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Effect of Nitrate and Ammonium Nitrogen on the Growth and Mineral Composition of Crassulacean Acid Metabolism Plants[1]

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Abstract. **Effects of 2 N forms and 5 N levels on the growth and mineral composition of 3 species of crassulacean acid metabolism (CAM) plants were studied. Plants supplied with N produced more top dry weight and contained higher chlorophyll and N concentrations than those grown without N. CAM plants appeared to utilize NO_3^--N and NH_4^+-N equally well as measured by dry weight except in *Sedum telephoides,* where fertilization with NH_4^+-N reduced leaf growth. Leaf area and chlorophyll concentrations were generally higher in plants supplied with NO_3^--N than in those supplied with NH_4^+-N. Ammonium nutrition increased total N concentration in some plant parts of *S. telephoides* and *Kalanchöe verticillata.* Moreover, the leaf and stem mineral composition were not significantly affected by the rate and form of N or their interaction. Apparently the N requirements of CAM seedlings is relatively low on calcareous arid region soils in Iran. Furthermore, NH_4^+-N fertilization generally suppressed growth of CAM plants as compared to those fertilized with NO_3^--N.**

Nitrogen has long been recognized as one of the most important plant nutrients. In crop production plants respond differently to N form. Plants can absorb NO_3^--N, NH_4^+-N, urea or simple organic N molecules. In well-aerated soil, NO_3^--N is the predominant form of available N, but the ammoniacal form of N should be the desirable form since it is not as easily leached and denitrified as is NO_3^--N.

The relative merits of NO_3^--N and NH_4^+-N as sources of N for plant growth have been reviewed. Several workers (1, 13, 14, 15, 16, 18, 19, 20) have reported that NH_4^+-N reduced plant growth as compared to NO_3^--N. In contrast, other reports (4, 8, 26) show that young seedlings respond more favorably to NH_4^+-N nutrition. Hewitt (11) and Street and Sheat (29) concluded that the response to N form may vary with species, environmental conditions, soil conditions such as pH and other factors. For instance, Greidanus et al. (10) observed that ammonium was essential for the growth of cranberry (*Vaccinium macrocarpon* Ait.). Spratt and Gasser (27) showed that kale (*Brassica oleracea* L.) utilized NO_3^--N more efficiently than NH_4^+-N but that N-forms were not as critical for wheat (*Triticum aestivum* L.) and ryegrass (*Lolium multiflorum* L.). In a subsequent study, Spratt and Gasser (28) stated that in a semiarid climate, ammoniacal fertilizer-N for wheat may be as good as, or even better than NO_3^--N but in a humid climate, nitrate may be better, provided that it is not lost by leaching or denitrification. Schrader et al. (24) observed that corn (*Zea mays* L.) grew more rapidly on NH_4^+-N plus NO_3^--N than on NO_3^--N or NH_4^+-N alone. The same results were reported by Weissman (30) in sunflower (*Helianthus annuus* L.). However, Morris and Gidden (18) noted no significant differences in plant growth between NH_4^+-N and NO_3^--N for cotton (*Gossypium hirsutum* L.), grain sorghum (*Sorghum bicolor* (L.) Moench) and coastal bermudagrass (*Cynodon dactylon* L. Pers.).

Ammonium has an effect quite different from that of NO_3^--N on plant growth, development, chemical composition, and metabolism. Ammonium-N suppresses cation absorption (1, 5, 7, 12, 13, 14, 19, 20, 32), enhances anion uptake (5, 7, 13, 20), inhibits water uptake (19), lowers the activity of several enzymes (31) and decreases organic acid content (14).

In recent years, the increasing use of crassulacean acid metabolism (CAM) plants as ornamentals has demonstrated a need for integrated studies concerning their growth and nutritional requirements. However, the growth response of these plants to N rate and form has not been studied. This experiment was undertaken to evaluate the influence of N form at several levels on the growth and mineral composition of 3 CAM plant species.

