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A Physiological Basis for Different Patterns of Nitrate Accumulation in Two Spinach Cultivars[1]

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Abstract. **'America' spinach (*Spinacia oleracea* L.) is a savoy-leafed cultivar and tends to accumulate NO_3^- in its leaf blades, petioles, and roots when the level of NO_3-N nutrition is relatively high. 'Hybrid 424' spinach is smooth-leafed, larger in size, and accumulates much less NO_3^- than 'America' especially when NO_3-N nutrition is high. A greater NO_3^- reductase activity in Hybrid 424, especially in its leaf blades, may account for its lower NO_3^- content compared to that of 'America'.**

Humans are continually exposed to small amounts of nitrates and nitrites which usually cause no harm, but which in high concentrations or under special circumstances may cause illness or even death (9). Since vegetables provide a major portion of our dietary intake of NO_3^-, it is important that their NO_3^- contents be maintained at as low levels as possible consistent with yield and quality of produce.

Certain vegetable cultivars tend to accumulate high levels of NO_3^- (1, 2, 3). Cantliffe (3) found that several spinach cultivars and plant introductions differed in NO_3^- accumulation according to leaf type. A reasonable assumption is that the differences in NO_3^- concn in these plants is due to their differential capacities to absorb or to reduce and assimilate NO_3^-. Support for the view that cultivars differ in ability to assimilate NO_3^- comes from the work of Hageman and Flesher (4), who demonstrated an inverse relationship between NO_3^- accumulation and NO_3^- reductase activities in 2 lines of corn (*Zea mays* L.). The present study attempts to account for the wide differences in NO_3^- concn found in savoy-leafed and smooth-leafed forms of spinach on the basis of NO_3^- reductase activity and distribution within the plants and on the nitrogenous constituents found in xylem exudate from the roots.

Materials and Methods

Spinach cultivars America, savoy-leafed and a NO_3^- accumulator, and Hybrid 424, smooth-leafed and a non-accumulator, were used in this study. Seeds were planted in plastic 15 x 11 cm pots holding 2200 g of a 1:1 (w/w) mixture of coarse and fine quartz sand and were watered with deionized water until germination. Following emergence, seedlings were thinned to 4 per pot and supplied with 10, 50, or 250 ppm NO_3-N daily as a surface drench of 100 ml. Other plant nutrients except Cl^- which varied inversely with NO_3^- concn were held constant at the concentrations of full-strength Hoagland's No. 1 solution (5). Plants were grown on these treatments for 6 weeks when severe N deficiency symptoms developed on the plants receiving 10 ppm NO_3-N. Yield, NO_3^- determination, and xylem exudate studies were from plants grown in a greenhouse at 24 ± 3° (day) and 16 ± 3°C (night) with daylength about 13 ± 1 hr. Plants used in the studies of NO_3^- reductase were grown in growth chambers using a 12-hr photoperiod (1800 ft-c) and temp of 24 ± 1° (light period) and 16 ± 1°C (dark period). Comparative studies (12) showed no significant differences between NO_3^- concn and NO_3^- reductase activities in spinach plants grown with the same nutritional regimes in the greenhouse and growth chambers.

Yields. At harvest, fresh wt of roots, petioles, and leaf blades was determined, and after drying at 65°C in a forced-draft oven for 48 hr, dry wt was determined.

NO_3^- determination. The dried plant tissue was pulverized with a mortar and pestle. Nitrate concn was determined with the phenoldisulfonic acid procedure of Humphries (6) and expressed on a fresh wt basis.

Assay for NO_3^- reductase activity. Samples of fresh plant tissue were collected, weighed, and frozen immediately at −15°C for at least 30 minutes. Extraction and assay were conducted according to the method of Wallace and Pate (15) except that the medium was 10^{-2} M in cysteine to provide maximum protection of the enzyme, particularly in root extracts, and 0.625 μmole of NADH was added to the assay mixture to obtain maximum enzymatic activity. Activity of enzyme was calculated as nanomoles of NO_2^- formed per hr per g fresh wt and total fresh wt of plant tissue.

Sap analysis. Shoots of 40 plants were excised at the surface of the sand. The first drop of sap was discarded, and xylem exudate was collected continuously for 6 to 8 hr following excision by allowing the sap to bleed into glass tubes attached to the decapitated plants with latex rubber tubing (13). Samples were bulked according to treatment at the end of the collection period at which time the number of bleeding plants, volume of exudate, and collection period were recorded. The samples were frozen at −15°C until analyzed. Nitrate was determined on 0.5 ml portions of sap evaporated to dryness in a 50 ml beaker placed in an oven at 60°C. After cooling, 4.5 ml of 0.35% $(Ag)_2SO_4$ solution were added followed by 0.5 ml of 1.0 M NaH_2PO_4 (pH 6.5). Detection of NO_3^- was by the phenoldisulfonic acid procedure (6). Ammonia was determined by a modification of the microdiffusion technique of Kenten (8) using 0.1 ml portions of sap. Amino acids and amides were determined by gas-liquid chromatography after conversion to their N-trifluoracetyl-*n*-butyl esters and trimethyl silyl derivitives, respectively. The amino acid and amide analyses were performed by Analytical Biochemistry Laboratories, Columbia, Missouri. Total N was determined on 0.1 ml portions of sap using a micro-Kjeldahl procedure modified by the addition of salicylic acid to include NO_3-N (6).

