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# Stress Alters Ammonia and Arginine Metabolism

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## INTRODUCTION

Stress-induced changes in the level and composition of the soluble nitrogen pool in plants are well-documented. While the changes that occur are the result of significant alterations in intermediary and secondary nitrogen metabolism, the underlying biochemical events and key enzymes influenced by specific environmental stresses remain to be demonstrated. In most cases, attention has been focused on the correlation between the capacity of some plants to accumulate nitrogenous compounds, such as putrescine, proline, and glycine-betaine, and their ability to tolerate environmental stresses. The metabolic logic for the accumulation of these compounds is unknown, and it is not clear which, if any, of these compounds function as actual mechanisms of stress tolerance or are simply symptoms of stress (10, 28).

In my laboratory, we have been investigating the hypothesis that increased production of ammonia in response to a number of abiotic stresses is a key factor causing the changes in intermediary and secondary nitrogen metabolism during stress, and that removal of ammonia through increased *de novo* biosynthesis of arginine represents a homeostatic mechanism important to plant stress tolerance.

In the first part of this communication, the results of experiments quantifying changes in leaf $NH_3$-$NH_4^+$ content and arginine metabolism involved in plant responses to environmental stresses are reported. In the second part, $NH_3$-$NH_4^+$ and arginine metabolism are contrasted in stress-tolerant and -sensitive plants. Evidence is presented suggesting that the generalized sequence of metabolic events $NH_3 \rightarrow$ arginine $\rightarrow$ polyamines might be related to the tolerance/sensitivity of plants to abiotic stresses. The results, thus far, are consistent with our proposal that in a stress-tolerant plant, ammonia production increases in response to stress, ammonia is removed through increased activity of the *de novo* arginine biosynthetic pathway, and arginine is converted to putrescine and subsequently to other polyamines. In contrast, the *de novo* arginine biosynthetic pathway is inhibited in a stress-sensitive plant, and ammonia levels increase, while polyamine titers decrease.

## CHANGES IN LEAF $NH_3$-$NH_4^+$ AND ARGININE METABOLISM IN RESPONSE TO ABIOTIC STRESSES

### Mineral Nutrient Deficiencies

Most macro- and many micronutrient deficiencies in vascular plants result in altered nitrogen metabolism (28). The accumulation of arginine, citrulline, and ornithine is associated with a number of mineral nutrient deficiencies in a variety of higher plant species: K, P, Fe, Cl, and Zn (24). In addition, agmatine, the intermediate compound formed in the conversion of arginine to putrescine, accumulates during deficiencies of K (10-fold), P (3-fold), Ca (3-fold), S (3-fold), Mg (2-fold), and Mn (2-fold) (27). Putrescine content increases in response to a number of mineral nutrient deficiencies, of which K and Mg deficiencies are the best studied (10).

It is well documented that arginine, citrulline, and ornithine accumulate during phosphorus deficiency in many vascular plant species (8,11,21,24). Under conditions of low P fertilization, free arginine levels were 10 to 50 times higher in leaves of four commercially important citrus rootstocks than in control plants receiving an adequate supply of P (21,24). In addition, the arginine content of the protein fraction increased 2- to 4-fold in P-deficient leaves of the citrus rootstocks (24). P deficiency also caused an increase in the free arginine content of leaves of banana (4-fold) (8), alfalfa (4- to 20-fold) (11), and summer squash (6-fold) (25).

We (25,26) have previously demonstrated that arginine accumulates in leaves of P-deficient plants due to increased activity of the *de novo* arginine biosynthetic pathway in response to the increased ammonia content of the leaves. The studies employed four rootstocks of commercial importance to the citrus industry in California. Plants were transferred at age 2 weeks to sterile sand containing 1 μg extractable P/g (–P) or phosphate-sufficient sand containing 100 μg $Ca(H_2PO_4)_2$ /g sand (+P). All plants were watered two times a week with half-strength Hoagland's nutrient solution without P. The rootstocks were selected for their ability to take up P and represented the range of P dependencies observed for citrus rootstocks: (a) high-rough lemon (*Citrus limon*); (b) moderate-'Carizzo' and 'Troyer' citrange (*Citrus sinensis* x *Poncirus trifoliata*), and (c) low-trifoliate orange (*P. trifoliata*) (3). Summer squash (*Cucurbita pepo*) plants transferred at age 5 d from hydroponic culture in Shive's nutrient solution containing 1 mM $KH_2PO_4$ and 2 mM $K_2SO_4$ (+P) to Shive's nutrient solution from which the 1 mM $KH_2PO_4$ was omitted and 2.5 mM $K_2SO_4$ was added (–P) were included to contrast an herbaceous annual with the woody perennials.

