Accumulation of myo-Inositol in Actinidia Seedlings Subjected to Salt Stress

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Seedlings of kiwifruit (Actinidia delicosa (A. Chev.) C. F. Liang et A. R. Ferguson var delicosa) and A. arguta (Sieb. et Zucc.) Planch. ex Miq. grown in hydroponic nutrient solutions with elevated salt (MgSO₄ and KCl) concentrations showed visible signs of stress at salt concentrations of 50 mm and above. The polyol myo-inositol accumulated in leaf tissue when the salt was added to 15 mm or more, with increases being similar in the two species. The increase in concentration of myo-inositol was approximately linear with rising salt. At any given salt concentration an increase in myo-inositol was linear with time from application of salt. myo-Inositol concentrations increased within the first 24 h of salt treatment, and declined again as quickly once the stress was removed. Sucrose also increased with salt stress, accumulating only once plants showed physical signs of stress. Accumulation of myo-inositol was negatively correlated to fructose and glucose.

Keywords: Actinidia arguta, Actinidia delicosa, kiwifruit, leaf tissue, myo-inositol, salt stress, sucrose.

INTRODUCTION

In response to water stress induced by high salt concentrations in the root zone, many plants synthesize and accumulate osmotically active, low molecular weight compounds including polyols (Bohnert, Nelson and Jensen, 1995). Polyols, because of their highly hydroxylated nature, may maintain functional hydration of enzymes and membrane integrity during water deficits (Bieleski, 1982).

The polyol myo-inositol is commonly found in higher plants (Loewus and Loewus, 1983). It is synthesized from glucose 6-phosphate via 1R-myoinositol 1-phosphate. Established physiological functions for myo-inositol and its derivatives include a role in membrane biosynthesis, membrane protection, plant signalling and as a precursor of cell wall components, gums and mucilages. myo-Inositol may act as an osmolyte, osmoprotectant, or serve as a storage carbohydrate under stress, as has been postulated for other polyols (Loewus and Loewus, 1983; Bohnert et al., 1992).

In most plants, myo-inositol is present at low levels, amounting to less than 5% of the major soluble carbohydrates (Bieleski, Clark and Klages, 1997). However, in kiwifruit, [Actinidia delicosa (A. Chev.) C. F. Liang et A. R. Ferguson var delicosa] pools of myo-inositol representing 20% of the major soluble carbohydrate pool (14 mg g⁻³ d. wt) have been found in fruit during early stages of development. In leaves, concentrations were similar (12 mg g⁻³ d. wt) throughout most of the season (Klages et al., 1998). High concentrations have also been found in several other species of Actinidia (H. Boldingham, unpubl. res.). In Actinidia arguta (Sieb. et Zucc.) Planch. ex Miq., myo-

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MATERIALS AND METHODS

Plant material

Seeds of A. delicosa (A. Chev.) C. F. Liang et A. R. Ferguson var delicosa and A. arguta (Sieb. et Zucc.) Planch. ex Miq. were germinated in acid-washed sand and the seedlings grown in individual containers in continuously aerated nutrient solution (Smith et al., 1989). The quantity of nutrients added for successive 3 to 4 d growth periods was calculated assuming logarithmic dry weight increases with time and the concentrations of the various elements required in leaves to sustain that growth (Smith et al., 1989). Treatments were applied 5 to 7 weeks after seedlings were placed in the nutrient solutions. All plant harvests were carried out at the same time of day. Three experiments were carried out as follows:

