Growth and solute accumulation in 3-week-old seedlings of *Agropyron elongatum* stressed with sodium and potassium salts

Ralph Weimberg


*Agropyron elongatum* [Host. (Beauv.)] cv. Arizona Glendale, was grown in liquid medium salinized with either NaCl, KCl, or a 50:50 mixture of these two salts at osmotic potentials ranging from 0 to −1.6 MPa. The amount of growth in 21 days was measured, and extracts were made of the shoots at this time. The extracts were assayed for low-molecular-weight organic compounds (glucose, fructose, sucrose, betaine, proline) and inorganic solutes (Na⁺, K⁺, Cl⁻, P). The purpose was to determine if there was any correlation between the harmful effect of salinity on growth and the concentrations of solutes in tissues. Growth inhibition of *A. elongatum* was roughly proportional to the osmotic potential of the growth medium and was independent of the ionic composition of the salinizing salts. Total monovalent cation (the sum of Na⁺ and K⁺) concentrations and the ratio of these two cations in leaves were mainly a function of the ionic composition of the salt in growth media, and, to a lesser degree, of osmotic potentials. At an osmotic potential of −0.2 MPa, total monovalent cation in leaves was the same as in non-stressed plants. However, if the salinizing salt contained NaCl, there was an increase in foliar Na⁺ with a balancing decrease in K⁺. At stress levels between −0.4 and −1.6 MPa, and, if the media were salinized with either 100% NaCl or a 50:50 mixture of NaCl and KCl, total monovalent cation concentrations remained constant at a value that was twice that in non-stressed plants. Although total monovalent cation concentrations were equal in plants grown under these two salinity conditions, the K⁺/Na⁺ ratios shifted from a value of 1:2 in plants grown in 100% NaCl to 3:1 in plants subjected to the 50:50 mixture. If 100% KCl was used to salinize media, total monovalent cation was 80% of its concentration in NaCl-treated plants in the range of −0.4 to −1.2 MPa. At −1.6 MPa due to 100% KCl, total monovalent cation was double that in plants subjected to −0.4 MPa. In the range of osmotic potentials from −0.2 to −1.2 MPa, the chloride:cation ratio was 1:2. At −1.6 MPa the ratio changed to 3:4. Proline started accumulating in leaves of *A. elongatum* when the tissue concentration of total monovalent cation exceeded 200 µmol (g fresh weight)⁻¹. Above this threshold value of total monovalent cation, the proline concentration of leaves was 6% of the amount of total monovalent cation that exceeded 200 µmol (g fresh weight)⁻¹.

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

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Introduction

Osmotic adjustment is defined as the net accumulation of solutes in cells of higher plants in response to external water deficits or salinity (Turner and Jones 1980). Osmotic adjustment has long been considered to be a tolerance or protective mechanism of plants when stressed by salinity or drought (Maas and Nieman 1978, Radin 1983). Presumably such a build-up of solutes in cells prevents wilting of the plants by changing the solute to solvent ratio (i.e. the water activity of the solvent) inside cells to match a similar change in the external envi-
environment in order to prevent net water movement outward across the cell plasma membrane (Borowitzka 1981). There have been numerous studies to identify and quantitatively measure the compounds whose concentrations increased in stressed plant tissues to try to obtain experimental evidence for a relationship between osmotic adjustment and salt or drought tolerance. (The following references are restricted to saline-stressed plants: Briens and Larher 1982, 1983, Coughlan and Wyn Jones 1982, Gorham et al. 1981, Voetberg and Stewart 1984, Weimberg et al. 1984. See also Greenway and Munns 1980, Wyn Jones and Gorham 1983 and references therein.) The compounds usually found in increased quantity in the tissues were inorganic ions, proline, betaine and sugars. A few other compounds have also been reported on occasion. Their relationship to growth inhibition by stress (or salt tolerance of the plant), however, has never been proven.