For the treatment durations presented in Table I, young, fully expanded leaves used in the analyses exhibited no visible symptoms of P deficiency. (To eliminate the secondary effects of senescence, young, fully expanded leaves were used in all our research unless otherwise stated). Withholding P resulted in a reduction in total biomass and plant height that became significantly greater as the deprivation continued. Rough lemon, the most P-dependent rootstock, exhibited the greatest reduction in growth (Table I).

**Table I.** *Effects of Phosphorus Deprivation on Plant Growth and Leaf Nitrogen Metabolism Expressed as P-Deficient/P-Sufficient X100%*[a]

| Plant (Species) | Treatment Duration | Plant Growth | Total N[b] | $NO_3^-$[c] | $NH_3$-$NH_4^+$[d] | Arginine Biosynthesis[e] | Total Arginine[f] | Total Amino Acids Less Arginine[g] |
|---|---|---|---|---|---|---|---|---|
| Rough lemon | 6 wk | 55 | 88 | 146 | 119 | 993 | 194 | 86 |
| (*Citrus limon*) | 12 wk | 24 | 129 | 337 | 177 | 1,300 | 425 | 77 |
| Carrizo citrange | 6 wk | 81 | 97 | 147 | 237 | 300 | 115 | 97 |
| (*Citrus sinensis x* | 12 wk | 59 | 82 | 152 | 106 | 740 | 295 | 73 |
| *Poncirus trifoliate*) | 28 wk | 15 | 131 | 300 | 216 | 445 | 495 | 86 |
| Troyer Citrange (*C. sinensis x P. trifoliata*) | 28 wk | 15 | 128 | 319 | 107 | 318 | 370 | 93 |
| Trifoliate orange (*P. trifoliata*) | 12 wk | 48 | 91 | 161 | 60 | 271 | 150 | 92 |
| Summer squash (*Cucurbita pepo*) | 10 d | 81 | 95 | 209 | 108 | 219 | 113 | 91 |

[a]Data used to calculate P-deficient/P-sufficient x 100% were taken from Rabe and Lovatt (24 -26). [b]Average value ± SD. for the P-sufficient control plants was 28.6 ± 2.8 and 49.2 ± 1.6 mg N per g dry wt leaf tissue for the citrus rootstocks and *C. pepo*, respectively. [c]Values for P-sufficient control plants ranged from 200 to 1,000 µg $NO_3^-$ per g dry wt leaf tissue for the citrus rootstocks and 2,500 ± 330 µg $NO_3^-$ per g dry wt for *C. pepo*. [d]Average value for P-sufficient control plants was approximately 750 µg $NH_3$-$NH_4^+$ per g dry wt leaf tissue. [e]Incorporation of $NaH^{14}CO_3$ into arginine plus urea per g fr wt leaf tissue during a 3-h incubation at 30°C ranged from 14 to 40 nmol for leaves from P-sufficient plants. [f]Total arginine content of leaves from P-suffcient control plants was approximately 75 µmol per g dry wt leaf tissue. [g]Leaf content of total amino acids, excluding arginine, was 1,371 ± 129 (X ± SD.) µmol per g dry leaf tissue from P-sufficient citrus rootstocks and 2,130 µmol per g dry wt for *C. pepo*.

