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Changes in growth and water-soluble solute concentrations in *Sorghum bicolor* stressed with sodium and potassium salts

Ralph Weimberg, H. R. Lerner and A. Poljakoff-Mayber

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Sorghum bicolor L. Moench, RS 610, was grown in liquid media salinized with NaCl, KCl, Na_2SO_4, K_2SO_4 or with variable mixtures of either NaCl/KCl or Na_2SO_4/K_2SO_4 at osmotic potentials ranging from 0 to –0.8 MPa. The purpose was to study the effects of different types and degrees of salinity in growth media on growth and solute accumulation. In 14-day-old plants the severity of leaf growth inhibition at any one level of osmotic potential in the medium increased according to the following order: $NaCl < Na_2SO_4 < KCl = K_2SO_4$. Inhibition of growth by mixtures of Na^+ and K^+ salts was the same as by K^+ salts alone. Roots responded differently. Root growth was not affected by Na^+ salts in the range of 0 to –0.2 MPa while it was stimulated by K^+ salts. The major cation of leaves was K^+ because *S. bicolor* is a Na^+-excluder, while Na^+ was the major cation in roots except at low Na^+/K^+ ratios in media. Anions increased in tissues linearly in relation to total monovalent cation, but not with a constant anion/cation ratio. This ratio increased as the cation concentrations in tissues increased. Sucrose in leaf tissue increased 75 fold in Chloride-plants (plants growing in media in which the only anion of the salinizing salts was Cl^-) and 50 fold in Sulphate-plants (the only anion of the salinizing salts was SO_4^{2-}). Proline increased 60 and 18 fold in Chloride- and Sulphate-plants, respectively, as growth media potentials decreased from 0 to –0.8 MPa. The concentrations of both sucrose and proline were directly proportional to the amount of total monovalent cation in the tissue. Sucrose concentrations began increasing when total monovalent cations exceeded 100 μmol (g fresh weight)$^{-1}$ (the monovalent cation level in non-stressed plants), but proline did not start accumulating until monovalent cation concentrations exceeded 200 μmol (g fresh weight)$^{-1}$. Therefore, sucrose seemed to be the solute used for osmotic adjustment under mild conditions of saline stress while proline was involved in osmotic adjustment under more severe conditions of stress. Concentrations of inorganic phosphate, glucose, fructose, total amino acids and malic acid fluctuated in both roots and leaves in patterns that could be somewhat correlated with saline stress and, sometimes, with particular salts in growth media. However, the changes measured were too small (at most a 2–3 fold increase) to be of importance in osmotic adjustment.

Additional key words – Osmotic adjustment, salt-stress, solutes.

Ralph Weimberg (reprint requests), U.S. Salinity Lab., Agricultural Research Service, USDA, 4500 Glenwood Drive, Riverside, CA 92501, U.S.A.; H. R. Lerner and A. Poljakoff-Mayber, Dept of Botany, The Hebrew Univ. of Jerusalem, 91904 Jerusalem, Israel.

Introduction

Plants react to saline or water stresses by a reduction in growth and, in most plants, by an accumulation of water-soluble solutes in tissues. Although plants respond in this manner, there are quantitative differences in the degrees of response. The degree of growth response of plants to salinity, expressed as yield decrease under saline conditions compared to non-saline conditions, is called the plant's "salt tolerance" (Maas and

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Hoffman 1977), and the ability to accumulate increased amounts of solutes is known as "osmotic adjustment" (Turner and Jones 1980). It is believed that plants adjust osmotically to reduce the water potential in plant cells so that it is lower than the external environment and, thus, to insure a flow of water into the plant.

A number of inorganic and organic compounds have been identified which increase in concentration in tissues of higher plants subjected to saline stress (Ackerson 1981, Borowitzka 1981, Coughlan and Wyn Jones 1980, Flowers and Hall 1978, Gorham et al. 1980, Greenway and Munns 1980, Hellebust 1976, Jones et al. 1980, Matile 1978, Munns et al. 1982, Weimberg et al. 1982). One of these solutes, proline, is such a common organic compound in stressed plants that it is the sole subject of three recent reviews (Aspinall and Paleg 1981, Stewart 1981, Stewart and Hanson 1980). Although proline appears to be a ubiquitous solute for osmotic adjustment, it does not start accumulating until the plant is subjected to moderate to severe levels of stress (Downton and Loveys 1981, Huber and Schmidt 1978, Jefferies et al. 1979, Storey and Wyn Jones 1979, Weimberg et al. 1982, Wyn Jones and Storey 1978). Indeed, in two species where it has been studied (Voetberg and Stewart 1983, Weimberg et al. 1982), proline does not begin to increase in concentration until the monovalent cation concentration of cells crosses a threshold value of 200 μmol (g fresh weight)$^{-1}$, and this threshold value is not reached until the plants are at least moderately stressed by salinity. In *Sorghum bicolor* it has been further shown that there is an apparent specific ion effect of K^+ on proline accumulation because this threshold was reached in leaves of plants at lower levels of salinity if the salinizing salt was KCl than if the salinizing salt was NaCl (Weimberg et al. 1982).