Statistical analysis. The experimental design was completely random with 5 treatment replications in all experiments except where the xylem exudates were bulked. The *t*-test was used to determine the significance of the difference between means.

Results

Plant growth. Marked differences in plant growth occurred when the plants were grown at 10, 50, or 250 ppm NO_3-N (Table 1). Growth and green color increased with increases in N in the nutrient solution but 'Hybrid 424' was relatively more

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Table 1. Fresh wt and nitrate concn of spinach grown on 3 levels of nitrate-N nutrition.

NO_3-N nutrition level (ppm)	Plant part	Fresh wt (g/pot)		NO_3-N concn (ppm fr wt)	
		America	Hybrid 424	America	Hybrid 424
10	Blades	3.61	3.33	1.2	5.8*
	Petioles	0.44	0.57*	3.1	4.1
	Roots	4.28	2.57*	33.6	7.0*
50	Blades	12.76	19.97*	2.3	11.4*
	Petioles	2.40	5.45*	10.2	13.5
	Roots	13.48	18.58*	103.8	31.2*
250	Blades	30.78	53.23*	394.2	121.8*
	Petioles	6.52	21.87*	1302.0	458.1*
	Roots	13.23	26.89*	814.7	313.4*

*Mean fresh wt or NO_3^- concn of 'Hybrid 424' significantly different from that of 'America' at 5% level.

responsive to increases in N than 'America'. At the higher levels of N, 'Hybrid 424' was much larger in size than 'America'. The dry matter contents as a percentage of fresh wt in blades, petioles, and roots, respectively, were 10.3, 8.4, and 7.1 for 'America' and 11.4, 9.1, and 8.8 for 'Hybrid 424'. Percentage dry matter did not vary with NO_3-N nutritional level although the plants receiving 250 ppm NO_3-N appeared more succulent than the others.

Nitrate concentrations. The NO_3-N concn in the tissues of both cultivars remained low in plants grown on 10 or 50 ppm NO_3-N with the roots being the major site of accumulation (Table 1). With 250 ppm NO_3-N in the nutrient solution, NO_3-N concn in the tissues were elevated with petioles accumulating the highest concn of NO_3-N followed by roots and blades in descending order. At 250 ppm the NO_3-N concn in 'America' was about 3 times that of 'Hybrid 424' in all tissues.

Nitrate reductase activity. In general, an overall increase in NO_3^- reductase activity occurred with increases in NO_3-N in the medium (Table 2), and enzyme induction with increases in NO_3-N was much greater in blades than in petioles or roots. The NO_3^- reductase activity per g fresh wt of blades of 'Hybrid 424' was significantly greater at 250 ppm NO_3-N nutrition than that of 'America'. 'Hybrid 424' with adequate N nutrition is inherently a much larger plant than 'America', and if the activity of the enzyme per unit wt of tissue is factored by the total wt of tissue, the difference in assimilatory capacity between the cultivars becomes more apparent. At 250 ppm NO_3-N nutrition,

Table 2. Nitrate reductase activities in spinach grown on 3 levels of nitrate-N nutrition.

NO_3-N nutrition level (ppm)	Plant part	Nitrate reductase activity			
		Specific (nmoles NO_2^-/hr/g fr wt)		Total (nmoles NO_2^-/hr/plant fr wt)	
		America	Hybrid 424	America	Hybrid 424
10	Blades	4425	1700*	3989	1410*
	Petioles	5035	2009*	550	283*
	Roots	325	178*	349	115*
50	Blades	9352	11943*	29808	59625*
	Petioles	4131	3307*	2483	4546*
	Roots	724	194*	2441	900*
250	Blades	10939	16277*	84000	214460*
	Petioles	5389	5333	8871	29365*
	Roots	492	488	1638	3366*

*Mean of specific or total nitrate reductase activity of 'Hybrid 424' significantly different from that of 'America' at 5% level.

Table 3. Major nitrogenous constituents of bleeding sap collected from spinach.

Cultivar	NO_3-N nutrition level (ppm)	Nitrogenous constituent				
		Amino-N	Amide-N	NH_4-N	NO_3-N	Total
		μgN/hr/plant				
America	10	1.38	0.16	0.04	4.67	6.25
	50	5.48	7.84	0.54	24.93	38.79
	250	11.32	15.06	1.74	58.29	86.41
		% of total N				
	10	22	3	1	74	100
	50	14	20	2	64	100
	250	13	17	2	68	100
		μgN/hr/plant				
Hybrid 424	10	0.39	2.09	0.08	5.75	8.31
	50	2.81	15.16	0.73	32.48	51.18
	250	5.50	20.88	6.08	69.63	102.09
		% of total N				
	10	5	25	1	69	100
	50	6	29	1	64	100
	250	5	21	6	68	100

total NO_3^- reductase activities in blades, petioles, and roots of 'Hybrid 424' exceeded those in 'America'. This was also true for blades and petioles at the 50 ppm level of NO_3-N nutrition. At the lowest level of NO_3-N nutrition which severely limited growth of both cultivars and especially 'Hybrid 424', 'America' had slightly greater NO_3^- reductase activities in all tissues. Higher enzymatic activity was also noted in the roots of 'America' at the 50 ppm level of NO_3-N nutrition.