Total nitrogen content of leaves did not increase until growth of the plants was severely restricted by P deprivation. However, the $NO_3^-$ content of the leaves increased significantly with only a 20% decrease in plant biomass (Table I). Concomitant with the increase in leaf $NO_3^-$ content during P deficiency, there was an increase in leaf $NH_3$-$NH_4^+$ content and/or an increase in the activity of the arginine *de novo* biosynthetic pathway, consistent with the increased availability of ammonia (Table I). Leaf $NH_3$-$NH_4^+$ content and/or rate of arginine biosynthesis increased sooner and to a greater degree in rough lemon, the most P-dependent rootstock, than in trifoliate orange, the least P-dependent rootstock. The two citranges, which are *Citrus* x *Poncirus* hybrids, exhibited intermediate responses. For all the rootstocks, leaf $NH_3$-$NH_4^+$ content and/or activity of the arginine *de novo* biosynthetic pathway increased in a manner that paralleled the severity of the stress and the accumulation of arginine (Table I).

The activity of the arginine *de novo* biosynthetic pathway was assessed in intact cells of young, fully expanded leaves by measuring the incorporation of $NaH^{14}CO_3$ into the combined pool of arginine plus urea (16). It should be noted that this method does not employ inhibitors of urease or arginine decarboxylase and thus underestimates the activity of the arginine biosynthetic pathway. The activities of arginase and arginine decarboxylase were either unaffected or accelerated up to twofold during P deficiency (24). Our observation of increased arginine decarboxylase activity is consistent with a previous report that agmatine accumulates during P deficiency (27).

The specific activity of nitrate reductase was unaffected in leaves of the P-deficient citrus rootstocks, but was accelerated twofold in squash (25). Given the significant increase in available nitrate during P deprivation, nitrate reduction probably contributed to the increasing pool of free $NH_3$-$NH_4^+$. In addition, the reduction in plant biomass would be expected to have a concentrating effect on all components of the soluble nitrogen fraction of the leaf. The fact that the pool of total amino acids excluding arginine remains the same or decreases during P deprivation (Table I) suggests that amino acid turnover may also contribute to the increased availability of $NH_3$-$NH_4^+$ and provides evidence that arginine is preferentially synthesized over most other amino acids. The protein content of –P leaves decreased 30% after 7 months of P deprivation.

To confirm that the increased activity of the pathway for the *de novo* biosynthesis of arginine was dependent on the increased availability of $NH_3$-$NH_4^+$, the responsiveness and sensitivity of the *de novo* arginine biosynthetic pathway to changing leaf $NH_3$-$NH_4^+$ levels was tested. The petioles of detached leaves were immersed in aerated solutions of $NH_4Cl$ at final concentrations ranging from 10 to 50 mM for 3 to 15 h. *De novo* arginine biosynthesis increased with increasing concentration and duration of exposure (Table II).

It is well documented that ammonia assimilation in plants proceeds by the combined activities of glutamine synthetase and the glutamate synthase cycle (GOGAT) (19). The availability of glutamate and glutamine would both impact arginine *de novo* biosynthesis. Glutamate is the precursor of ornithine. While

**Table II.** *De Novo Arginine Biosynthesis in Ammonium-Treated, Young, Fully Expanded Leaves from P-sufficient Control Plants Expressed as $NH_4^+$-Treated ÷ Control x 100%*

| $NH_4Cl$ Concentration | Duration of Treatment | De novo Arginine Biosynthesis[a] | |
|---|---|---|---|
| | | Rough Lemon | Squash |
| 30 | 3 | 278 | 218 |
| 10 | 15 | 425 | 368 |
| 50 | 3 | 512 | 840 |
| 50 | 15 | 600 | 1,198 |

[a]Average value ± SD. (N = 5 experiments) for basal incorporation of $NaH^{14}CO_3$ in arginine plus urea per g fr wt leaf tissue during the 3-h incubation at 30°C for detached leaves of P-sufficient plants immersed in aerated solutions for up to 15 h was 9.3 ± 1.4 nmol for rough lemon and 4.0 ± 0.7 nmol for squash. Calculated from data presented in Rabe and Lovatt (25).

the evidence is inconclusive in vascular plants, glutamate is probably converted to ornithine via the acetylglutamate cycle (30). Glutamine serves as the source of amide nitrogen for the synthesis of carbamylphosphate. Both ornithine and carbamylphosphate are substrates of ornithine carbamyltransferase in the synthesis of citrulline. An additional amide group would be donated by aspartate at the targininosuccinate synthase step in the conversion of citrulline to arginine (30).