While the theory that plants need to accumulate solutes to prevent wilting under stress conditions seems valid, there is also a growing body of evidence that indicates that solute accumulation inhibits growth, and that saline damage to glycophytes is due to “excessive ion accumulation” (Greenway and Munns 1980, Jeschke 1984). A comparison of the relative degrees of growth inhibition of varieties of a glycophytic species or closely related species by salinity shows that the more tolerant forms are the plants that are better “Na-excluders” (Läuchli 1984, Lütte 1983).

The responses of halophytes to salinity are somewhat different from those of glycophytes (Jefferies and Rudnik 1984, Munns et al. 1983, Stewart and Ahmed 1983). Leaf cells of non-stressed halophytes usually contain much higher levels of inorganic ions than non-stressed glycophytes. Salinity, especially NaCl, is needed by a number of halophytic plants at low to moderate concentrations (ca 50–125 mM) for optimal growth. The compounds that accumulate in halophytes subjected to saline stress are the same as in glycophytes (inorganic ions, proline, betaine, etc.). Because of the high concentrations of inorganic ions even in non-stressed halophytic plants, this investigation was undertaken to study the dynamics of Na⁺ and K⁺ accumulation in leaves of Agropyron elongatum, an halophyte, grown in media salinized with NaCl and/or KCl, and the consequences of the accumulation of these cations on the concentrations of other solutes. The quantitative responses of A. elongatum to K⁺ was compared to its responses to Na⁺ to determine if there was any decrease in the tolerance of the plant to salinity due to K⁺ as has been described for the glycophytes S. bicolor (Weimberg et al. 1984) and mung beans (Salim and Pitman 1983), and a few halophytes (Munns et al. 1983).

A. elongatum was chosen for this investigation mainly because it is a halophytic member of the Triticeae, an economically important group of plants. Salt tolerance studies on other species in this tribe have been reported recently (Chauhan et al. 1980, Gorham et al. 1984, 1985, Wyn Jones et al. 1984). Some detailed information is available on salinity effects on cultivars and accessions of A. elongatum (Elzam and Epstein 1969, Shannon 1978). Nomenclature of the Triticeae is in a state of flux (Dewey 1983, McGuire and Dvorak 1981); therefore, in this report it was decided to use the name for this plant that is most familiar to plant scientists, Agropyron elongatum.

**Abbreviation** – TotM, the sum of K⁺ and Na⁺.

**Materials and methods**

**Growth of plants**

Seeds of *Agropyron elongatum* [Host. (Beav.)] cv. Arizona Glendale, were obtained from a commercial seed company. This species is a decaploid organism. Approximately 20 g of seeds were germinated by first soaking them in aerated water at room temperature for 6 h and then spreading them on moist cheese-cloth supported by stainless-steel screens over a solution of 0.5 mM Ca(NO₃)₂, in 41 pots. The pots were covered so that the seeds germinated in the dark in a high humidity atmosphere at 25°C. After 4 days of germination, the seedlings were transferred to aerated 1/2 strength Hoagland’s growth medium (Weimberg et al. 1982). The plants were grown in a growth chamber kept at 25°C with 14 h in the light, and 10 h in the dark. The light source was a bank of GE fluorescent lamps, Code 32773, and 100 W incandescent bulbs providing an intensity of 58 W m⁻² at plant height. (Mention of company names or products is for the benefit of the reader and does not imply endorsement, guarantee, or preferential treatment by the USDA or its agents.)

**Beginning on the 5th day, salt solutions of either NaCl, KCl, or a 50:50 mixture of the two on a molar basis were added to media in amounts sufficient to lower the osmotic potential of the growth medium by 0.2 MPa. This salinization procedure was repeated at 24 h intervals until the desired osmotic potential was reached. Volumes of growth media lost by evaporation and transpiration were replaced with fresh water.**

Plants were harvested on the 21st day of growth after the lights had been on for 6 h. Growth of leaves and roots were measured as fresh weight by excising them from the seeds and weighing them separately. The roots were blotted on paper towels before being weighed.