Since proline is found only in moderately and severely stressed sorghum plants, it means that there are probably other compounds that increase in concentration in slightly or mildly stressed plants; i.e., before proline accumulation begins. What these compounds might be is currently unknown. Therefore, a survey of concentrations of a number of inorganic and organic low-molecular-weight, water-soluble compounds in non-stressed and stressed plants of *S. bicolor* was undertaken in order to obtain an overview of the osmotic adjustment patterns of this plant. In addition, the effects of sodium and potassium chloride or sulphate on these osmotic adjustment patterns was studied. The purpose was to try to identify any compounds possibly involved in osmotic adjustment, to ascertain the relationships of these compounds to one another, and to determine if the cations or anions of the salinizing salts modified the amounts of these compounds in a manner similar to the effect of K^+ on proline accumulation.

Abbreviations – Cl-plants (Su-plants), plants from media in which the only anion of the salinizing salt was chloride (sulphate); K_f^+, K_r^+, Na_f^+, Na_r^+, the content of potassium and sodium ions in the leaves (foliar) and roots, respectively; TotM, the sum of K^+ and Na^+; ψ_w, water potential of the growth medium.

Materials and methods

Growth of plants

Sorghum bicolor, RS 610, seeds were germinated and allowed to grow for 4 days as described previously (Weimberg et al. 1982). On the 4th day, seedlings were placed over aerated media composed of 3 m*M* KNO_3, 2.5 m*M* $Ca(NO_3)_2$, 1.5 m*M* $MgSO_4$, 0.14 m*M* KH_2PO_4, 0.07 m*M* K_2HPO_4, 0.045 m*M* $MnSO_4$, 0.23 m*M* H_3BO_3, 7.6×10^{-4} m*M* $ZnSO_4$, 3.2×10^{-4} m*M* $CuSO_4$, 1.2×10^{-4} m*M* H_2MoO_4, and 2.5×10^{-2} g ferric sequestrene (containing 10% Fe^{3+}) l^{-1}. The plants were grown in a growth chamber kept at 29°C for 14 h in the light (58 Wm^{-2} at plant height) and then at 25°C for 10 h in the dark. Beginning on the 6th day, plants were subjected to salinity stresses by adding solutions of either KCl or K_2SO_4 to growth media in amounts sufficient to lower the Ψ_w (water potential of a growth medium) by an increment of 0.2 MPa at 24 h intervals, until the desired level of K^+ salt was reached (–0.1 MPa in cases where only this amount of K^+ was needed). A potential of –0.1 MPa equals 23 m*M* KCl or NaCl or 15 m*M* K_2SO_4 or Na_2SO_4. If the amount of K^+ salt added did not lower the Ψ_w of the particular medium to that needed for the selected growth condition, the salinization was continued by the addition of the Na^+ salt of the same anion until the sum of K^+ and Na^+ salts produced the Ψ_w desired.

Plants were harvested on the 14th day of growth after the lights had been on for 6 h. Before excision of the tissues, roots were washed in three fresh changes of distilled water to remove occluded salts. After excision, the roots were blotted on paper towels. Growth was measured as the fresh weight of tissue immediately after the tissue had been cut from the plant.

Extraction and assay of solutes

Water-soluble solutes in roots and leaves were extracted with a water-toluene system (Weimberg et al. 1981, 1982). All solutes were measured in the crude extract. The cations, K^+ and Na^+, were assayed by atomic absorption spectrometry. Appropriate physical or chemical methods were used to assay for Cl^- (Cotlove 1963), SO_4^{2-} (Farber 1976), P_i (Taussky and Shorr 1953), amino acids (Spies 1957) and proline (Bates et al. 1973). Enzymatic methods of assay were used to detect glucose (Slein 1965) and fructose (Klotzsch and Bergmeyer 1965). Sucrose was hydrolyzed with invertase, assayed as glucose, and its concentration was calculated as the increased amount of glucose present after invertase hy-

drolysis. Malate was also measured enzymatically (Hohorst 1965) except that the system of glutamic-oxalacetic acid transaminase at pH 7 was used as the oxalacetate-trapping agent instead of hydrazine.

Replication

The growth experiment was replicated at three different times. The solute extraction experiment was replicated twice with plants grown at different times. Each extraction step was done in duplicate, and all solutes in each crude extract were assayed at least twice. Standard deviations are summarized in the figures for the sake of clarity.

Results

Effect of saline-stress on growth

Weights of leaves from saline-stressed plants of *S. bicolor* decreased with increasing external salinity in the root zone. A Ψ_w of –0.2 MPa resulted in a 35–50% decrease in fresh weight, depending on the salt, and at –0.8 MPa fresh weights were only 43–15% of those of non-stressed plants (Fig. 1A). The inhibitory effect of the individual four salts was not equal but increased according to the following order: NaCl < Na_2SO_4 < K_2SO_4 = KCl. If mixtures of NaCl/KCl or Na_2SO_4/K_2SO_4 were used to salinize the media, the inhibition of growth was the same as shown for K^+ salts alone in Fig. 1A, regardless of the Na^+/K^+ ratio (data not shown). Thus, it appears that media with 23 m*M* or more of K^+ were more inhibitory to growth than basic media, which contains only 3.5 m*M* K^+, salinized with Na^+ salts.