Taken together, the changes in nitrogen metabolism induced by P deprivation in the two species and two hybrids of citrus rootstocks, and in squash, strongly suggest the following sequence of biochemical events leading to the accumulation of arginine: reduced plant growth, $NO_3^-$ accumulation, increased $NH_3$-$NH_4^+$ production, and accelerated arginine *de novo* biosynthesis. Recently, Larson (13) demonstrated that this sequence of events occurs during Mg-deficiency, resulting in the accumulation of not only $NO_3^-$, $NH_3$-$NH_4^+$, and arginine, but also putrescine.

Two-day-old dark-germinated seedlings of summer squash (*Cucurbita pepo*) were transferred to hydroponic culture in Shive's nutrient solution with and without Mg and grown under continuous illumination at 380 $\mu\Sigma/m^2 \cdot s$ at 30°C for 11 d. Growth of the first leaf of the Mg-sufficient plants was linear from day 3 through day 11 of the treatment. For the Mg-deficient plants, growth of the first leaf stopped at day 6 of Mg deprivation, attaining only 25% of the biomass of the Mg-sufficient control leaf. This resulted in dramatic increases in the levels of $NO_3^-$, $NH_3$-$NH_4^+$, free arginine, and putrescine in the Mg-deficient leaf (Table III).

## Low-Temperature and Water-Deficit Stress

In *Citrus*, flowering is recurrent and floral intensity is low under tropical and subtropical conditions (20), unless synchronized into a well-defined period of

**Table III.** Effects of Magnesium Deprivation on Leaf Growth and Nitrogen Metabolism Expressed as Mg-Deficient + Mg-Sufficient X 100%[a]

| Treatment Duration | Leaf Growth[b] | $NO_3^-$[c] | $NH_3$-$NH_4^+$[d] | Free Arginine[e] | Free Putrescine[f] |
|---|---|---|---|---|---|
| 5 | 68 | 117 | 97 | 200 | 475 |
| 7 | 59 | 263 | 157 | 490 | 600 |
| 11 | 24 | 250 | 263 | 900 | 800 |

[a]Data used to calculate Mg-deficient/Mg-sufficient x 100 were taken from Larson (13). The methods are the same as Rabe and Lovatt (25). Putrescine was determined according to the method of Flores and Galston (5). [b]Average value ± SE (N = 20) for the Mg-sufficient control plants was 1.9 ± 0.05 gfw at day 11. [c]Average value for three separate experiments for the Mg-sufficient control plants was 3,100 μg $NO_3^-$ per g dry wt from day 5 through 11. [d]Average value for three separate experiments for the Mg-sufficient control plants ranged from 3,060 μg per g dry wt at day 5 (20% of full leaf expansion) to 816 μg per g dry wt at day 11 (90% of full leaf expansion). [e]Average value for two separate experiments for the Mg-sufficient control plants was 1 μmol arginine per gfw from day 5 through day 11. [f]Average value for two separate experiments for the Mg-sufficient control plants was 20 μmol putrescine per g fr wt from day 5 through 11.

concentrated bloom by external conditions. Flower formation in *Citrus* is promoted by drought or low temperature, followed by restoration of climatic conditions favorable for growth (18, 20). Thus, stress applied in a quantitative manner provides a controlled system during which metabolic changes can be monitored in relation to flower initiation.

In my laboratory, we (17, 18) monitored the relationship between nitrogen metabolism in young, fully expanded leaves and floral intensity in experiments employing: (a) low-temperature stress to induce flowering in 5-year-old rooted cuttings of the 'Washington' navel orange and (b) water-deficit stress to induce flowering in commercially grown 16-year-old 'Frost Lisbon' lemon trees on 'Troyer' citrange rootstock.

