COMPARISON OF SOME ASPECTS OF NITROGEN METABOLISM OF AVOCADO WITH CITRUS

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Abstract

Comparison of NH_3-NH_4^+ metabolism during low temperature stress-promoted flowering in the Hass avocado and *Citrus* species identified differences in nitrogen metabolism between avocado and *Citrus*: (1) the basal level of NH_3-NH_4^+ was 10-fold lower in Hass avocado leaves than in leaves of the Washington navel orange; (2) concomitantly, the basal activity of the pathway for the *de novo* biosynthesis of arginine was 10-fold lower in avocado leaves; (3) avocado leaves developed symptoms of ammonia toxicity at lower NH_3-NH_4^+ concentrations than the Washington navel orange; and (4) there was virtually no urea uptake by Hass avocado leaves in response to a foliar application of urea that significantly raised the leaf NH_3-NH_4^+ content of the Washington navel orange. The results suggested that leaves of avocado scions may have a limited role in nitrate reduction and ammonia assimilation compared to the roots of avocado rootstocks. This interpretation is supported by the preliminary results presented here demonstrating that the activities of nitrate reductase and glutamine synthetase are frequently greater in roots of avocado rootstock varieties than in the leaves of avocado scion varieties.

1. Introduction

In recent years, research in my laboratory has examined the effects of various environmental stresses on NH_3-NH_4^+ metabolism, especially in relation to flowering in citrus and avocado (Lovatt *et al.*, 1988a,b; Nevin and Lovatt, 1989). We have demonstrated that environmental stresses that inhibit shoot growth or leaf expansion cause leaf concentrations of NH_3-NH_4^+ to increase. For tropical and subtropical tree crops low temperature and water-deficit stress promote flowering. Thus, we applied these two stresses in a quantitative and reproducible manner to provide a controlled system during which we monitored changes in NH_3-NH_4^+ metabolism in relation to flower initiation in citrus and avocado. The results identified differences in NH_3-NH_4^+ metabolism in the leaves of the Hass avocado versus the leaves of the Washington navel orange and Frost Lisbon lemon. These observed differences in metabolism prompted us to undertake additional research to characterize key aspects of nitrogen metabolism in avocado.

For tree crops, the preferred external source of inorganic nitrogen is nitrate. Thus, a mechanism must be available to reduce nitrate to NH_3-NH_4^+ for assimilation into amino acids and subsequently proteins. Some amino acids donate N for the synthesis of nucleotides which are, in turn, used to make DNA and RNA. Nitrate can be reduced to NH_3-NH_4^+ in roots and/or leaves. The major site of nitrate reduction in tree crops is believed to be the roots. The rate limiting enzyme in nitrate reduction is nitrate reductase (NR). The NH_3-NH_4^+ formed is assimilated by the collaborative activity of two enzymes, glutamate dehydrogenase and glutamine synthetase. In this communication, the results of recent research comparing protein content and activities of nitrate reductase and glutamine synthetase in leaves and roots of avocado scion and rootstock varieties are reported.

2. Materials and Methods

Youngest fully expanded leaves and young root tips were used in all assays. Leaves and roots were harvested, respectively, from the avocado variety collection and a rootstock trial at South Coast Field Station, Irvine, CA. Samples from six trees for each variety were pooled; samples were assayed in duplicate.

2.1 Nitrate reductase activity in cell-free extracts

Cell-free extracts were prepared from 1 g fresh leaf tissue ground with a mortar and pestle in 5 ml 100 mM K-phosphate (pH 7.5) containing 1 mM EDTA, 1 mM cysteine, and 3% (W/V) casein and centrifuged at 10,000g for 10 min at 4°C. The supernatant fraction served as the source of enzyme. The activity of nitrate reductase was measured according to the method of Scholl *et al.* (1974) in a 2-ml reaction mixture of the following composition: 5 μmol K-phosphate (pH 7.5), 20 μmol KNO_3, 0.8 μmol NADH, and 50 to 200 μl enzyme. After incubation at 30°C for 15 min, the reaction was stopped with the addition of 50 μmol/ml zinc acetate. Phenazine methosulfate (15 nmol/ml) was added as a post assay treatment to oxidize excess NADH.