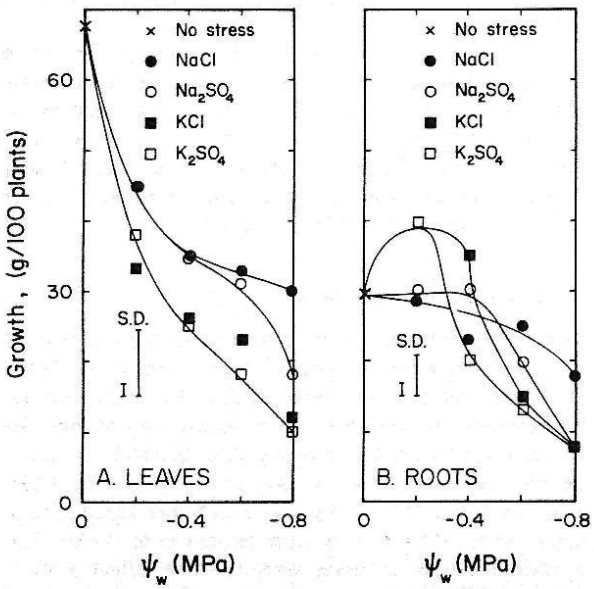

Fig. 1. Effect of several different salts on growth. Plants were harvested on the 14th day after germination. Leaves and roots were weighed separately. Vertical lines are the minimum and maximum SD observed with data in this figure.

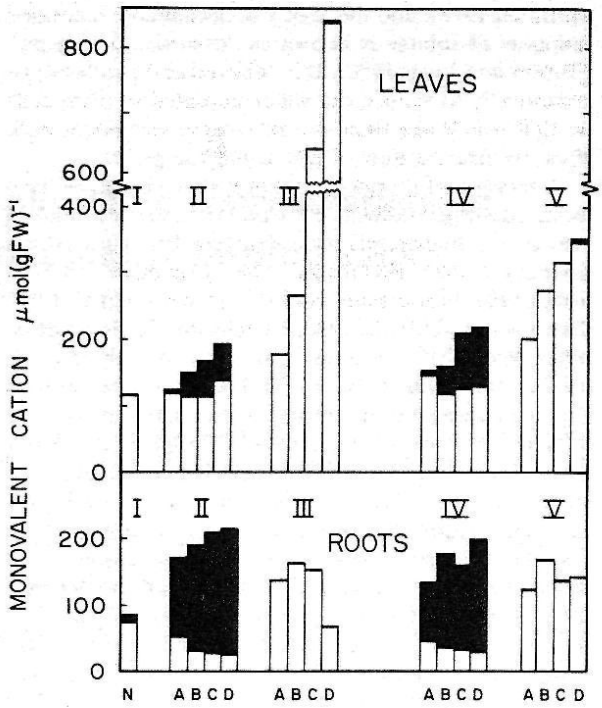

Fig. 2. Accumulation of Na^+ and K^+ in leaves and roots of plants grown in salinized media at potentials ranging from 0 to –0.8 MPa. Set I, non-stressed plants; Set II, media salinized with NaCl; Set III, media salinized with KCl; Set IV, media salinized with Na_2SO_4; Set V, media salinized with K_2SO_4; N, no salt added; A, –0.2 MPa; B, –0.4 MPa; C, –0.6 MPa; D, –0.8 MPa. White bars, K^+; black bars, Na^+. Range of SD for K^+ in leaves, ± 1.5 to 30; for K^+ in roots, ± 0.2 to 7.5; for Na^+ in leaves; ± 0.3 to 11; for Na^+ in roots, ± 0.3 to 10.

Lowering of Ψ_w to –0.2 and –0.4 MPa with K^+ salts actually stimulated root growth in comparison to non-salinized plants while Na^+ salts had little or no effect (Fig. 1B). All salts inhibited growth when Ψ_w was lowered to –0.6 and –0.8 MPa, but NaCl was less inhibitory than the other salts. The results were the same with salt mixtures as with a K^+ salt alone with the same anion (data not shown).

Effect of salinity on solute concentrations

I. *Solutes in leaves*

A. *Sodium, potassium and TotM (total monovalent cation).* Na^+ was absent in non-stressed plants because control growth media contained no Na^+. Levels of K_f^+ (foliar K^+) in Na^+-stressed plants (due either to NaCl or Na_2SO_4) were more or less equal under all growth conditions and equal to the amounts in non-stressed plants (Fig. 2). In these same plants, Na_f^+ (foliar Na^+) increased with increasing stress, but under the most severe conditions of Na^+-salt stress studied (–0.8 MPa),

TotM (the sum of K^+ and Na^+) of leaves had increased by only 50–60% over that of non-stressed plants.

In plants subjected to KCl or K_2SO_4 stress, K_f^+ increased equally in Cl-plants and Su-plants grown in media at –0.2 and –0.4 MPa (Fig. 2), and, at –0.4 MPa, the concentration of this cation was twice that in non-stressed plants. As Ψ_w was lowered further, K_f^+ increased but by different patterns in Su- and Cl-plants. In Su-plants, K_f^+ concentrations increased to a value of 345 μmol (g fresh weight)$^{-1}$ and further increases in K_2SO_4 in the media did not result in higher concentrations of K_f^+. In Cl-plants no such maximum in K_f^+ was observed. At –0.8 MPa, K_f^+ was 7.5 times higher in Cl-plants than in non-stressed plants, and the data suggest that the concentration might go even higher if the plants were subjected to even more severe levels of stress due to KCl. These differences in concentrations of Na_f^+ and K_f^+, depending on the cation of the salinizing salt, permit one to conclude that *S. bicolor* is a Na^+-excluder.