Five-year-old rooted cuttings of the 'Washington' navel orange, induced to flower by low-temperature stress, were subjected to an 8-h day (500 $\mu\Sigma/m^2 \cdot s$) at 15 to 18°C, 16-h night at 10 to 13°C. After 4, 6, or 8 weeks of low-temperature treatment, trees were transferred to 12-h day (500 $\mu\Sigma/m^2 \cdot s$) at 24°C, 12-h night at 19°C. Maximum flower number, or peak bloom, occurred 4 weeks after the trees were transferred to the warm temperature. Control trees maintained at the warm temperature throughout the experiment did not flower. Low-temperature stress did not cause water-deficit stress. The average predawn water potential of the trees during the 8 weeks of low-temperature treatment was the same as for the warm-temperature control trees: $-0.7 \pm 0.2$ MPa.

Neither leaf total nitrogen nor $NO_3^-$ content changed during or after the low-temperature treatment. However, there was a significant increase in leaf $NH_3$-$NH_4^+$ content during the low-temperature stress. $NH_3$-$NH_4^+$ accumulated in a

manner that paralleled the duration of the stress. The amount of $NH_3$-$NH_4^+$ that accumulated was directly proportional to the number of flowers produced by the tree. Tree $NH_3$-$NH_4^+$ status had no effect on the number of vegetative shoots the tree produced. This is a significant observation because it provides strong evidence that $NH_3$-$NH_4^+$ is not simply increasing budbreak. If this were the case, the number of vegetative shoots would also have increased with increasing tree $NH_3$-$NH_4^+$ content. Thus, $NH_3$-$NH_4^+$ is specifically influencing flower formation. These results are summarized in Table IV.

To determine if there was a cause-and-effect relationship between leaf $NH_3$-$NH_4^+$ content and flower initiation and floral intensity, we subjected trees to only 4, 6, or 8 weeks of low-temperature stress and artificially raised the $NH_3$-$NH_4^+$ status of the trees by foliar application of low-biuret urea at a rate of 1.5 g/tree. Foliar application of low-biuret urea increased the leaf $NH_3$-$NH_4^+$ content and resulted in a concomitant and equal increase in flower number per tree (Table V).

For the more than 100 trees used in the many experiments conducted, including trees subjected to 2, 4, 6, or 8 weeks of low-temperature stress and trees treated with foliar applications of low-biuret urea, leaf $NH_3$-$NH_4^+$ content ranged from 389 to 2,639 μg/g dry wt leaf tissue. The corresponding number of flowers for each tree was 4 to 3065. These results indicate that there is a threshold level of $NH_3$-$NH_4^+$ below which flowering will not take place in *Citrus*. That value is approximately 400 μg/g dry wt leaf tissue.

We have recently examined the effect of low-temperature stress on the activity of the *de novo* arginine biosynthetic pathway. The incorporation of $NaH^{14}CO_3$ into arginine plus urea in leaves of low-temperature-treated trees increased with

**Table IV.** *Linear Regression Analyses of Stress, $NH_3$-$NH_4^+$, and Flowering in Citrus*

| Stress | Independent Variable x | Dependent Variable y | Probability p | Coefficient of linear Correlation r |
|---|---|---|---|---|
| Low-Temperature[a] | Duration of stress | $NH_3$-$NH_4^+$ | $p < 0.01$ | 0.605 |
| | $NH_3$-$NH_4^+$ | Flowers per tree | $p < 0.0001$ | 0.803 |
| | $NH_3$-$NH_4^+$ | Floral shoots per tree | $p < 0.01$ | 0.413 |
| | $NH_3$-$NH_4^+$ | Vegetative shoots per tree | NS[b] | 0.077 |

[a]μg $NH_3$-$NH_4^+$ per g dry wt leaf tissue collected at the end of the first week in the warmer temperature after completion of the low-temperature treatment.
[b]Not significant at $p < 0.10$. Reprinted with permission from Proc. 6th Int. Citrus Congr. 1:475-483.

the duration of the low-temperature treatment and in a manner that paralleled the accumulation of $NH_3$-$NH_4^+$ in the leaves (Table V).