2.2 Glutamine synthetase in cell-free extracts

Glutamine synthetase activity was assayed by formation of γ-glutamyl hydroxamate by a modification of the method of McCormack *et al.* (1982). The addition of 0.5 ml of cell-free extract prepared in 0.2 M Tris-maleic buffer (pH 7.2) initiated the reaction which proceeded in a 1-ml reaction mixture of the following composition: 50 mM Tris-maleic buffer (pH 7.2), 15mM $MgCl_2$, 5 mM ATP, 40 mM glutamate, and 10 mM hydroxylamine. Reactions were incubated for 60 min at 30°C and terminated by the addition of 0.3 ml of a solution containing equal volumes of 10% ferric chloride in 0.2N HCl (W/V), 24% TCA, and 6N HCl. Reaction mixtures were centrifuge at 10,000g for 10 min and the OD_{540nm} of the supernatants measured. An OD_{540nm} of 0.1 corresponded to 0.225 μmol γ-glutamyl hydroxamate (Lovatt, 1983).

2.3 Protein content

The protein content of the supernatant used as the source of enzyme in each assay was determined according to Bradford (1976) using bovine serum albumin as the standard. The buffer used in each enzyme assay served as the reagent blank.

3. Results and discussion

3.1 Previous research

Hass avocado trees on clonal Duke 7, 2 years from budding, and 5-year-old rooted cuttings of the Washington navel orange of comparable size were induced to flower by subjecting the trees to 4 and 8 weeks of low temperature. The basal level of leaf NH_3-NH_4^+ in the warm temperature control trees was 10-fold lower for avocado than for the Washington navel orange (Table 1). Avocado trees subjected to 4 or 8 weeks of low temperature stress exhibited a maximum (3.5-fold) increase in leaf NH_3-NH_4^+ content 2 weeks after the initiation of the low temperature treatment, accumulating approximately 100 μg NH_3-NH_4^+ per g dry wt.

Table 1 - Changes in NH_3-NH_4^+ content and *de novo* arginine biosynthesis in leaves of Hass avocado and Washington navel orange during low temperature stress.

Duration of Stress (weeks)	AVOCADO		CITRUS	
	Ammonia -[z] ammonium	Arginine[y] biosynthesis	Ammonia -[x] ammonium	Arginine[y] biosynthesis
0 - control (basal level)	42	0.5	456	5
2	149	1.9		
4			559	7
8			672	27

[z] Leaf ammonia-ammonium was determined using fresh leaf tissue and the average value of the final results converted to μg/g dry wt. for comparison (Nevin and Lovatt, 1989).
[y] Values are nmol $NaH^{14}CO_3$ incorporated into arginine plus urea per g fr. wt. leaf tissue during a 3-hour incubation period from Nevin and Lovatt (1989) for avocado and Lovatt (unpublished) for citrus.
[x] Values are μg/g dry wt. (Lovatt *et al.*, 1988a).

In contrast, NH_3-NH_4^+ accumulated in the leaves of the Washington navel orange in a manner that paralleled the duration of the stress, so that the highest concentration was observed at the end of the 8-week low temperature treatment (Table 1). Expressed as a percent of the warm temperature control, the change in leaf NH_3-NH_4^+ content of trees subjected to 4 or 8 weeks of low temperature stress was less dramatic than for the Hass avocado, representing an increase of 22% and 47%, respectively. This was, however, an increase of approximately 100 μg per g dry wt during each 4 week period.

The basal activity of the pathway for the *de novo* biosynthesis of arginine was 10-fold lower in the Hass avocado leaves than in the Washington navel orange (Table 1). For both the avocado and citrus, the activity of the *de novo* arginine biosynthetic pathway increased coincidently with the increased pool of available NH_3-NH_4^+ (Table 1).

For commercially producing, 16-year-old Frost Lisbon lemon trees induced to flower by water-deficit stress, the NH_3-NH_4^+ content of the leaves and floral intensity increased in a manner that paralleled the severity of the stress (Table 2). Basal levels of NH_3-NH_4^+ in leaves of Frost Lisbon lemon and Washington navel orange were similar: 519 and 456 μg per g dry wt, respectively.