**Extraction and assay of solutes**

The leaves were extracted with a water-toluene treatment as described previously for sorghum (Weimberg et al. 1981). After the leaves were weighed, 4 g of the top 5 cm of the leaves were cut off, rinsed quickly in distilled water to remove any surface salts, and placed in 50 ml H₂O at 25°C. To this was added 1 ml of toluene. The containers were gently shaken for 1 h. The liquid was
decanted, and the extraction repeated two more times. The three supernatants were pooled, filtered, and diluted to 150 ml with water. All solutes were measured in the crude extract. The cations Na\(^+\) and K\(^+\) were assayed by atomic absorption spectrometry. Appropriate chemical methods were used to measure Cl\(^-\) (Cotlove 1963), P, (Tauskyy and Shorr 1953), proline (Bates et al. 1973) and betaine (Grieve and Grattan 1983). Enzymatic methods were used to detect glucose (Slein 1965), fructose (Klotzsch and Bergmeyer 1965), sucrose (Weimberg et al. 1984) and malic acid (Hohorst 1965).

**Replication**
The entire experiment was performed in duplicate. The second set of plants was grown ca six months after the first set.

**Results**

**Growth**
Seedlings of *A. elongatum* could survive stresses due to salinities down to -2.0 MPa for the 3-week growth period of these experiments. However, tissues were assayed for solutes in 3-week-old plants stressed with salinity only down to -1.6 MPa because the plants at -2.0 MPa were too small and difficult to handle.

Non-stressed seedlings produced 33 g per 100 plants of leaf tissue (Tab. 1A) and 25.5 g per plants of roots (Tab. 1B) in a 3-week-growth period starting from germination of seeds. At -1.6 MPa, shoot growth was only one-third of that of unstressed plants, and, undoubtedly, most of that growth occurred during the salinization period. The degree of growth inhibition at each level of osmotic potential was the same for all three salinity conditions.

**Inorganic ions**
Total monovalent cations (TotM) in non-stressed plants was around 220 μmol (g fresh weight)\(^{-1}\) with almost all of it due to K\(^+\) (Tab. 2). Stress levels of -0.2 MPa had little effect on TotM regardless of the composition of the stressing salts. However, if NaCl was present in the medium the amount of Na\(^+\) in leaves increased with a balancing loss of K\(^+\). At stress levels ranging from -0.4 to -1.6 MPa, foliar concentrations of Na\(^+\) and K\(^+\) and their sums were a function mainly of the ionic composition of the salts used to salinize growth media. Therefore, the data obtained at these stress levels in Tab. 2 are best analyzed as follows:

I. Plants grown in media salinized with 100% NaCl. At -0.4 MPa, the TotM concentration was approximately twice that in non-stressed plants, but TotM then remained constant despite further decreases in media osmotic potentials to values as low as -1.6 MPa. The K\(^+\)/Na\(^+\) ratio was 1:2 and it also remained constant at all stress levels in the range of -0.4 to -1.6 MPa.

II. Plants grown in media salinized with a 50:50 mixture of NaCl and KCl. TotM values were equal to those in plants grown in 100% NaCl, but the K\(^+\)/Na\(^+\) ratios were not. The ratio was constant but, because the concentration of K\(^+\) in leaves was greater than in plants grown in 100% NaCl, the ratio in these plants was 3:1.

III. Plants grown in 100% KCl. TotM also increased in plants grown in KCl at -0.4 MPa, but its concen-

<table>
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<tr>
<th>Osmotic potential (MPa)</th>
<th>g FW (100 plants)(^{-1})</th>
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<tbody>
<tr>
<td>No stress</td>
<td>100% NaCl</td>
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<tr>
<td>A. Shoots</td>
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<td>0</td>
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<td>(LSD(_{0.05}))</td>
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<td>B. Roots</td>
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<td>0</td>
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tration was only 80% of that in plants grown in NaCl. TotM remained constant at greater stress levels but only to -1.2 MPa. At -1.6 MPa, there was a doubling in the concentration of TotM compared to its concentration in plants grown at -0.4 to -1.2 MPa. A K'/Na' ratio in plants grown in KCl is, of course, meaningless.

