantitatively measured in 100% acetone extract by spectrophotometry using the re-determined extinction coefficients (Lichtenthaler et al., 2005).

Lycopene determination

Extraction was performed according to Fish et al. (2002). Samples were first chopped and homogenized in a laboratory homogenizer. Approximately 0.3-0.6 g samples were weighed and 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol and 10 mL of hexane were added. The absorbance of the hexane layer (upper layer) was measured at absorbance 503 nm spectrophotometry using the re-determined extinction coefficients (Perkins et al., 2004).

pH measurements

The fruit juice pH levels were measured with a pH

meter (SI Analytics ProcessLine pH electrodes, Germany)

In vivo chlorophyll fluorescence measurements

In vivo chlorophyll fluorescence was measured on the upper surface of the third fully expanded leaf, after being left for 30 min to dark adaptation, at room temperature. The chlorophyll fluorescence induction curve was monitored by a Plant Analyzer (PEA, Hansatech Ltd King's Lynn, Norfolk, England) with 600Wm^{-2} of red (630nm) light intensity (excitation intensity) (Ouzounidou et al., 2008). The initial fluorescence intensity (Fo) when all reaction centers (RCs) are open, Fm maximal fluorescence intensity when all reactions centers (RCs) are closed, F_v variable fluorescence and the maximal photochemical efficiency of photosystem II (PSII) photochemistry in the dark-adapted state (F_v/F_m), were calculated. The indicators were measured at room temperature on intact leaves of four replicate plants from the six treatments.

Gas exchange measurements

Gas exchange was measured on the second fully expanded leaf with a Li-6200 portable photosynthesis meter (LiCor, Inc. Lincoln, NE) supplied with IRGA (Li-6250). The CO_2 analyzer was calibrated with two standard CO_2/air mixtures. Laminas of two leaves were used per measurement, enclosed vertically within a fan-stirred 1/4 l cuvette. The mean CO_2 concentration and leaf-to-air vapor pressure deficit for all measurements were 350 μmol mol^{-1} and 20 mbar, respectively. Measurements were made at 25°C under a photon flux density of about 750 μmol^{-2} s^{-1}. Calculations of A (assimilation rate), E (transpiration rate), gs (stomatal conductance) and Ci (intercellular concentration of CO_2) from gas exchange measurements were according to von Caemmer and Farquhar (1981).

Determination of lipid peroxidation

The level of lipid peroxidation in shoots/leaves after five days of chilling stress and 3 days of recovery treatment was measured as malondialdehyde (MDA) content determined by reaction with 2-thiobarbituric acid (TBA) reactive substances as described by Giannakoula et al. (2012). The tissue was homogenized in 0.3% TBA in 10% trichloroacetic acid (TCA) at 4°C. The concentration of MDA was calculated from the difference of the absorbance at 532 nm and 600 nm using the extinction coefficient of 155 mmol^{-1}cm^{-1} and expressed as nmol (MDA) g^{-1} of fresh weight.

Determination of proline and carbohydrates

Shoots/leaves were cut into small pieces, weighed, placed separately in glass vials containing 10mL of 80% (v/v) ethanol, and heated at 60°C for 30 min. The extract was then filtered and diluted with 80% (v/v) ethanol 6 up to 20 mL (Khan et al., 2000). The shoot/leaf concentrations of free proline and carbohydrates were determined in this extract following the acid ninhydrin reagent method and the anthrone method, respectively (Khan et al., 2000; Plummer, 1987). Two mL of the aqueous alcohol extract was transferred into test tubes and 2 mL of acid-ninhydrin was added. With glass marbles on top, to minimize evaporation, test tubes were maintained at 95°C for 60 min in a water bath and then allowed to cool at room temperature. 4 mL toluene was added to each replicate and thoroughly mixed. After separation of solution layers, the toluene layer was carefully removed, placed in glass cuvettes, and absorption was determined at 518 nm. Ethanol extracts, as used for the proline assay, were diluted 10 times with 80% (v/v) ethanol for the assay of carbohydrates. The diluted extract was added drop-by-drop in 2 mL anthrone reagent in test tubes in an ice bath and left to mix the content. Fully mixed samples were incubated in a water bath at 90°C for 15 min, cooled and absorbance was read at 625 nm.