When mixed Na^+ and K^+ salts were used to salinize media, Na_f^+ decreased greatly and K_f^+ increased as Na^+/K^+ ratios in media decreased, and consequently, K_f^+ accounted for almost all the TotM in plants grown in media with mixed salts.

B. *Chloride.* Hoagland's solution contains no chloride salts; therefore, leaves of non-stressed and Su-plants contained little or no Cl^-. Chloride increased linearly in leaves of Cl-plants with increasing TotM and, as with cations, there did not seem to be any maximum amount that the tissues could accumulate. Cl^- increased at a faster rate relative to TotM in NaCl-treated plants than in KCl-treated ones (Fig. 3), but, because *S. bicolor* is a Na^+-excluder, there was more Cl- and cation in leaves of KCl-treated plants. Interestingly, the chlorides in leaves from plants grown in media with mixed salts increased with increasing salinity as if the media contained only KCl. Cl^- concentrations increased linearly in relation to TotM, but, proportionately, this anion increased faster than cation. The anion/cation ratio increased from 1:2 at a stress level of –0.2 MPa to 3:2 at –0.8 MPa in KCl-treated plants and to 2:1 in NaCl-treated plants. A ratio of 1:1 occurred only when foliar TotM equalled 170 μmol (g fresh weight)$^{-1}$ in NaCl-stressed plants or 325 μmol (g fresh weight)$^{-1}$ in plants stressed with KCl.

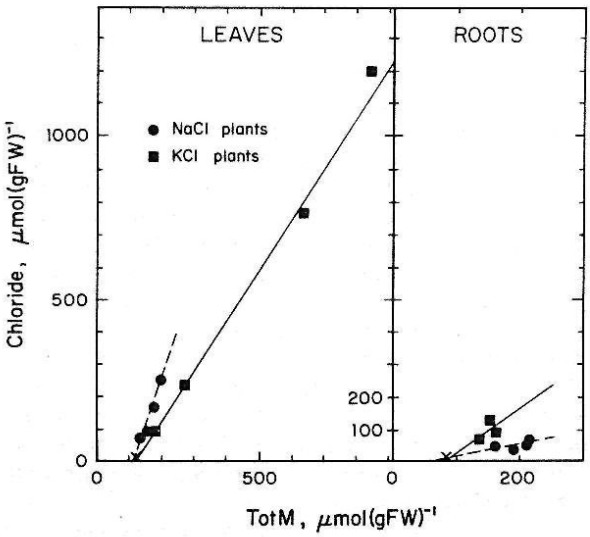

Fig. 3. Accumulation of chloride in leaves and roots of Cl-plants as a function of TotM. Solid lines, linear regression lines calculated from data (closed squares) for plants grown in the presence of KCl at media potentials ranging from 0 to –0.8 MPa (equation of line for leaves, y = 1.6x – 197; for roots, y = 1.2x – 100); dashed lines, linear regression lines from data (closed circles) of NaCl-treated plants at media potentials ranging from 0 to –0.8 MPa (equation of line for leaves, y = 2.8x – 305; for roots, y = 0.4x – 28.1); x, data from non-stressed plants.

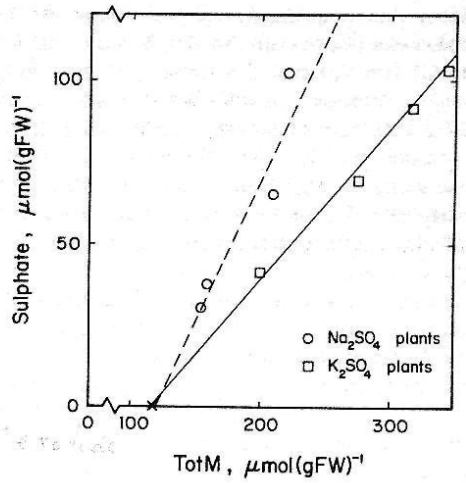

Fig. 4. Accumulation of sulphate in leaves of Su-plants as a function of TotM. Solid and dashed lines are linear regression lines calculated from data from plants grown at media potentials ranging from 0 to –0.8 MPa. Squares, data from plants grown in the presence of K_2SO_4 (equation of line, y = 0.46x – 52); circles, data from plants grown in the presence of Na_2SO_4 (equation of line, y = 0.85x – 100); x, data from non-stressed plants.

C. *Sulphate.* Hoagland's solution contains 1.5 m*M* SO_4^{2-}. Nevertheless, SO_4^{2-} was not found in leaves of non-stressed plants nor of Cl-plants. Sulphate increased linearly in Su-plants as TotM increased but, again, because *S. bicolor* is a Na^+-excluder, rates were dependent upon whether the media were salinized with Na_2SO_4 or K_2SO_4 (Fig. 4). The modifying effect of Na^+-exclusion on limiting the amount of anion was not as great in Su-plants as its effect in reducing Cl^- concentrations in Cl-plants. A maximum concentration of SO_4^{2-} in leaf tissue of 110 μmol (g fresh weight)$^{-1}$ was observed in K_2SO_4-treated plants. A similar value was measured in Na_2SO_4-treated plants subjected to a stress of –0.8 MPa, but it is not

clear from the data whether or not this would be the maximal concentration for Na_2SO_4-treated plants also. As with Cl^- in Cl-plants, the amounts of SO_4^{2-} in leaves of Su-plants subjected to salinities due to mixed cation salts increased with increasing salinity as if the media contained only K_2SO_4. Under both Na_2SO_4- and K_2SO_4-salinity conditions, the anion/cation ratio increased with increasing TotM but the ratio never reached the value of 1:2 for balanced inorganic anion and cation.