The concentration of Cl⁻ in shoots under all three salinizing conditions from -0.2 to -1.2 MPa was 55% that of TotM, on an average. However, at the most severe level of stress, the relative concentration of Cl⁻ increased to 70–88%. These relative proportions of Cl⁻ were independent of the composition of the salinizing medium.

The change in inorganic phosphate concentrations was also independent of the salinizing salts. Inorganic phosphate in leaves increased but by very small amounts as the level of stress was increased.

**Sugars**

The levels of sucrose, glucose and fructose were constant and very low under all growth conditions. Their average concentrations were 3.5, 15.5, and 11.0 μmol (g fresh weight)⁻¹, respectively (data not presented).

**Betaine and malic acid**

No betaine could be detected in plants grown under any growth condition at stress levels between 0 and -1.2 MPa. Some betaine was measured in plants grown at -1.6 MPa but the amounts were low and the results erratic among three replicated experiments. The concentrations of betaine in leaves of plants grown in 100% NaCl in each of the replicated experiments were 0, 0.8, and 1.9 μmol (g fresh weight)⁻¹ respectively. Similar fluctuations in values were obtained for plants grown under the other two salinity conditions.

Malic acid was present in only trace quantities, at most, in plants under all conditions of growth (data not presented).

**Proline**

Proline was present in leaves of all plants, including non-stressed plants, and its concentrations were directly related to TotM. It was calculated that there would be a threshold value of 200 μmol (g fresh weight)⁻¹ of TotM before proline would begin to accumulate (Fig. 1). However, this threshold does not exist in A. elongatum because non-stressed plants already contained more TotM than this threshold amount and, also, these plants contained proline. In stressed plants, proline increased in balance with increasing TotM. Thus, its concentration was a constant 6% of that part of TotM that exceeded the threshold amount and, also, these plants contained proline. In stressed plants, proline increased in balance with increasing TotM.
Proline accumulates in numerous plants undergoing saline and other types of stress (see Greenway and Munns 1980, Weimberg et al. 1984, Wyn Jones 1981 for further references). Jefferies et al. (1979) measured proline concentrations in two halophytes, Limonium vulgare and Triglochin maritima, exposed for 2 weeks to levels of artificial sea water ranging from −0.05 to −2.4 MPa. Subjecting their data to linear regression analysis showed that proline accumulation roughly followed the same pattern in these two species as reported here for A. elongatum and previously for S. bicolor (Weimberg et al. 1984). TotM in S. bicolor increased with decreasing media osmotic potentials and reached a value of 200 μmol (g fresh weight)^{-1} when the stress was severe enough to cause a 40–50% reduction in growth. In A. elongatum, a stress level of −0.2 MPa had no effect on either growth of the plant or TotM. However, if the growth medium contained NaCl, there was an increase in foliar Na^+ and a balancing decrease in K^+ so that their sum remained the same as in non-stressed plants.

Significant changes in growth and solute concentrations began when the osmotic potential of the medium was lowered to −0.4 MPa, but the pattern of these changes in response to increasing levels of salinity were not parallel to one another. As stated above, growth inhibition was a function of the osmotic potential of the medium and was not related to the specific ions of the salt. Changes in solute concentrations, in contrast, were related to the ionic composition of the salts used for salting the medium and were almost independent of the osmotic potentials of the media. At stress levels ranging from moderate to severe, Na^+ : K^+ ratios in leaf tissues were determined by the ionic composition of the medium, but with one exception their sums remained almost unaffected by either ionic composition or osmotic potential. The exception was plants grown at −1.6 MPa due to 100% KCl. This growth condition caused a doubling of TotM over its concentration in plants grown under milder conditions of stress. Chloride concentrations were closely related to TotM values. The Cl^− /TotM ratio was 1:2 under all salinity conditions at stress levels ranging from −0.2 to −1.2 MPa. At −1.6 MPa, the amount of Cl^− increased relative to TotM so that the ratio was 3:4.