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A. deliciosa

In expt 1, conductivities of nutrient solutions were adjusted by adding stock solutions of MgSO₄ (2 M) and KCl (2 M) in a 1:4 ratio to final concentrations of 0:3:1:2, 1:25:5, 2:5:10, 3:75:15, 5:20 and 10:40 mm (approx. 400, 870, 1360, 1970, 2400 and 4100 μS). The choice of MgSO₄ and KCl instead of NaCl in all experiments was based on earlier findings (Lionakis, 1985; Smith, Asher and Clark, 1987) where irrigation water containing 10 mm sodium resulted in severe damage to kiwifruit vines. Chlorine, on the other hand, showed no adverse effects at elevated concentrations. Further, soils in the natural habitat of kiwifruit in China are moderately high in potassium (Li et al., 1985) and high in chloride (Smith et al., 1988). The 1:4 ratio of MgSO₄ and KCl used here was derived from the ratio in which these two salts are added in the plant nutrient solution. Plants were grown for a further 10 d. Each treatment was replicated six times. The conductivities of the nutrient solutions were monitored (Triac digital conductivity meter) and the nutrient feeding regime maintained over the course of the experiment. On day 10 of the salt treatments leaf stomatal conductances were measured using a steady state porometer (LICOR LI-1600) and the plants were harvested. From each plant the youngest fully expanded leaf plus the adjacent basal and distal leaf were harvested, combined, weighed and immediately frozen in liquid N₂. Samples were lyophilized, re-weighed and ground to a fine powder prior to starch and sugar analyses.

In expt 2, stock solutions of MgSO₄ (2 M) and KCl (2 M) were added in a 1:4 ratio to nutrient solutions (approx. 350 μS) of half the plants to give a 50 mm (10 mm MgSO₄; 40 mm KCl) (approx. 4000 μS) salt treatment. The other plants were maintained at about 350 μS (control). Over the following 10 d plants were destructively harvested and leaf samples from six plants of each treatment were taken every second day and treated as described previously. Tissue samples were processed and analysed as above. Fresh and dry weights of the remaining leaves, root and shoot (stem and petiole) from each plant were determined.

A. arguta

Seedlings of A. arguta (expt 3) were germinated and grown in hydroponic solution as described above. Prior to commencing treatments, the conductivities of nutrient solutions were approx. 410 μS (control). For salt treatments, nutrient solutions were adjusted by increasing concentrations of MgSO₄ and KCl (1:4) to 50 mm (low salt) and 100 mm (high salt) which increased conductivities to approx. 3700 μS and 6400 μS, respectively. Five days after salt treatments had been applied, hydroponic solutions of all plants were replaced with fresh solutions as follows: control plants and the former high salt plants were returned to solutions with conductivities of approx. 410 μS (‘control’ and ‘high salt/recovery’ treatments, respectively). The former low salt plants were placed in solutions to which stock solutions (2 M) of MgSO₄ and KCl (1:4) were added to give a 100 mm treatment (‘low salt/high salt’ plants). The choice of a 5 d period for the first salt treatment was based on previous experiments in which A. deliciosa plants showed clear signs of stress after 5 d of salt treatment and changes in carbohydrate partitioning were apparent. Periodically throughout the treatment periods (total of 20 d of treatments), six replicate plants of each treatment were harvested and leaves analysed for starch and sugar concentrations as described above.

Carbohydrate analyses

Ground samples were re-lyophilized and a subsample of 0.1 g was analysed as described previously (Klages et al., 1998).

RESULTS

Plant growth

All salt-treated A. deliciosa plants in expt 1 were in similar condition to control plants by day 10. There was no significant effect on plant growth or on stomatal conductance (data not shown).

After 10 d of treatment in expt 2, A. deliciosa plants exposed to salt (50 mm) showed outward signs of stress. In these plants stomatal conductance decreased from the first day after imposition of the treatment and declined to around 45% of the controls by day 10 (Table 1). Salt treatment resulted in reduced plant dry matter by day 10 (Table 1). The decreases in dry matter accumulation by whole plants were closely reflected in dry matter accumulation in the root tissue alone (Table 1).