D. *Phosphate.* Phosphate concentrations increased with decreasing Ψ_w but were independent of ionic composition of the salinizing salts. On the average, P_i concentrations in leaves of both Cl- and Su-plants were 10.5±0.4, 12±0.3, 16±1.0, 22±2.7, and 28±3.1 µmol (g fresh weight)$^{-1}$ at Ψ_w of 0, –0.2, –0.4, –0.6 and –0.8 MPa, respectively.

E. *Sucrose.* Sucrose increased linearly as TotM increased in both Cl-plants and Su-plants (Fig. 5). The only exception to this linear relationship was in leaves of Su-plants grown at –0.8 MPa stress. These particular values were higher than would be expected from the calculated regression line. There is no apparent reason for these exceptional results. Sucrose accumulated at a slightly faster rate in comparison to TotM in Su-plants (26%) than in Cl-plants (22%). There were no separate Na^+ and K^+ effects other than that the total amount in leaves of plants subjected to –0.8 MPa was higher in KCl-treated plants than in NaCl-treated plants. This result, undoubtedly, is a consequence of the fact that

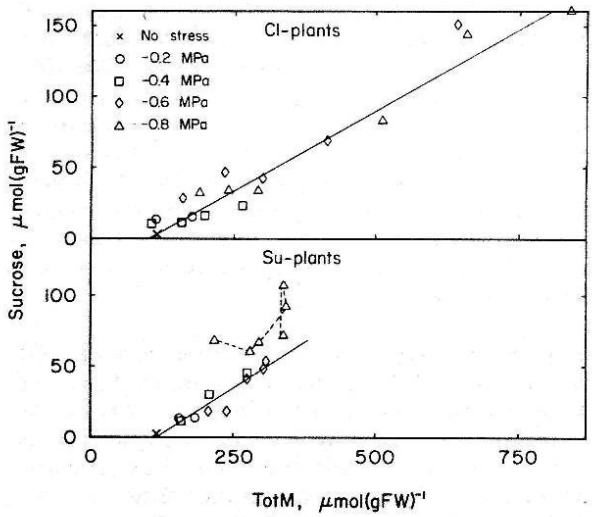

Fig. 5. Sucrose concentrations in leaves of stressed plants as a function of foliar TotM. Solid lines, linear regression lines (equation of line for Cl-plants, y = 0.22x – 19.7; for Su-plants, y = 0.26x – 30.9); dashed line, sucrose content in Su-plants grown in media with increasing proportions of K^+ at –0.8 MPa (these data are presented separately because they were not used in the calculation of the linear regression line for Su-plants); x, data from non-stressed plants.

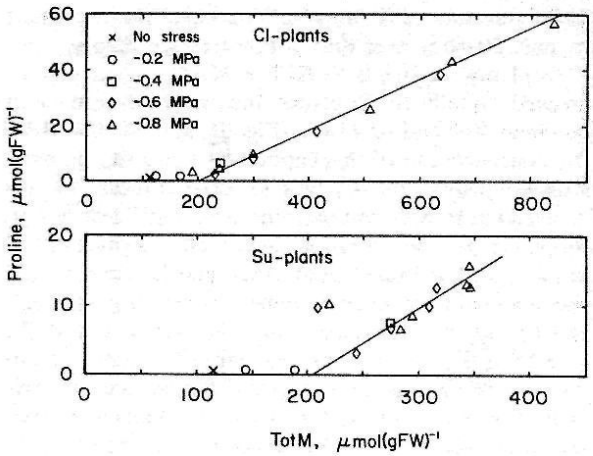

Fig. 6. Proline concentration in leaves of stressed plants as a function of foliar TotM. Symbols: same as Fig. 5. Solid lines, calculated linear regression lines except that data for the conditions of either 23 m*M* KCl in media for Cl-plants or 15 m*M* K_2SO_4 in media for Su-plants when total leaf water potential equalled –0.6 and –0.8 MPa were omitted because of their poor correlation with the rest of the data. (Equation of line for Cl-plants, y = 0.088x – 16.9; for Su-plants, y = 0.11x – 23.6).

KCl-treated plants contained a higher content of TotM. The calculated threshold of TotM for sucrose accumulation to begin was 100–110 µmol (g fresh weight)$^{-1}$ in both Cl- and Su-plants. This is close to the observed value of TotM in non-stressed plants (Fig. 2).