Water-deficit stress was also used to induce flowering in 16-year-old 'Frost Lisbon' lemon trees on 'Troyer' citrange rootstock under commercial production in the San Joaquin Valley to produce a second bloom in the fall that yields a crop of summer "verdelli" lemons. This is in addition to the winter crop that is harvested from the normal spring bloom. The summer crop of "verdelli" lemons is highly desirable because it has a greater market value than the winter crop. Trees were subjected to water-deficit stress during July and August and rewatered at the end of August to give maximum bloom by the end of September. Trees were subjected to water-deficit stress treatments of different severity. The treatments and results are summarized in Table VI.

One set of lemon trees was subjected to moderate stress. The trees were maintained at $\psi$= -2 MPa for 50 d by deficit irrigation. Half of the trees received a foliar application of low-biuret urea at the rate of 0.1 kg N per tree. Foliar application of low biuret urea raised the leaf $NH_3$-$NH_4^+$ content of the trees to 863 µg/g dry wt and increased the number of flowers per tree 2.7-fold over trees not receiving a foliar application of urea. This degree of floral intensity was not significantly different at the 5% level from the number of flowers produced by trees receiving the maximum stress. For lemon trees induced to flower by water-deficit stress, the average $NH_3$-$NH_4^+$ content of the leaves during the

**Table V.** *Effect of Low-Temperature Stress and of Foliar Application of Urea on the Leaf $NH_3$-$NH_4^+$ Content and on the Flowering of 'Washington' Navel Orange (*Citrus sinensis*)*

| Weeks of Low-Temp Stress | Number Flowers per Tree[d] | | Leaf $NH_3$-$NH_4^+$ Content[a,d] | | $NaH^{14}CO_2$ Incorporated into Arginine + Urea[a,e] |
|---|---|---|---|---|---|
| | Without Urea[b] | With Urea[c] | Without Urea[b] | With Urea[c] | Without Urea[b] |
| | *µg/g dry wt* | *%* | *µg/g dry wt* | *%* | *nmol/g fr wt•3 h* |
| 0 | 6 a | - | 456 a | -- | 5 |
| 4 | 117 b | 194 | 559 b | 166 | 7 |
| 6 | 131 b | 230 | 583 b | 215 | 11 |
| 8 | 347 c | 126 | 672 c | 134 | 27 |

[a]Determined during the first week after transfer to the warmer temperature. [b]Values within a column followed by different letters are significantly different at $p < 0.05$ Duncan's multiple range test. [c]Low-biuret urea was applied at the rate of 1.5 g per tree at the end of the low-temperature treatment. Data are expressed as a percent of the control trees not treated with urea. [d]Reprinted with permission from Israel J Bot **37:** 181-188. [e]Data are the average values from four separate experiments; each experiment did not include all treatments.

**Table VI.** *Effect of Water-Deficit Stress and Foliar Urea on Leaf $NH_3$-$NH_4^+$ Content and Flowering of Lemon (*Citrus limon*)*[a]

| Treatment | Number of Flowers per Tree | Average Leaf $NH_3$-$NH_4^+$ Content During Stress |
|---|---|---|
| | | *μg/g dry wt* |
| Control - no water stress (> -1 MPa) | 14 b | 519 c |
| Severe water stress of short duration (-3 MPa in 30 d) | 53 b | 646 b |
| Severe water stress (-3 MPa in 20 d) followed by moderate water stress for 40 d (-2 MPa) | 611 a | 728 b |
| Moderate water stress (-2 MPa for 50 d) | 156 b | 683 b |
| Moderate water stress (- 2 MPa for 50 d) with foliar urea (0.1 kg of nitrogen per tree) | 426 a | 863 a |

[a]Values within a column followed by a different letter are significantly different at $p < 0.05$ by Duncan's Multiple Range Test. Reprinted with permission from Israel J Bot 37: 181-188.

stress was significantly correlated with the number of flowers per tree ($p < 0.05$; $r = 0.56$).