In A. arguta (expt 3), stomatal conductance gave a clear indication of the stress status of the plants and reflected the conductances in the nutrient solutions (Table 2). Stomatal conductances immediately decreased in both salt treatments. In low salt/high salt plants the initial dosage of salt resulted in only a slight decrease in stomatal conductance. Upon change to high salt treatment on day 5 stomatal conductance declined rapidly, and remained low throughout the re-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of treatment</th>
<th>Stomatal conductance (m s⁻¹)</th>
<th>Root d. wt (g)</th>
<th>Whole plant d. wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>277 ± 45.6</td>
<td>13 ± 0.2</td>
<td>48 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>304 ± 26.4</td>
<td>10 ± 0.2</td>
<td>39 ± 0.7</td>
</tr>
<tr>
<td>(350 μS)</td>
<td>4</td>
<td>297 ± 18.1</td>
<td>16 ± 0.2</td>
<td>66 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>381 ± 42.9</td>
<td>24 ± 0.3</td>
<td>90 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>257 ± 38.6</td>
<td>30 ± 0.5</td>
<td>109 ± 1.7</td>
</tr>
<tr>
<td>Salt</td>
<td>1</td>
<td>169 ± 1.4</td>
<td>10 ± 0.2</td>
<td>39 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>130 ± 24.3</td>
<td>11 ± 0.2</td>
<td>47 ± 0.7</td>
</tr>
<tr>
<td>(4000 μS)</td>
<td>4</td>
<td>210 ± 51.3</td>
<td>15 ± 0.3</td>
<td>62 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>219 ± 41.5</td>
<td>17 ± 0.2</td>
<td>75 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>116 ± 14.1</td>
<td>16 ± 0.3</td>
<td>75 ± 1.6</td>
</tr>
</tbody>
</table>

Data represent measurements of six plants per treatment per day ± SEM.
TABLE 2. Stomatal conductances, root and whole plant dry weights in response to salt stress in A. arguta control (410 μS) plants, plants subjected to a low salt (3700 μS; 50 mM)/high salt (6500 μS; 100 mM) treatment, and plants subjected to a high salt (6500 μS; 100 mM)/recovery (410 μS) treatment over a 20 d treatment period.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day of treatment</th>
<th>Stomatal conductance (m s⁻¹)</th>
<th>Root d. wt (g)</th>
<th>Whole plant d. wt (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1</td>
<td>219 ± 7</td>
<td>0.7 ± 0.03</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>229 ± 26</td>
<td>0.8 ± 0.1</td>
<td>3.1 ± 0.3</td>
</tr>
<tr>
<td>(410 μS)</td>
<td>6</td>
<td>348 ± 32</td>
<td>0.9 ± 0.1</td>
<td>4.2 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>150 ± 19</td>
<td>1.7 ± 0.3</td>
<td>8.7 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>182 ± 25</td>
<td>1.6 ± 0.2</td>
<td>7.3 ± 1.6</td>
</tr>
<tr>
<td>Low salt/high salt</td>
<td>1</td>
<td>184 ± 20</td>
<td>0.7 ± 0.1</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>289 ± 32</td>
<td>0.8 ± 0.1</td>
<td>3.3 ± 0.6</td>
</tr>
<tr>
<td>(3700/6500 μS)</td>
<td>6</td>
<td>40 ± 12</td>
<td>1.0 ± 0.1</td>
<td>4.8 ± 1.2</td>
</tr>
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<td>17</td>
<td>61 ± 16</td>
<td>0.7 ± 0.1</td>
<td>6.0 ± 0.9</td>
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<td></td>
<td>20</td>
<td>55 ± 21</td>
<td>0.5 ± 0.1</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>High salt/recovery</td>
<td>1</td>
<td>85 ± 10</td>
<td>0.6 ± 0.1</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>77 ± 18</td>
<td>0.7 ± 0.1</td>
<td>3.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>131 ± 26</td>
<td>0.6 ± 0.1</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>(6500/410 μS)</td>
<td>17</td>
<td>139 ± 23</td>
<td>1.1 ± 0.1</td>
<td>6.4 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>124 ± 27</td>
<td>1.7 ± 0.2</td>
<td>7.7 ± 1.4</td>
</tr>
</tbody>
</table>

Data represent measurements of six plants per treatment per day ± SEM.