F. *Proline.* Exposure of sorghum plants to salinity resulted in the accumulation of proline in leaves as a function of TotM but only after TotM exceeded 200 µmol (g fresh weight)$^{-1}$ (Fig. 6). The rates of increase measured in these experiments were 9% in Cl-plants and 11% in Su-plants, about twice the rate reported in a previous experiment (Weimberg et al. 1982). The explanation for the lower amounts of proline in NaCl-treated plants compared to KCl-treated plants observed in this and the earlier work was undoubtedly that there is less TotM in tissues in NaCl-treated plants. There was a good correlation with all proline data with the calculated regression line except those for the concentrations of proline in plants grown at stresses of –0.6 or –0.8 MPa due to either NaCl or Na_2SO_4. Under these conditions, proline concentrations were higher than would be expected. The meaning of these results is not apparent from the data.

G. *Glucose and fructose.* Fructose values in leaves were approximately 50% of those for glucose but the pattern of change of these sugars was otherwise very similar. The concentration of glucose in leaves of non-stressed plants was 22.3±4.0 µmol (g fresh weight)$^{-1}$. At –0.8 MPa, glucose had increased to 36.6±1.9 µmol (g fresh weight)$^{-1}$ in plants stressed with NaCl, to 67.5±6.3 in plants stressed with KCl, but to 82.4±7.8 and 106±9.0

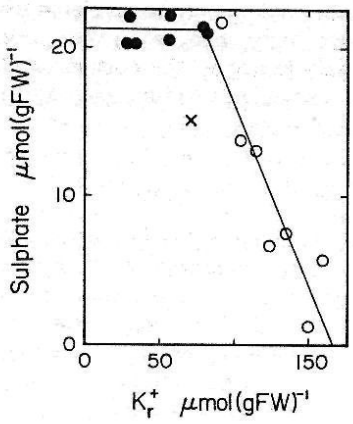

Fig. 7. Sulphate ion concentrations in roots of Cl-plants as a function of K_r^+. Symbols: closed circles, data used to calculate linear regression line when K_r^+ was less than 80 μmol (g fresh weight)$^{-1}$ (equation for line, $y = 0.002x + 21.1$); open circles, data used to calculate linear regression line when K_r^+ equalled or was greater than 80 μmol (g fresh weight)$^{-1}$ (equation of line, $y = -0.25x + 41.5$); x, sulphate level in non-stressed plants.

μmol (g fresh weight)$^{-1}$ in plants stressed with Na_2SO_4 and K_2SO_4, respectively. At Ψ_w greater than –0.8 MPa, glucose and fructose concentrations were lower by amounts that were, more or less, in proportion to the stress.

H. *Malate.* The content of malate in leaves of both Cl- and Su-plants varied between 1 and 10 μmol (g fresh weight)$^{-1}$ in no distinctive pattern.

I. *Amino acids.* In non-stressed plants, the amino acid concentration was 13.3±0.5 μmol (g fresh weight)$^{-1}$. In Su-plants, the amino acid concentrations in plants treated with Na_2SO_4, K_2SO_4 or any mixture of the two at –0.8 MPa could be averaged at 25.4±2.1 μmol (g fresh weight)$^{-1}$ because amino acid levels appeared to be independent of the K^+/Na^+ ratio. In Cl-plants grown at –0.8 MPa, the amino acid concentration in plants exposed to NaCl was 23.3±1.5 μmol (g fresh weight)$^{-1}$ and it increased with increasing K^+/Na^+ ratios until the concentration was 43.3±6.3 μmol (g fresh weight)$^{-1}$ in plants subjected to 100% KCl.

II. *Solutes in roots*

A. Sodium, potassium and TotM. The concentrations of Na^+ and K^+ were lower in roots than leaves and the major cation was Na^+. The changes that occurred as growth conditions were changed were more or less the same in Cl- and Su-plants. In Na^+-treated plants, K_r^+ (K^+ in roots) decreased about 50% as Ψ_w decreased while Na_r^+ (Na^+ in roots) increased 20-fold compared to non-stressed plants (Fig. 2). In K^+-treated plants there was, of course, practically no Na_r^+. K_r^+ in K^+-treated plants increased in amounts similar to the pattern of increase in Na_r^+ in Na^+-treated plants. However, at –0.8 MPa, there was a dramatic decrease in the amount of K_r^+ in Cl-plants, as if the root tissue had lost its ability to store this cation. This was balanced with decreased anion in this tissue as will be mentioned later. It should be remembered, though, that this was also the condition for a large increase in K_r^+.

When media contained mixed salts, K_r^+ increased and Na_r^+ decreased as the proportion of K^+ in media increased. However, they seemed to change in balance with one another at any one level of Ψ_w so that changes in TotM were small.

B. *Chloride.* The pattern of change in Cl^- concentration was dependent on whether media were salinized with NaCl or KCl (Fig. 3). If plants were grown in media with mixed salts, Cl^- levels in roots increased with increasing salinity as if the media contained only KCl. An exception to this pattern was in Cl-plants subjected to –0.8 MPa stress. As mentioned above, roots seemed to lose their ability to store K^+ and Cl^- as the K^+/Na^+ ratio increased. Since these Cl^- values at 0.8 MPa and high K^+/Na^+ ratios are obviously out of line with results obtained at other growth conditions, it was considered prudent to omit them from the evaluation of the data in Fig. 3. Although the anion/cation ratio in roots increased with increasing TotM, the ratios never reached the theoretical value of 1:1.