The results provide evidence that $NH_3$-$NH_4^+$ and/or the activity of the *de novo* arginine biosynthetic pathway increased during phosphorus and magnesium deficiencies and during low-temperature and water-deficit stresses. It is important to note that for all the experiments conducted in the author's laboratory, plants received no ammonium fertilizer. Thus, the $NH_3$-$NH_4^+$ that accumulated was generated endogenously. Our work is not the first to report the accumulation of $NH_3$-$NH_4^+$ during stress. In 1978, Frota and Tucker (9) reported that bean plants (*Phaseolus vulgaris*) receiving $(^{15}NH_4)_2SO_4$ accumulated $^{15}NH_4^+$ when the plants were subjected to salinity or water-deficit stress. Nilson and Muller (22) reported that $NH_4^+$ accumulated in the roots of *Lotus scoparius* during water-deficit stress. Flores and Galston (4) added a note in proof that free $NH_4^+$ titers increased in oat seedlings allowed to wilt by withholding water. Our work is the first to examine changes in the activity of the *de novo* arginine biosynthetic pathway during abiotic stresses.

## CHANGES IN LEAF $NH_3$-$NH_4^+$ AND ARGININE METABOLISM IN STRESS-TOLERANT VERSUS STRESS-SENSITIVE PLANTS

Demonstration that $NH_3$-$NH_4^+$ production increases during a number of abiotic stresses and that the activity of the arginine *de novo* biosynthetic pathway

increases during stress and in response to increasing tissue concentrations of $NH_3$-$NH_4^+$ suggests that these events are related to polyamine biosynthesis through the generalized sequence of events: $NH_3 \rightarrow$ arginine $\rightarrow$ polyamines. Tissue levels of putrescine also increase during a number of the same abiotic stresses (10) and in response to $NH_4^+$ feeding (1, 10, 12, 14). In addition, the comprehensive study of Flores and Galston (6, 7) demonstrated that for cereals subjected to water-deficit stress conditions, the putrescine that accumulates appears to be synthesized predominantly from arginine via arginine decarboxylase: (a) arginine decarboxylase activity, but not ornithine decarboxylase, was demonstrated to increase during water-deficit stress induced by various osmotica (4) or by withholding water (7); (b) experiments with α-difluoromethylornithine, an analogous-specific inhibitor of ornithine decarboxylase, provided evidence that arginine decarboxylase, but not ornithine decarboxylase, participated in putrescine production during stress (6); (c) osmotic stress resulted in a significant increase in glutamine (7) [glutamine levels increase with increasing availability of ammonia (19) and glutamine is a substrate essential for *de novo* arginine biosynthesis in plants (30)]; and (d) a lack of available arginine was shown to be the cause of reduced putrescine synthesis when osmotically stressed oat leaves were placed in the dark (7).

The putative role of putrescine as a plant protectant during stress is equivocal (10, 28). There is considerable evidence in the literature to support a role for putrescine in the tolerance of plants to abiotic stresses (10, 28), but there is also accumulating evidence associating increased putrescine content with the development of the symptoms of stress (1, 12, 29).

Thus we have initiated experiments examining the relationship between $NH_3$ $\rightarrow$ arginine $\rightarrow$ polyamines in stress-tolerant versus stress-sensitive plants.

## Salinity Stress

Transferring 7-d-old summer squash (*Cucurbita pepo*) and muskmelon (*Cucumis melo*) plants hydroponically cultured in Shive's nutrient solution (control) to Shive's nutrient solution plus 30 or 60 mM NaCl-$CaCl_2$ (2:1 molar ratio, the salt was added at the rate of one-third the final concentration every other day) resulted in a significant reduction in the growth of summer squash at the end of only 10 d of 30 mM salt treatment (Table VII). In contrast, the muskmelon (*Cucumis melo*) exhibited no reduction in growth after 21 d at 60 mM salt.

Salt stress reduced the $NO_3^-$ content of the leaves of both plants. Despite the decrease in $NO_3^-$ levels, leaf $NH_3$-$NH_4^+$ content increased in the salt-sensitive squash plants concomitant with inhibition of the arginine *de novo* biosynthetic pathway (Table VII). In contrast, the activity of the arginine biosynthetic pathway was stimulated in the salt-tolerant muskmelon consistent with the increased production of ammonia during salt stress, but $NH_3$-$NH_4^+$ did not accumulate (15).