Hydrogen peroxide

For the determination of H_2O_2, the samples were extracted according to Giannakoula et al. (2007, 2010). Hydrogen peroxide was measured spectrophotometrically after reaction with KI. The reaction mixture consisted of 0.5 mL 0.1% trichloroacetic acid (TCA),

root extract supernatant, 0.5 mL of 100 mM K-phosphate buffer and 2 mL reagent (1 M KI (w/v) in fresh double-distilled H_2O). The blank probe consisted of 0.1% TCA in the absence of root extract. The reaction was developed for 1 h in darkness and absorbance was measured at 390 nm. The amount of hydrogen peroxide was calculated using a standard curve prepared with known concentrations of H_2O_2.

Experimental design, data collection and statistical analyses.

Thirty-nine plots (three replications for each treatment) were set up using a randomized complete design. Each plot contained 21 single plants in 3 rows with 7 plants per row spaced 50 cm apart within each row. The distance between rows was 60 cm, and between plots 100 cm. Cultural practices were carried out according to the recommended production practices for the area. Data were subjected to analysis of variance (ANOVA) using the SPSS 11.0.1 for Windows statistical package (SPSS, Chicago, USA). For comparison of the means, Duncan's multiple range tests ($p \leq 0.05$) were employed.

RESULTS AND DISCUSSION

Effect of salinity and drought on the level of photosynthetic pigment

Plants that are subjected to environmental stress often suffer oxidative damage (Scandalios, 1993). The use of growth, physiological and biochemical criteria has been recommended to achieve salinity stress to plants (Ashraf, 2004, 2009). Long-term growth responses of tomato plants to salinity stress and the possible ameliorative role of Si to salt stress were analyzed. During development, a series of changes in color took place, indicating changes in the photosynthetic pigments. A concentration-dependent response of photosynthetic pigment to salinity stress was observed. The contents of chlorophylls (Chl) in the plants were significantly affected by NaCl and drought treatment (Table 4) and decreased from 12.8 mg g^{-1} f.w. total Chl in the control to 10.9 mg g^{-1} f.w. and 7.0 mg g^{-1} f.w in plants grown at 150 mg NaCl and drought + 150 mM NaCl, respectively. The lower Chl*a*+b at a high salinity level also indicates stress and damage to the photosynthetic apparatus. A decline in the level of photosynthetic pigments may be attributed to salinity-induced inhibition of chlorophyll biosynthesis (Khan, 2006) that may be caused by the induced nutrient deficiency.

On the other hand, tomato color is another important factor affecting consumers' tomato preferences. The color of a ripe tomato is determined by the ratio of two pigments, lycopene and β-carotene (Hobson and Grierson 1993). Lycopene, a carotenoid, is formed during fruit ripening and determines the degree of fruit redness. The red color of the fruit originates from lycopene (Tepic et al. 2006). As shown in Table 2, lycopene content differs significantly between the control and salt treatments. Moreover, the lycopene content seemed to increase significantly, especially in the NaCl and Si treatments.

Effect of salinity and drought on the pH level, fruit fresh and dry weight

The fruit characteristics of the control tomato plants (0 NaCl) were compared with those of tomato plants treated with NaCl and no irrigation. The results showed that the fruit fresh weight was significantly influenced by NaCl (Table 1). We observed a reduction of 26% in plants treated with NaCl. The fruit dry weight content (% percentage) of treated and untreated tomato plants varied between 4.6% and 5.8%, as shown in Table 2. More specifically, the dry weight of the control plants was significantly higher than those of the plants treated with salt and no irrigation.