C. *Sulphate.* Although there was no sulphate in leaves of Cl-plants, there was some in roots. The SO_4^{2-} level in roots of Cl-plants was dependent not on TotM but on K_r^+. As long as K_r^+ was less than 80 μmol (g fresh weight)$^{-1}$, SO_4^{2-} was constant at slightly more than 20 μmol (g fresh weight)$^{-1}$ (Fig. 7). It should be noted that K_r^+ in non-stressed plants was 70 μmol (g fresh weight)$^{-1}$. As K_r^+ increased above 80 μmol (g fresh weight)$^{-1}$, SO_4^{2-} concentrations decreased until there was no more SO_4^{2-} in the tissue when K_r^+ exceeded 160 μmol (g fresh weight)$^{-1}$.

Sulphate in roots of Su-plants increased in balance with TotM, and the anion/cation ratio was constant at 1:3. The same ratio in non-stressed plants was 1:5.

D. *Phosphate.* Phosphate levels in roots fluctuated within a narrow range [from 5 to 9 μmol (g fresh weight)$^{-1}$] under all growth conditions but generally decreased as the K^+/Na^+ ratio increased.

E. *Sucrose.* The sucrose concentration was 7.0±2.0 μmol (g fresh weight)$^{-1}$ in roots of non-stressed plants. It increased to 22.7±2.4 and 13.9±1.9 μmol (g fresh weight)$^{-1}$ when growth media was at –0.8 MPa caused by 100% NaCl or Na_2SO_4, respectively. The sugar levels decreased as the K^+/Na^+ ratio increased until the sugar concentration was zero, or nearly so, when the medium contained 100% K^+.

F. *Proline.* As in leaves, little proline was found in roots containing less than 200 μmol (g fresh weight)$^{-1}$ of TotM. Only roots from plants grown at –0.8 MPa stress due to either NaCl or Na_2SO_4 contained that much TotM and, therefore, it was only in these roots in which proline was found in amounts [ranging from 1 to 1.5 μmol (g fresh weight)$^{-1}$] greater than that in non-stressed plants.

G. *Glucose and fructose.* As in leaves, fructose values were approximately 50% of those for glucose. Glucose concentrations in roots of plants treated with NaCl or Na_2SO_4 remained relatively constant at all levels of stress and were in general equal to the amount in non-stressed plants of 11.5±0.7 μmol (g fresh weight)$^{-1}$. However, at each level of stress, as the K^+/Na^+ ratio increased, glucose concentrations decreased. As an example, at –0.8 MPa in plants subjected to 100% KCl or K_2SO_4 stress, glucose concentrations were 0 and 1.8±0.8 μmol (g fresh weight)$^{-1}$, respectively.

H. *Malate.* Malate concentrations varied from 3 to 6 μmol (g fresh weight)$^{-1}$ in Cl-plants as salinity levels increased and from 3 to 12 μmol (g fresh weight)$^{-1}$ in Su-plants. In general, at each level of salinity, malate decreased as the K^+/Na^+ ratio increased.

I. *Amino acids.* As salinity increased, free amino acids generally increased to a maximum of about twice that of non-stressed plants which was 12.1±0.7 μmol (g fresh weight)$^{-1}$. However, at each level of stress, the amino acid concentrations decreased as the K^+/Na^+ ratio increased.

Discussion

General features of solute accumulation in roots and leaves

There were significant differences between roots and leaves in the patterns of change in solute concentrations due to salinity. One difference was that Na^+ was the predominant monovalent cation of roots (except when the ratio, Na^+/K^+, was 1:1 or less in the growth medium) while in leaves K^+ always exceeded Na^+ by large amounts. Another difference between roots and leaves was in the quantitative and relative amounts by which K^+ and Na^+ changed in tissues as the proportion of K^+ salt in growth media was increased and Na^+ salt decreased to keep Ψ_w constant. Basically, at any one constant level of salinity, greater proportions of K^+ in media resulted in large increases in foliar TotM – and almost all of it due to K_f^+ – while, in roots, K_r^+ increased and Na_r^+ decreased more or less in balance with one another so that there were only small changes in TotM of the roots. A third difference was that organic solute concentration changes in roots, except for proline, were small and seemed to be a response to media osmotic potentials rather than to any accumulated tissue constituent. Because major changes in solute concentrations occurred mainly in leaves, the portions of the Discussion dealing with solutes will be limited to results obtained with leaf tissue.

Inorganic solutes

Of all solutes studied in *S. bicolor,* foliar concentrations of the inorganic ions increased by the largest amounts in response to salinity. However, lesser amounts accumulated in leaves of plants subjected to Na^+ stresses compared to amounts in plants subjected to K^+ stresses, especially if the anion was chloride. This is indoubtedly due to the fact that sorghum is a Na^+-excluder.

Cations and anions increased in leaves in a linear relationship to one another but also with a continuing change in their relative proportions as conditions of stress became more severe. In Su-plants, the cation/anion ratio approached but did not reach the value of 1:2 for balanced concentrations of inorganic anion and cation. In Cl-plants, the ratio increased with increasing salinity such that it reached 3:2 in KCl-treated plants and 2:1 in NaCl-treated plants at stress levels of –0.8 MPa.