Hass avocado on clonal Duke 7 rootstocks, 1 and 2 years from budding, were subjected to water-deficit stress under controlled environmental conditions in a growth chamber. At the initiation of the experiment, the basal level

Table 2 - Changes in NH_3-NH_4^+ content (μg/g dry wt.) in leaves of Hass avocado and Frost Lisbon lemon during water-deficit stress.

Xylem pressure potential (MPa)	AVOCADO[z]		CITRUS[y]
	Young trees (growth chamber experiment	Mature trees (field experiment)	Mature trees (field experiment)
-0.5	1508	163	519
-2.0	2061		683
-3.0		268	728

[z] (Nevin and Lovatt, 1987).
[y] (Lovatt *et al.*, 1988a).

of NH_3-NH_4^+ in these trees was extremely high (1508 ± 102 μg per g dry wt leaf tissue) increasing 36% in trees with pre-dawn xylem potentials less than -2.0 ± 1.0 MPa after 21 days of stress. This high concentration of NH_3-NH_4^+ has not been observed for avocado in any subsequent experiments (Table 2). Water-deficit-stressed avocado trees exhibited browning of young shoot tips and necrosis of the leaf tip and margin, which progressed across the blade of the leaf to the petiole. Leaf abscission was considerable. Mature Hass avocado trees in the field subjected to water-deficit stress also accumulated NH_3-NH_4^+ and exhibited the symptoms described above (Nevin and Lovatt, 1987) (Table 2).

These results demonstrate the disconcerting fact that a specific concentration of NH_3-NH_4^+ may cause leaf tip burn and necrosis of the leaf margin in one case but not in another, suggesting that other factors are involved in the expression of the symptoms of NH_3-NH_4^+ toxicity. Recently, Corey and Barker (1989) provided evidence that the symptoms of NH_3-NH_4^+ toxicity in tomato are related to increased ethylene biosynthesis. Whether this is also the case in avocado remains to be determined. Given the variation in response, establishing a threshold value for NH_3-NH_4^+ that results in leaf damage and shoot tip die back in a field situation may prove difficult.

Despite the variation in leaf NH_3-NH_4^+ observed for young Hass avocado trees, comparison of the basal leaf of NH_3-NH_4^+ in leaves from mature field-grown trees representing several avocado scion varieties demonstrated remarkable similarity in this parameter (Table 3).

Table 3 - Basal NH_3-NH_4^+ content of leaves (μg/g dry wt.).

	SPRING (March / April)	SUMMER (July / August)	
Variety	X ± STD DEV (N=5)	Expt. 1	Expt. 2
Hass	48 ± 6	34	29
Bacon	58 ± 4	29	26
Fuerte		30	23
Pinkerton	60 ± 10	28	31
Gwen	50 ± 4	29	33

Taking the results of all experiments thus far, including those for trees subjected to stress, the range in leaf NH_3-NH_4^+ content for Hass avocado is 25 to 2061 μg per g dry wt. However, the upper end of this range is a single observation; one rarely observes values exceeding 1000 μg per g dry wt for field-grown trees. The lower end of the range for avocado is significantly lower than that of the Washington navel orange for which a range of 389 to 2636 μg NH_3-NH_4^+ per g dry wt has been observed (Lovatt *et al.* 1988b). This range is not toxic to the navel orange. The range for Washington navel orange also includes trees that received a foliar application of low biuret urea which successfully increased the NH_3-NH_4^+ content of the leaves (Lovatt *et al.* 1988a,b). An identical foliar application of low biuret urea to Hass avocado trees of similar size failed to increase leaf NH_3-NH_4^+ content (Nevin and Lovatt, 1989).

Taken together, the low basal level of free NH_3-NH_4^+, the concomitantly low activity of the pathway for the *de novo* biosynthesis of arginine and the sensitivity of avocado leaves to free NH_3-NH_4^+ suggests that the leaves of avocado scions may have a limited role in nitrate reduction and NH_3-NH_4^+ assimilation compared to the roots of commercial rootstocks.

3.2 Current research

Due to the emerging role of leaf NH_3-NH_4^+ in the response of plants to stress and in flowering, it was important to identify the relative contribution of the leaves of avocado scions versus the roots of avocado rootstocks to the reduction of nitrate and the assimilation of NH_3-NH_4^+ in the tree. To this end, we compared the activities of nitrate reductase and glutamine synthetase.

