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Development of Biochemical Indices of Stress Tolerance for Rapid Initial Screening of Large Numbers of Avocado Rootstock and Scion Varieties

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The overall objectives of this two-year study are: (i) to test drought and salt tolerance of commercially available rootstocks and scion varieties and the progeny of wild avocado species that grow and produce well on barren hillsides of Guatemala without benefit of irrigation; (ii) to develop a biochemical index that can be used to rapidly screen avocado rootstocks or scion varieties for drought and salinity tolerance; *(iii)* to demonstrate rootstocks tolerant to drought salinity impart tolerance to the stress; *i.e.,* actually improve the performance of the scion during stress.

The objective for the first year of research was to identify the site of nitrate reduction, NH_3-NH_4 + production and assimilation, and amino acid biosynthesis in avocado; *i.e.,* leaves *versus* roots, and to characterize differences in the rates at which these metabolic events proceed in various avocado scion and rootstock varieties.

Abiotic stresses cause changes in nitrogen metabolism which result in increased NH_3-NH_4^+ production and accumulation to toxic levels in stress-sensitive plants. Thus, it is essential to identify the important site(s) of nitrogen reduction and assimilation as the first step in identifying a set of biochemical characteristics to use in screening stress-tolerant rootstock and scion varieties.

Table 1. 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		
Duke7	95	17
Topa Topa	33	30
G755	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

For tree crops, the preferred external source of inorganic nitrogen is NO_3^-. Thus, a mechanism must be available to reduce NO_3^- to NH_3-NH_4^+ so that it can be assimilated into amino acids which are subsequently used to make proteins. Some amino acids donate N for the synthesis of nucleotides which are, in turn, used to make DNA and RNA. NO_3^- can be reduced to NH_3-NH_4^+ in roots and/or leaves. The major site of NO_3^- reduction in tree crops is believed to be the roots. The rate limiting enzyme in nitrate reduction is nitrate reductase (NR). We assessed the activity of this enzyme in both roots and leaves of several avocado rootstock and scion varieties, respectively. The NH_3-NH_4^+ formed is assimilated by collaborative activity of two enzymes, glutamine synthetase (GS) and glutamine (amide): 2-oxoglutarate aminotransferase (oxido reductase NADP) (GOGAT). GS catalyzes the first step in collaboration. We assessed the activity of GS in roots and leaves of several avocado rootstock and scion varieties, respectively.

Nitrate reductase was variable in both roots and leaves (Table 1). 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.

Glutamine synthetase activity was variable in both roots and leaves (Table 2). 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 2. Glutamine synthetase activity in roots and leaves of avocado rootstock and scion varieties respectively.

Variety (tissue)	nmol glutamyl hydroxamate synthesized/mg/protein/hr EARLY SUMMER (June / July)	EARLY FALL (September / October)
Rootstocks		
Duke7	10435	17533
Topa Topa	4766	6926
G755	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 3). The average protein content of roots from the various rootstocks tested, 1.0 + 0.5 (x ± STD.DEV. N = 6) was 4-tbld 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 3. Protein content.

Variety (tissue)	X ± STD DEV mg protein/g fr. wt. (n = number of experiments)
Rootstocks	
Duke7	0.6 ± 0.5 (n = 4)
Topa Topa	0.7 ± 0.2 (n = 5)
G755	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)
Scions	
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)

The low protein content of avocado leaves is consistent with our previous observations of lower rates of basal metabolism when comparing the activities of metabolic pathways in avocado leaves with those of other plant species. For example, the basal rate of the pathway for the *de novo* biosynthesis of arginine was 0.5 nmoles $NaH^{14}CO_3$ incorporated into arginine plus urea per g fresh weight Hass avocado leaf tissue. This is one tenth the basal rate of this pathway in leaves of the Washington navel orange.

To look at the efficiency with which NH_3-NH_4^+ formed is converted to protein, we looked at the basal level of leaf NH_3-NH_4^+ content to determine if there was a high background of NH_3-NH_4^+ that was not being converted to amino acids, and thus to protein.

The basal levels of NH_3-NH_4^+ in avocado leaves of the several scion varieties examined were very similar and extremely low (Table 4). The average basal NH_3-NH_4^+ level for citrus leaves from several varieties was approximately 10-fold greater. The leaves of avocado are much more sensitive to toxicity, exhibiting leaf tip burn and necrosis of the leaf margin at lower concentrations of endogenous NH_3-NH_4^+ than citrus. The reason for these differences is not known at this time.

Table 4—Basal NH_3-NH_4^+ content of leaves (μg/g dry wt.).

	SPRING (March / April)	SUMMER (July / August)	
Variety	X±STDDEV(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

The principle investigator was on sabbatical leave during part of the fiscal year 1988-89; thus, the initiation of the project did not coincide with the start of the fiscal year. This report, therefore, covers less than a full year's activity. Grant support for this project from the California Avocado Commission was for a partial year. Please note the results of additional experiments performed after the presentation of the final report on October 20, 1989, have been included in this communication.

While further documentation is necessary, the results thus far demonstrate that the avocado rootstock may be a more important factor in the nitrogen nutrition of the tree than the scion variety. This does not appear to be the case with citrus, which has high levels of NR activity in the leaves, as well as high levels of nitrate. Basal levels of nitrate range from 200 to 1000 μg per g dry weight leaf tissue for several citrus varieties. For leaves of avocado scion varieties, levels of nitrate rarely exceed 100 μg per g dry weight. This is consistent with the reduction and assimilation of nitrate in the roots of avocado and transport of nitrogen to the leaves as amino acids.

The fact that avocado roots are probably the major site of nitrogen reduction and assimilation emphasizes the importance of good root health to avocado production.