**Table VII.** *Effect of Salinity Stress on Growth and $NO_3^-$ $NH_3$-$NH_4^+$ and Arginine Metabolism of Cucurbits*[a]

| Species | Duration and Severity of the Stress | Growth[b] | $NO_3^-$ [c] | $NH_3$-$NH_4^+$ [d] | Arginine Bio-synthesis[e] |
|---|---|---|---|---|---|
| | | *+ salt/control x 100%* | | | |
| Summer squash | 10 d | | | | |
| (*Cucurbita pepo*) | 30 mM | 39 | 74 | 126 | 69 |
| | 60 mM | 38 | 49 | 133 | 61 |
| Muskmelon | 21 d | | | | |
| (*Cucumis melo*) | 30 mM | 90 | 59 | 93 | 170 |
| | 60 mM | 105 | 37 | 89 | 160 |

[a]Data used to calculate +salt/control x 100% were taken from Lovatt (15). [b]The average value for the control plants was 960 mg dry wt per plant for *C. pepo* and 590 mg dry wt per plant for *C. melo*. [c]The average value for the control plants was 3,183 μg $NO_3^-$ per g dry wt leaf tissue for *C. pepo* and 9,600 μg $NO_3^-$ per g dry wt leaf tissue for *C. melo*. [d]The average value for the control plants was 750 μg $NH_3$-$NH_4^+$ per g dry wt leaf tissue for both *C. pepo* and *C. melo*. [e]The average value for the control plants was 23 and 7 nmol $NaH^{14}CO_3$ incorporated into the combined pool of arginine plus urea per gfw leaf tissue • 3 h for *C. pepo*.

## Osmotic Stress

We (23) have examined the effect of 14 d of osmotic stress on epicotyl cells of alfalfa (*Medicago sativa*) in suspension culture. The studies employed two populations of alfalfa. The cultivar Mesilla (Mes-0) is drought sensitive and water-use inefficient; while the third-cycle population (Mes-3) resulting from phenotypic recurrent selection for productivity under less optimum moisture, is drought tolerant and water-use efficient (2). Alfalfa cultures were challenged with increasing concentrations of polyethyleneglycol from 0 to 32% (w/v) to simulate water-deficit stress ranging from -0.5 to -3.0 MPa. The drought-tolerant population maintained low titers of $NH_3$-$NH_4^+$ and accumulated putrescine under stress. In contrast, the drought-sensitive population accumulated high levels of $NH_3$-$NH_4^+$, and putrescine titers decreased, suggesting that the activity of the *de novo* arginine biosynthetic pathway might be inhibited in the sensitive populations during osmotic stress. This possibility was tested in subsequent experiments using 16% (w/v) PEG. After 14 d, growth of the drought-sensitive population, Mes-0, was inhibited 40%, the activity of the arginine *de novo* biosynthetic pathway was inhibited 80%, and tissue concentrations of $NH_3$-$NH_4^+$ approximately doubled. In contrast, growth of the drought-tolerant population, Mes-3, was unaffected, the activity of the *de novo* arginine biosynthetic pathway was unaffected or accelerated, and $NH_3$-$NH_4^+$ did not accumulate.

Thus in two very different systems, employing three different species, whole plants versus cells in suspension culture, and two different stresses, the plants that were sensitive to stress were characterized by inhibition of the *de novo* arginine biosynthetic pathway and high tissue levels of $NH_3$-$NH_4^+$. We are currently identifying the source of ammonia that accumulates during each stress and localizing the site(s) of inhibition of the *de novo* arginine biosynthetic pathway in the stress-sensitive plants. While there is much work to be done to determine if the removal of ammonia through increased activity of the *de novo* arginine biosynthetic pathway and/or the resulting increased provision of arginine for polyamine biosynthesis are important factors in the tolerance of plants to abiotic stresses, the results thus far are encouraging.

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