Nitrate reductase was variable in both roots and leaves (Table 4). For the various rootstocks tested, NR activities ranged from a low 12.5 nmol nitrate reduced per mg protein for roots of Thomas to a high 95 nmol for roots of clonal Duke 7. The range in NR activities for leaves was lower than in roots, from <1 to 27 nmol nitrate reduced per mg protein.

Table 4 - Nitrate reductase activity in roots and leaves of avocado rootstock and scion varieties, respectively.

Variety (tissue)	EARLY SUMMER (June / July)	EARLY FALL (September / October)
Rootstocks		
Duke 7	95	17
Topa Topa	33	30
G 755	27	17
Borchard	36	30
Thomas	32	13
Toro Canyon	15	64
Scions		
Hass	7	3
Bacon	7	2
Fuerte	12	4
Pinkerton	10	27
Gwen	20	<1

Glutamine synthetase activity was variable in both roots and leaves (Table 5). For the various rootstocks tested, GS activities ranged from a low of 747 nmol glutamyl hydroxamate synthesized per mg protein for roots of G755 to a high of 45603 nmol synthesized for roots of Thomas. As observed for NR, leaves exhibited less variation in GS activity.

Table 5 - Glutamine synthetase activity in roots and leaves of avocado rootstock and scion varieties respectively.

	nmol glutamyl hydroxamate synthesized/mg/protein/hr	
Variety (tissue)	EARLY SUMMER (June / July)	EARLY FALL (September / October)
Rootstocks		
Duke 7	10435	17533
Topa Topa	4766	6926
G 755	747	2086
Borchard	7022	7318
Thomas	14757	45603
Toro Canyon	2396	12503
Scions		
Hass	338	5867
Bacon	<1	<1
Fuerte	5773	2130
Pinkerton	2008	9476
Gwen	932	6374

The generally greater capacity of roots of avocado rootstocks for nitrate reduction and glutamine synthesis relative to leaves of scion varieties is consistent with the roots of a grafted tree being the more important site of nitrogen assimilation. It is possible that at certain times of the year, the leaves of some scion-rootstock combinations would be more important in NH_3-NH_4^+ metabolism than the roots.

The predominant end product of nitrogen fertilization is protein. The protein content of both avocado roots and leaves was very low compared to that of *Citrus* species (Table 6). The average protein content of roots from the various rootstocks tested, 1.0 ± 0.5 (x ± STD.DEV, N = 6) was 4-fold lower than the average protein content of the leaves of various scion varieties examined, 4.2 ± 0.5 (x ± STD.DEV, N = 5). Roots of avocado had levels of protein less than or equal to those of the roots of *Citrus* species. However, the protein content of leaves from avocado scion varieties was approximately one eighth the protein content of leaves of citrus scion varieties determined by the same method.

Table 6 - Protein content.

Plant (tissue)	X ± STD DEV mg protein/g fr. wt. (n = number of experiments)
Avocado rootstocks (roots)	
Duke 7	0.6 ± 0.5 (n = 4)
Topa Topa	0.7 ± 0.2 (n = 5)
G 755	1.7 ± 0.2 (n = 5)
Borchard	0.6 ± 0.2 (n = 5)
Thomas	0.6 ± 0.2 (n = 5)
Toro Canyon	1.6 ± 0.2 (n = 5)
Avocado scions (leaves)	
Hass	4.0 ± 0.5 (n = 5)
Bacon	4.2 ± 0.3 (n = 5)
Fuerte	4.7 ± 0.3 (n = 5)
Pinkerton	3.5 ± 0.4 (n = 5)
Gwen	4.8 ± 0.9 (n = 5)
Citrus rootstocks (roots)	
Rough lemon	2.9 ± 0.3 (n = 3)
Carrizo citrange	1.7 ± 0.3 (n = 3)
Citrus scions (leaves)	
Washington navel orange	33.0 ± 4.0 (n = 4)
Valencia sweet orange	31.4 ± 1.2 (n = 4)
Lemon	30.4 ± 2.2 (n = 4)
Grapefruit	27.8 ± 3.5 (n = 4)

While further documentation is necessary, these preliminary results provide evidence that the avocado rootstock may be a more important factor in nitrogen nutrition of the avocado tree than the scion variety and thus emphasize the importance of good root health to avocado production.

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