Proline was the only organic solute found to accumulate in stressed plants of A. elongatum, and its concentrations were directly proportional to TotM under all conditions of growth. One can calculate that there is a threshold value of 200 μmol (g fresh weight)^{-1} before proline should begin to accumulate. This is the same threshold reported previously for S. bicolor (Weimberg et al. 1984) and barley (Voetberg and Stewart 1983). However, in A. elongatum this threshold is only of theoretical interest because non-stressed plants contained more than 200 μmol (g fresh weight)^{-1} of TotM. Consequently, one should expect to find, and one does find, proline in shoots of non-stressed plants.

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![Fig. 1. Proline content of shoots as a function of total monovalent cations. Open symbols are data points for Experiment I; closed symbols are data points for Experiment II.](image-url)
et al. 1984). In Simmondsia chinesis, the concentration of proline ranged from 3–5% of TotM (Tal et al. 1979), and in Mesembryanthemum nodiflorum, proline concentrations were 2% of Na⁺ in plants subjected to 150–400 mM NaCl and increased to 4% in plants at 500 mM NaCl (Treichel 1975). Unfortunately, the data in the latter two reports do not permit a determination of a threshold value. Thus, these observations lend further support to the theory that organic solutes such as proline are probably located in the cytoplasm in concentrations adequate to balance a low osmotic potential of the vacuole where most of the inorganic solutes are stored.

The other two organic solutes found in many salt-stressed plants are sugars, particularly sucrose, and betaine. Sugars are at too low a concentration in A. elongatum to be important for osmotic adjustment and their concentrations are not affected by subjecting the plant to saline stresses. Flowers et al. (1977) claims that sugars play a minor role in osmotic adjustment in halophytes, and many halophytes have been characterized as utilizing mainly nitrogenous compounds for osmotic adjustment (Briens and Larher 1982, Jefferies and Rudnik 1984). Jefferies et al. (1979) found no significant changes in sugar concentrations in leaves of the halophytes they studied. On the other hand, Shannon (1978) found enough sucrose in 32 lines of tall wheatgrass to permit him to suggest that sugars might be used as markers of salt tolerance in this taxonomic group. Clearly, though, A. elongatum ev. Arizona Glendale, is not a sugar accumulator and, therefore, it fits the category of being a nitrogenous accumulating species.

The absence of betaine in extracts of plants used in this study, except at low concentrations in plants grown under the most severe saline conditions used, is puzzling. There is ample evidence in the literature that betaine is accumulated by members of the Triticeae when plants are subjected to saline stress (Gorham et al. 1984, 1985, Wyn Jones et al. 1984). We have found betaine in other cultivars of A. elongatum (R. Weimberg, unpublished results). However, these studies were done with plants that were grown in the field and were several months old or even older. The plants used in the experiment reported here were very young (3 weeks old) when harvested, grown in a growth chamber under controlled conditions, and were grown in liquid media. It is not known if age and growth conditions are factors to be considered in betaine accumulation. This problem will be investigated further.

The main conclusion to be drawn from the present investigation is that there is no relationship between osmotic adjustment in A. elongatum and its salt tolerance as measured by growth inhibition by salinity. In terms of growth, A. elongatum responded to increasing amounts of salinity in the root zone in the manner typical for numerous plants. However, as already described and discussed, no concomitant change occurred in foliar solute concentrations. This raises the question as to whether or not the concentrations of particular solutes in plant tissue can be used as markers or indicators of salt tolerance. A survey of the literature of osmotic adjustment patterns in glycophytes and halophytes gives no unambiguous indication that the ability of a plant to accumulate any one or group of solutes could be a measure of its salt tolerance. Indeed, the only biochemical parameter detected so far that appears to be reliably correlated with tolerance is the relative ability of glycophytes to exclude Na⁺ (or avoid ion excess). Certainly, much more work is needed to obtain an understanding of osmotic adjustment and the role of solutes in the water relations of the plant in order to elucidate the mechanisms of salt tolerance.

References
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