An explanation for these changing and increasing anion/cation ratios may be that the function of the anion is to maintain turgor as was suggested by Cram (1980) for beet. Cram concluded that increasing internal Cl^- acted as a homeostatic agent for maintaining constant turgor in disks of beet storage tissue exposed to increasing external concentrations of KCl. A similar function for Cl^- and, perhaps, SO_4^{2-} may be inferred from these experiments with sorghum. Such an explanation is compatible with the bioenergetics concept of the effect of salinity on cell growth (R. H. Nieman, personal communication) in that the cells could be expending so much of their energy increasing and then maintaining internal Cl^- or SO_4^{2-} (and, thus, turgor) that an inadequate amount of energy was left to support biosynthetic and growth processes.

Organic solutes

Of the organic solutes measured in leaves only the concentrations of proline and sucrose changed in parallel with increasing amounts of TotM. These organic solutes were absent, or nearly so, in non-stressed plants and, as the media osmotic potentials were decreased, the organic solutes increased by amounts large enough to be significant in osmotic adjustment. The other solutes (total amino acids, malate, glucose and fructose), on the other hand, were present in higher concentrations, compared to sucrose and proline, in non-stressed plants but their concentrations only approximately doubled under the most severe conditions of stress studied in these experiments. Because of these relatively small changes in concentration, these latter compounds could perhaps

play a role in osmotic adjustment, but only if they were confined to some small cellular compartment. The monosaccharides, glucose and fructose, might be of importance in general osmotic adjustment at very high salinity levels. Increases in sugars and proline have been reported in a number of other plants, both glycophytes and halophytes, when subjected to either water or saline stresses (Briens and Larher 1982, Flowers and Hall 1978, Gorham et al. 1980, 1981, Jefferies et al. 1979, Jones et al. 1980, Meyer and Boyer 1981, Munns et al. 1982, Rathert et al. 1981, Sharp and Davies 1979, Wyn Jones 1981). The degrees of change in organic solutes were not equal in all plants. Another solute known to accumulate in some stressed plants and believed to be involved in osmotic adjustment is betaine (Wyn Jones 1981). Betaine could not be detected in tissues of *S. bicolor* subjected to –0.8 MPa stress (results not presented). Hitz and Hanson (1980) reported betaine in water-stressed sorghum but at such low concentrations as not to be of value for osmotic adjustment.

The current theory of osmotic adjustment (amply discussed in the numerous reviews on this topic) states that solutes are contained in compartments of cells with a separation of inorganic solutes into the vacuole and organic solutes into the cytoplasm. The theory is that the organic solutes act as balancing osmotica for the low osmotic potential of the vacuole. (It should not be inferred however that there is a clear-cut separation of inorganic and organic solutes into these two compartments). Measured concentrations of proline in sorghum leaves are of the right order of magnitude to agree with this theory if the compound is, indeed, restricted to the cytoplasm. However, there is a threshold value of 200 μmol (g fresh weight)$^{-1}$ of TotM before any increased amounts of proline can be detected. Thus, proline does not start accumulating until a considerable amount of inorganic solutes is already present in cells and, presumably, in the vacuolar compartment. Sucrose might be the other solute of the cytoplasm that serves as the balancing osmoticum at stress levels less than those that stimulate proline accumulation, because it starts accumulating as soon as TotM exceeds 100 μmol (g fresh weight)$^{-1}$, the amount in non-stressed plants. However, if sucrose is restricted to the cytoplasm, the concentrations of sucrose would be too large to have this sole purpose. Thus, further work needs to be done to determine the function of solutes as well as to determine what are the signals in the environment, cytoplasm, vacuole or other compartment that initiate and regulate reactions leading to solute accumulation and compartmentation.

Correlation of solute concentration with growth

Growth inhibition by saline stress is commonly accepted to be due to a lowering of the water potential of growth media caused non-specifically by dissolved excess ions (Flowers et al. 1977, Greenway and Munns 1980). Most studies concerning the effects of salinity on growth have dealt with the effect of one salt, NaCl. The results reported here show that laboratory studies restricted to NaCl stress would not fully describe the span of the osmotic adjustment abilities of sorghum and, perhaps, other plants and the relationship of solute accumulation to growth. Roots and leaves did not respond equally to stress nor to the compounds used for the stressing salts. Leaf growth was inhibited more severely with K^+ salts than with Na^+ salts and was inhibited to some degree by both cations at even the mildest levels of stress. Root growth, on the other hand, was stimulated by mild stress caused by K^+ salts while growth was not significantly affected by Na^+ salts at the same concentrations. At Ψ_w lower than –0.4 MPa, both cations became inhibitory to root growth, but the degrees of inhibition by the same concentrations of salt were greater now with K^+. Stimulation of root growth by low levels of salinity (Flowers and Hall 1978, Hoffman et al. 1980, Hsaio and Acevedo 1974) and drought (Meyer and Boyer 1981, Sharp and Davies 1979) has been observed in other plants also. Thus, sorghum can be described as being salt-tolerant when growing in media salinized with NaCl but salt-sensitive when treated with K^+ salts. It is obvious that there is a correlation between growth inhibition and the concentration of solutes in leaf tissue. The correlation shows that under conditions in which *S. bicolor* is salt-tolerant, leaf tissue contains less water-soluble solutes, both inorganic and organic, than it does under conditions in which the plant is salt-sensitive. It may be concluded, then, that the ability to accumulate larger concentrations of water-soluble solutes (i.e., K^+ in this study) in leaves is not a beneficial mechanism in terms of growth for this plant.

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