Sap analysis. Analysis of the sap showed that NO_3^-, glutamic acid, and glutamine were the principal nitrogenous constituents of xylem exudate. Ammonia and other amino acids were also present; however, asparagine was absent from the xylem sap. For clarity, these are grouped in Table 3 as *amino-N* which includes alanine, valine, glycine, isoleucine, leucine, proline, threonine, serine, methionine, homoserine, phenylalanine, aspartic acid, glutamic acid, tyrosine, lysine, histidine, and arginine, *amide-N* which is glutamine only, *NH_3-N,* and *NO_3-N.* The total N, NO_3-N, and reduced N (amino, amide and NH_3-N) exported from the roots of 'Hybrid 424' exceeded that of 'America'. The proportion of NO_3-N in the exuded sap remained relatively constant with level of NO_3-N nutrition and between cultivars. Based on these observations, differences in xylem transport of NO_3^- between the cultivars are not apparent.

Discussion

'Hybrid 424' and 'America' spinach cultivars are easily distinguished from one another on the basis of their leaf morphology and plant size. 'Hybrid 424' (smooth leaf, larger plant) responds directly to increases in NO_3-N nutrition and does not grow well at low levels of NO_3-N nutrition. At levels of NO_3-N nutrition optimum for growth, 'Hybrid 424' accumulates relatively little NO_3^- in its tissues. 'America' (savoy leaf, smaller plant) also responds well to increases in NO_3-N fertilization but in contrast to 'Hybrid 424' accumulates substantial concentrations of NO_3^- in its tissues especially as the level of NO_3-N nutrition exceeds its needs (1, 10).

The difference in NO_3^- accumulation between the 2 cultivars appears to be related to the difference in NO_3^- reductase activity in the leaf blades. Nitrate reductase activity in plants, especially in leaves, is induced by the presence of NO_3^- (4, 7, 14, 15). Activity of this enzyme was induced by increases in NO_3^- in the culture medium to a far greater extent in leaf blades of 'Hybrid 424' than in those of 'America'. Specific activity of the enzyme per unit fresh wt of blade tissue was nearly 50% greater in 'Hybrid 424' than in 'America', and the total activity in the blades of 'Hybrid 424' was at least twice that found in 'Amer-

ica'. Although specific activities were not greater in the roots and petioles of 'Hybrid 424' than in 'America', 'Hybrid 424' had a greater total NO_3^- reductase activity in these tissues because of its larger size.

The qualitative and quantitative composition of bleeding sap from the 2 cultivars did not differ significantly. The proportions of NO_3-N and reduced N did not differ although it appeared that 'Hybrid 424' might transport slightly larger amounts of these constituents than those found in the bleeding sap of 'America'. No evidence was found to indicate that 'Hybrid 424' had a lesser capacity to absorb and translocate NO_3^- than 'America'. It is also clear that there was no dilution effect giving lower NO_3^- concn in 'Hybrid 424', for the total NO_3^- content in its leaves was less than that found in 'America' (see also 10).

Determination of the NO_3^- reductase activity is another factor, in addition to leaf morphology and growth rate, which will be useful in distinguishing between the tendencies of spinach cultivars to accumulate NO_3^-. The potentially higher growth rate, higher yields, higher protein contents, and lower NO_3^- contents appear to be interrelated features found in the smooth-leafed cultivar (12). Careful varietal selection based on this relationship and management of the nutritional regime (11) will virtually insure avoidance of any human health hazards connected with the consumption of spinace and should lead to production of a more nutritious product at lower costs.

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A Physiological Basis for Different Patterns of Nitrate Accumulation in Cucumber and Pea[1]

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Abstract. **Cucumber (*Cucumis sativus* L.) plants accumulate more NO_3^- than pea (*Pisum sativum* L.) plants. The differences in accumulation appear to be due to differences in the abilities of the two species to reduce NO_3^- in their roots. Only 2% of the NO_3^- reductase activity of cucumber was found in its roots, whereas nearly 92% of the activity was found in the blades. In pea, NO_3^- reductase activity was more evenly distributed throughout the plant; 67% of the activity was in the blades, 18% in the roots, and the remainder in the stems and petioles. Nitrate-N comprised 80% of the N present in bleeding sap of roots of cucumber plants from which the shoots had been excised. In contrast, NO_3-N constituted only 30% of the N in the sap from pea roots, the remaining 70% of the N consisting of amino acids and amides. Asparagine or aspartic acid was the major carrier of reduced N in pea, and glutamine was the major carrier in cucumber. The differences in N transport and assimilation appear to bear considerably on plant composition and efficiency of N usage.**

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