There was little difference in plant dry matter accumulation between the treatments for most of the treatment period (Table 2). However, plants which had received the low salt/high salt treatment showed a dramatic decrease in total dry matter between days 16 and 20, which was associated with loss of dry leaves at the lower stem end of the plants as plants showed severe leaf necrosis by this stage.

While root dry matter increase was not affected in high salt/recovery plants at any stage (Table 2), root appearance did change as they turned from white and healthy to brown and slimy by day 5. In low salt/high salt plants, however, the high salt treatment caused root growth to cease with a dry matter loss from the roots from day 10 onwards, indicating root tissue necrosis (Table 2). The necrosis was clearly visible, as was the new re-growth on roots of the high salt/recovery treatment (Fig. 1).

Non-structural carbohydrates

The major non-structural carbohydrates measured in A. deliciosa and A. arguta leaves were fructose, glucose, sucrose, myo-inositol and starch.

Addition of different salt concentrations ranging from no salt to 50 mM in expt 1 resulted in a linear increase in myo-inositol.
Sucrose concentrations remained steady (data not shown). Fructose and glucose concentrations steadily declined, and myo-inositol concentrations in Actinidia deliciosa leaves (Fig. 2), while fructose and glucose concentrations steadily declined, and sucrose concentrations remained steady (data not shown).

In expt 2, Actinidia deliciosa control plants showed little change in leaf myo-inositol (Fig. 3A). Salt treatment resulted in a linear increase in leaf myo-inositol over the treatment period (Fig. 3B) and by day 10 myo-inositol had increased by 60% from day 1. Glucose and fructose declined rapidly over the first 4 d of salt treatment. While the pattern of hexose decline differed from that of myo-inositol increase, the total increase in myo-inositol (502 mg g\(^{-1}\) d. wt) was very close to the total decrease in both hexoses (475 mg g\(^{-1}\) d. wt) over the total treatment period. In control plants, glucose and fructose increased while sucrose decreased over the treatment period. In these plants the combined overall increase in glucose and fructose was similar to the total decrease in sucrose. In salt-stressed plants sucrose concentrations remained stable over the 10 d. However, there was neither any difference between control and salt-stressed plants in total major sugars, nor over the treatment period (Fig. 4) within each set of plants. Starch was similar in all plants until the latter part of the treatment period, and by day 10 leaves of salt-stressed plants contained significantly less starch than leaves of control plants (Fig. 4).

Exposure to elevated salt in the root zone over the first 5 d of salt treatment resulted in an increase in myo-inositol (3.1 mg g\(^{-1}\) d. wt) in the leaves of Actinidia arguta (Fig. 5A–C), similar to the increase observed in Actinidia deliciosa leaves (1.52 mg g\(^{-1}\) d. wt). Plants subjected to the high salt treatment (100 mm) showed a slightly more rapid myo-inositol increase. However, by day 5, myo-inositol concentrations were similar in Actinidia arguta plants of both salt treatments. Upon increasing the salt concentration from 50 mm to 100 mm in the low salt/high salt plants on day 5, myo-inositol decreased temporarily (as pot solutions were changed over) and subsequently increased further until day 13 of treatment. By day 13 myo-inositol had increased by 70% (4.25 mg g\(^{-1}\) d. wt) from the onset of the salt treatment. Over the final 7 d of treatment, myo-inositol concentrations declined again, as leaf tissue became severely necrotic. Plants exposed to 100 mm salt over the first 5 d of treatment (high salt/recovery) showed a rapid and immediate decrease in myo-inositol upon removal of the salt stress on day 5. Myo-Inositol concentrations decreased until day 8 when they reached levels similar to those of control plants.
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Glucose and fructose decreased in plants of both salt treatments (Fig. 5B and C). Similar to *A. deliciosa*, the overall increase in myo-inositol to respective peak concentrations was close to the overall hexose decrease over the same time period in plants of the low salt/high salt treatment. However, as in *A. deliciosa*, the pattern of hexose decrease did not follow closely that of myo-inositol increase. Rather hexoses declined more rapidly in the first few days of salt treatment. In high salt/recovery plants, over the 5 d period of stress, hexoses decreased twice as rapidly as myo-inositol increased.