Additionally, the pH value also plays an important role in determining fruit quality characteristics (Turhan et al., 2011). Many studies focused on pH as a key element in tomato selection (Hong, Tsou 1998). Analysis results showed that the pH values of tomato fruit ranged between 4.8-4.2 (Table 2). In our study, pH values did not differ significantly between the treatments of tomato plants.

Table 1. Effects of drought, NaCl and silicium (Si) on carbohydrate (mol g^{-1} FW), proline (mol g^{-1} FW) lipid peroxidation (nmol g^{-1} FW) and H_2O_2 (nmol g^{-1} FW) concentrations of tomato plants. Significant difference at $P < 0.05$. The values are means ± SD of 15 plants.

Treatments	Salt concentration (mg l^{-1})	Proline	Carbohydrates	MDA	H_2O_2
Control	0	0.98 ± 0.2[a]	23.62 ± 2.1[a]	62.27 + 0.14[a]	6.4 ± 0.16[a]
No Irrigation	0	1.77 ± 0.2[b]	24.72 ± 0.1[a]	73.68 + 0.17[b]	7.7 ± 0.15[b]
No Irrigation+ NaCl	100	1.94 ± 0.01[b]	26.38 ± 1.0[b]	77.15 + 0.24[b]	8.0 ± 0.2[b]
No Irrigation+ NaCl	150	2.77 ± 0.01[b]	28.77 ± 1.4[b]	125 ± 4.3[c]	8.8 ± 0.14[c]
NaCl	100	3.15 ± 0.03[c]	31.81 ± 2.4[c]	139 ± 2.6[c]	8.9 ± 0.2[c]
NaCl	150	3.29 ± 0.07[c]	32.00 ± 0.9[c]	148± 2.8[d]	9.8 ± 0.15[c]
NaCl+ Si	150	1.63± 0.01[b]	24.0± 0.1[b]	73± 0.17[b]	7.1± 0.12[b]

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

Table 2. Effects of drought, NaCl and silicium (Si) on fruit weight (mg^{-1} g f.w.), dry weight (%), pH (%) and lycopene (mg^{-1} g f.w.) of tomato plants. Significant difference at $P < 0.05$. The values are means ± SD of 15 plants.

Treatments	Salt concentration (mg l^{-1})	Fruit weight (g/fruit)	Dry weight (%)	Lycopene (mg^{-1} g f.w.)	pH (%)
Control	0	185.3a	5.84a	89.6b	4.8a
No Irrigation	0	173.4a	5.01a	91.5c	4.61a
No Irrigation+ NaCl	100	155.8ab	4.81b	94.5b	4.45a
No Irrigation+ NaCl	150	134.5b	4.66b	98.3b	4.35a
NaCl	100	147.1b	4.85b	92.4c	4.23a
NaCl	150	138.3b	4.61b	98.6b	4.21a
NaCl+ Si	150	181.2a	5.21ab	107.5a	4.76a

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

Effect of salinity and drought on lipid peroxidation and H_2O_2

Malondialdehyde (MDA) content, a product of lipid peroxidation, has been considered as an indicator of oxidative damage. MDA is the decomposition product of polysaturated fatty acids of biomembranes and its increase shows plants under high-level antioxidative stress (Pan et al., 2006). The main site of attack by any redox active metal in a plant cell is usually the cell membrane. In our experiments, significant increases in MDA concentration were observed (Table 1). MDA concentration changed linearly with increased NaCl levels in the solution. Similar results were found with *Salicornia persica, S. europaea* and buckwheat (Aghaleh et al., 2009; Jovanović et al., 2011). Thus, the increased MDA indicates oxidative stress and this may be one of the possible mechanisms by which toxicity due to salinity could be manifested in the plant tissues. NaCl-treated tomato plants (150 mM) induced an increase in lipid peroxidation, revealed as 2-fold increase in MDA production compared to the control (Table 1). Additionally, the H_2O_2 concentration changed linearly with increased NaCl levels in the solution. The highest values of MDA and H_2O_2 were revealed with 150 mM NaCl. The application of Si in combination with NaCl had no effect on lipid peroxidation, whereas NaCl alone enhanced lipid peroxidation (Table 1). Thus, in our case, cell membrane stability can be utilized to differentiate

Table 3. Effects of drought, NaCl and silicium (Si) on gas exchange parameters of tomato plants. Significant difference at $P<0.05$. The values are means ± SD of 15 tomato plants.

Treatments	Salt concentration (mg l^{-1})	Amax	gs	Ci	E
Control	0	25.2 ± 0.01^{c}	0.5± 0.02^{c}	247± 0.01^{b}	3.7± 0.03^{b}
No Irrigation	0	20.1± 0.03^{b}	0.314± 0.04^{b}	199± 0.01^{a}	3.41± 0.01^{b}
No Irrigation+ NaCl	100	13.5± 0.03^{b}	0.214± 0.02^{b}	236± 0.02^{b}	3.23± 0.05^{b}
No Irrigation+ NaCl	150	9.45± 0.03^{a}	0.216± 0.01^{b}	294± 0.04^{c}	3.6± 0.01^{b}
NaCl	100	6.5± 0.03^{a}	0.5± 0.02^{c}	300± 0.01^{c}	2.19± 0.03^{a}
NaCl	150	5.90± 0.02^{a}	0.04± 0.03^{a}	156± 0.02^{a}	1.39± 0.01^{a}
NaCl+ Si	150	26.7± 0.03^{c}	0.44± 0.01^{c}	239± 0.03^{b}	3.59± 0.02^{b}

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

Table 4. Effects of drought, NaCl and silicium (Si) on chlorophyll fluorescence parameters, chla+b (mg^{-1} g f.w.) and lycopene (mg^{-1} g f.w.) of tomato plants. Significant difference at $P<0.05$. The values are means ± SD of 15 plants.

Treatments	Salt concentration (mg l^{-1})	Fo	Fm	Fv/Fm	Chla+b	Lycopene
Control	0	430a	2511b	0.829c	12.8c	89.6a
No Irrigation	0	684b	3048c	0.775b	13.4b	91.5a
No Irrigation+ NaCl	100	446a	1729a	0.742b	7.6b	94.5a
No Irrigation+ NaCl	150	897c	3380c	0.734b	7.0a	98.3b
NaCl	100	531a	1843a	0.712a	10.3b	92.4a
NaCl	150	1007c	3385c	0.683a	10.9a	98.6b
NaCl+ Si	150	519a	3360c	0.845c	14.5c	107.5c

Means followed by different letters in the same column for each treatment differ significantly ($p<0.05$).

sensitivity and resistance to salt as in many other cases (Aghaleh et al., 2009).

Effect of salinity and drought on proline and carbohydrates

Salt stress induces various biochemical and physiological responses in plants and affects almost all plant processes (Nemoto and Sasakuma, 2002). NaCl may act directly via osmotic or ionic sensory mechanisms, or it may act indirectly through mediators to affect existing metabolic pathways, gene expression, and result in a coordinated response to osmotic stress.

The amounts of proline and carbohydrates in grown tomatoes under drought stress and different concentrations of salinity are shown in Table 1. The treatment of the tomato plants with drought salinity increased proline and carbohydrate concentrations. The maximum proline accumulation was recorded at 150 mM NaCl (3.29). Accumulation of proline in response to excess NaCl has been described in several plants (Pan et al., 2006; Kwan, 2006).These fluctuations were greater and statistically more significant in the NaCl treatment. The maximum proline accumulation was recorded in plants treated with 150 mM NaCl.