Sucrose concentrations in leaves closely mirrored patterns of total major sugars (Fig. 6A and B). There was little change in sucrose over the first 8 d of treatment in any of the plants. From day 10 onwards however, leaf sucrose in plants of the low salt/high salt treatment increased rapidly throughout the remainder of the treatment period. By day 20 sucrose leaf concentration in these plants was 2.5-fold greater than on day 8. In plants of the high salt/recovery treatment sucrose declined to levels slightly below those of control plants (Fig. 6A).

Both salt treatments apparently affected leaf starch levels (Fig. 7). In plants which had received high salt treatment initially, starch declined transiently but started to rise again before the salt stress was relieved on day 5. From day 8 starch levels in these plants stabilized, but were over twice as high as in control plants. Towards the end of the recovery period starch declined again. Plants which had first received the low salt treatment showed no effect on starch levels until day 13 (8 d after beginning the high salt treatment), when starch had increased. Thus, the initial decline in starch observed in high salt/recovery plants was absent in low salt/high salt plants upon imposition of either salt treatment.

In plants of both salt treatments, total measured leaf carbohydrates were similar to one another and noticeably
higher than in control plants from day 10 onwards (Figs 6B and I). However, in the low salt/high salt plants, sugars represented 32% of the total carbohydrate vs. 18% in the high salt/recovery plants.

**DISCUSSION**

**Increase in myo-inositol in response to salt stress**

While the metabolic role of myo-inositol in *Actinidia* species is unknown, the results of the current experiments suggest involvement of myo-inositol in the response of these species to osmotic stress. In the two species there were similarities in the changes of leaf carbohydrate partitioning in response to salt stress. Increased accumulation of myo-inositol was measurable before plants showed any signs of salt stress such as wilting or stomatal closure (when the final concentration of salt added was at or below about 50 mM/4000 μS). The increase in myo-inositol concentration in leaves was gradual and occurred at approximately the same rate in both species. Upon removal of salt stress in *A. arguta*, a decline in myo-inositol concentrations was apparent after 1 d.

Polyols and sugar alcohols such as pinitol, mannotiol and sorbitol have been implicated as osmolectes in other plant species (Nguyen and Lamant, 1988; Keller and Ludlow, 1993; Everard et al., 1994; Loescher and Everard, 1996). Increases of myo-inositol in response to salt stress have been reported in several plants (Gorham, Hughes and Wyn Jones, 1981; Sacher and Staples, 1985; Raychaudhuri and Majumder, 1994; Ishitani et al., 1996; Sheveleva et al., 1997). Recent results (Nelson, Rammesmayer and Bohnert, 1998) have shown that myo-inositol plays a central role in metabolic responses leading to salt tolerance in *M. crystallinum*. In *Nicotiana tabacum* myo-inositol accumulates under salt stress but does not confer salt tolerance. However, in plants transformed with myo-inositol O-methyltransferase, D-ornithine was synthesized in response to drought or salt stress, and these plants showed increased resistance to both stresses (Sheveleva et al., 1997). On the other hand, overexpression of inositol 1-phosphate synthase in transgenic *Arabidopsis thaliana* did not increase the salt tolerance of the plants (Smart and Flores, 1997).

In spite of accumulating myo-inositol, *A. deliciosa* and *A. arguta* are not salt tolerant. However, myo-inositol may still provide some protection to the plant. It may act as a cytoplasmic osmoticum, may be involved in scavenging of oxygen radicals, and may act to store photosynthetic products which are not being utilized under stress conditions. While the amount change in myo-inositol was not large, it may nevertheless contribute to maintenance of osmotic balance under stress. The cellular location of myo-inositol in leaves is not known, and a shift of myo-inositol from the vacuole (where it has been speculated to serve as a long-term storage carbohydrate under normal conditions; Bieleski, 1982) to cytoplasm would increase its functionality in osmotic responses without dramatic increases in the total amount per cell.

The increase in myo-inositol does not appear due to retardation of growth in stressed plants of either *Actinidia* species as has been proposed for wild-type tobacco under salt stress (Sheveleva et al., 1997), since myo-inositol accumulation commenced some days before any reduction in plant weight gain was apparent. We are currently investigating whether accumulation of myo-inositol is due to a shift in carbohydrate partitioning with conversion of stored carbohydrate to myo-inositol or whether, under stress conditions, myo-inositol is a primary photosynthetic product in these species of *Actinidia*.

**Relationship between myo-inositol and other sugars**

As myo-inositol concentration increased upon salt treatment, fructose and glucose concentrations decreased in both *A. deliciosa* and *A. arguta*. However, the respective increase and decreases were not synchronous, suggesting that synthesis of myo-inositol is not directly at the expense of hexose sugars. An initial short-term increase in glucose and fructose concentrations was observed in the high salt treatment of *A. arguta* high salt/recovery plants. Such transient increases in the concentrations of hexose sugars have also been observed in salt-stressed tomato by Sacher and Staples (1985), and may represent an initial shock response or osmotic signal under sudden high salt concentrations in the plant’s environment. In both *Actinidia* species under stress, myo-inositol accumulation may reflect an increase in its precursor glucose/glucose 6-phosphate. Further, hexokinase-mediated sugar sensing may be involved in signalling during stress (Hare, Cress and Van Staden, 1998), and myo-inositol may provide a suitable sink.

Leaf sucrose increased in response to salt stress relative to control plants in both species. However, in both species, increases in sucrose were observed only in some stressed plants and only when plants showed physical signs of stress damage. While the absolute changes in sucrose were greater than those in myo-inositol, sucrose accumulation commenced only after several days of stress. It thus appears that a shift in partitioning between hexose sugars and myo-inositol, rather than accumulation of sucrose, is the primary carbohydrate response to salt stress in these two species.
Leaf starch and salt stress

In Actinidia species, leaf starch reserves declined under conditions of increased salt concentrations. A decrease in starch with a concomitant increase in sucrose and polyol has been documented in many studies of osmotic stress responses. Here, as in other studies (e.g. Keller and Ludlow, 1993), it is not known what proportion of the starch depletion is due to mobilization and what is simply a result of reduced photosynthetic activity. An initial decline in starch was observed only in Actinidia plants of the high salt treatment. This decline was concurrent with a sharp decline in stomatal conductance but not in plant growth. Thus, starch reserves may have been depleted over the first few days of stress, when photosynthesis had declined but growth was maintained. In contrast, in plants which had received the low salt treatment first, starch increased when plants were transferred to the high salt treatment. By that stage (day 5) some damage to the root tissue (and other tissues) had already occurred. The observed increase in starch in leaves may therefore be linked to the lack of functioning sinks to which carbohydrate could be exported. A similar situation was seen in plants upon recovery from the high salt, where starch levels increased significantly. In these plants, root tissue had been severely damaged and was showing signs of recovery only towards the end of the experiment when leaf starch concentrations declined once again.

CONCLUSION

Rapid accumulation of myo-inositol upon stress imposition and equally rapid decline upon stress relief in these two Actinidia species indicate that this is a primary carbohydrate response to salt stress, which is quickly adjustable to changes in environmental conditions. This appears to be at the expense of hexose accumulation. Considering myo-inositol's postulated properties as an osmolyte, as a storage carbohydrate and as a sink for glucose 6-phosphate in plants, its accumulation could be advantageous for both a metabolic and an osmotic requirement for growth under stress.

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LITERATURE CITED