The accumulation of proline in response to excess metals such as copper, cadmium, zinc and nickel has been described in several plants (Alia and Saradhi, 1995; Megateli et al., 2009). The suggestion that com-

patible solutes contribute to the detoxification of reactive oxygen species (Bohnert et al., 1995; Smirnoff, 1998) was confirmed recently when an elevated proline content was found to reduce free radical levels in response to osmotic stress in tobacco (Hong et al., 2000), and Pro and GB to reduce ROS in salt-stressed *Arabidopsis* roots (Cuin and Shabala, 2007).

In addition at the high concentration of NaCl (150 mM), the concentration of soluble sugars increased at a rate closely corresponding to the increase in fresh weight (Liu and van Staden, 2001) Zhang et al. (2000) and Roy and Bera (2002) have recorded increases in proline concentration when plants were grown in nutrient solutions containing excessive Cd, Hg or Mn concentrations, whereas Yelenosky and Vu (1992) have reported that both proline and carbohydrate concentrations increased during cold hardening of orange plants. Finally, proline and carbohydrates increased with an increase in NaCl and $CaCl_2$ salinity in *Catharanthus roseus* (Jaleel et al., 2007; Jaleel et al., 2008), in sorghum and wheat (Abdelasmad, 1993; Azooz et al., 2004). Similar results were observed in the groundnut (Girija et al., 2002).

Effect of salinity and drought on photosynthesis and chlorophyll fluorescent parameters

The diminution of the maximum quantum yield of PSII (Fv/Fm) means that the plants treated with an excess of NaCl (150 mM) were under stress conditions where molecular O_2 operates as an alternative acceptor for non-utilized electrons and light energy (Larsson et al., 1998; Cakmak and Romheld, 1997), resulting thus in the generation of reactive oxygen species (Cakmak, 1994). The ability of reactive oxygen species to cause photooxidative damages in organic molecules could probably explain the reductions of leaf chlorophyll, as well as the increase of proline and carbohydrate concentrations. Photosystem II (PSII) is believed to be the most stress sensitive. The *in vivo* chlorophyll fluorescence technique is a powerful non-destructive and fast method to detect changes in the photosynthetic activity in leaves influenced by changes in the environment. The ratio F_v/F_m has been shown to be reliable stress indicator. Drought stress is one of the most important abiotic stress factors that are generally accompanied by heat stress in dry season (Dash and Mohanty, 2001). Drought tolerant plants exposed to low water potential can be characterized by growth response, stomatal conductance of the leaves, by changes in water relations and ion accumulation of tissues, and by fluorescence induction parameters under water stress. In our results, chlorophyll fluorescence and gas exchange parameters differ significantly between control plants and plants treated with NaCl (Tables 3 and 4). More specifically, the assimilation rate had a significant reduction of about 73% between the control and the tomato plants treated with NaCl.

Additionally, soil drought and water deficits lead to a progressive suppression of photosynthetic carbon assimilation (Chaves 1991; Zlatev and Yordanov, 2004). We conclude that all gas exchange parameters (Table 3) and chlorophyll fluorescence parameters (Table 4) were strongly influenced by the high concentration of NaCl (150 mM) and no irrigation, whereas no changes took place in the treatment with NaCl and Si.

CONCLUSIONS

The lower level of lipid peroxidation in tomato plants treated with Si in combination with salt may explain an enhanced resistance to salinity compared to the other treatments including drought (no irrigation treatment). Our results suggest that Si supply has a significant role in contributing to the salt-stress resistance of tomato plants by improving the components of the antioxidant defense system. These data provide evidence of an internal mechanism of salt tolerance that increases the antioxidant system activity in order to limit cellular damages and is possibly linked to the salt and drought tolerance of the tomato plants. The simultaneous action of two factors (drought, salinity) aggravated the effects on the biochemical characteristics. The effect of NaCl stress seems to cause more changes at a concentration of 150 mmol. On the other hand, it apparently decreased the effect of salt stress as in the case of Si.

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