Materials and Methods

The soil used in this experiment was collected from the surface horizon of an alluvial calcareous silty clay from the vicinity of Badjgah Agricultural Experiment Station. This soil had a pH of 7.8 (saturated paste) and contained 1.10% organic matter, 0.05% total N and 11 ppm $NaHCO_3$-extractable P. Treatments were 5 N levels (0, 25, 50, 100 and 200 ppm), 2 N forms [$(NH_4)_2SO_4$ and $Ca(NO_3)_2$] and 3 CAM plant species, (*Sedum telephoides Michx., Kalanchöe laxiflora* Baker, and *Kalanchöe verticillata* Elliot). The experiment was arranged factorially in a completely randomized design with 3 replications. Five-week old cuttings with well-developed root systems were transferred into plastic pots, each containing 1000 g of air-dried soil. The soil was pretreated with 10 ppm nitrapyrin [2-chloro-6-(trichloromethyl)pyridine], a nitrification inhibitor. Characteristics of cuttings used in this experiment have been reported elsewhere (23). All pots were fertilized with 25 ppm of P as reagent grade $Ca(H_2PO_4)_2$. Plants were grown for 60 days in a greenhouse under 12-13 hours of daylight (16-28°C, 65% RH). The light intensity was 3.33-3.67 μ einsteins cm^{-2} min^{-1}. Natural light was supplemented by cool-white fluorescent tubes and incandescent bulbs. Pots were irrigated with distilled water to near field capacity by weight as needed.

At harvest, the above-ground portion of plants was cut at the soil surface and separated into stem and leaves. Roots were washed free of soil and other foreign particles. Chlorophyll concentration was determined by the Winterman's method as modified by Rouhani (22), and leaf area was measured using a graph paper method (25). Leaves, stems and roots were dried at 70°C, the dry weights were recorded, and the tissues were ground in a Wiley mill to pass a 40-mesh screen. Total N was determined by the micro-Kjeldahl method. For P, Ca, Mg, K and Na determinations, 500 mg samples were ashed at 450°C and the residues were taken up in dilute HCl. Phosphorus was determined colorimetrically by the ammonium molybdate-stannous chloride method (6). Other nutrients were measured with a Carl Zeiss Atomic Absorption Spectrophotometer.

Results and Discussion

Vegetative growth. The interaction of N form and level on dry weight was not significant (Table 1). Level of N from

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Table 1. Effect of N form and N rate on the dry weights of roots, stems and leaves of CAM plants.

N rate (ppm)	*S. telephoides*			*K. laxiflora*			*K. verticillata*		
	NO_3^--N	NH_4^+-N	Mean	NO_3^--N	NH_4^+-N	Mean	NO_3^--N	NH_4^+-N	Mean
				Root dry wt (g/pot)					
0	0.31	0.19	0.25	0.11	0.14	0.12	0.15	0.10	0.12
25	0.29	0.27	0.28	0.25	0.26	0.25	0.18	0.24	0.21
50	0.49	0.18	0.33	0.19	0.25	0.22	0.25	0.20	0.22
100	0.32	0.39	0.35	0.18	0.20	0.19	0.18	0.15	0.16
200	0.33	0.35	0.34	0.19	0.15	0.17	0.19	0.06	0.12
Mean	0.35	0.28		0.18	0.20		0.19	0.15	
LSD 5%									
N rate	NS			0.07			NS		
N form	NS			NS			NS		
Rate × form	NS			NS			NS		
				Stem dry wt (g/pot)					
0	0.77	0.71	0.74	0.84	1.09	0.96	0.51	0.47	0.49
25	1.16	1.27	1.21	2.03	1.70	1.86	0.72	0.83	0.77
50	1.42	1.08	1.25	1.54	1.80	1.67	1.00	0.87	0.93
100	1.10	1.27	1.18	2.06	1.96	2.01	0.75	0.62	0.68
200	1.25	1.00	1.12	2.02	1.66	1.84	0.81	0.45	0.63
Mean	1.14	1.06		1.70	1.65		0.76	0.65	
LSD 5%									
N rate	0.34			0.65			0.24		
N form	NS			NS			NS		
Rate × form	NS			NS			NS		
				Leaf dry wt (g/pot)					
0	2.01	1.45	1.73	1.30	1.30	1.30	1.67	1.51	1.59
25	2.15	2.02	2.08	1.39	1.41	1.40	2.30	2.59	2.44
50	2.90	1.97	2.43	1.43	1.41	1.42	3.33	2.64	2.98
100	2.60	2.44	2.52	1.33	1.20	1.26	2.74	2.46	2.60
200	3.20	1.88	2.54	1.16	1.15	1.15	2.92	1.70	2.31
Mean	2.75	1.95		1.32	1.30		2.60	2.18	
LSD 5%									
N rate	0.48			NS			0.76		
N form	0.30			NS			NS		
Rate × form	NS			NS			NS		

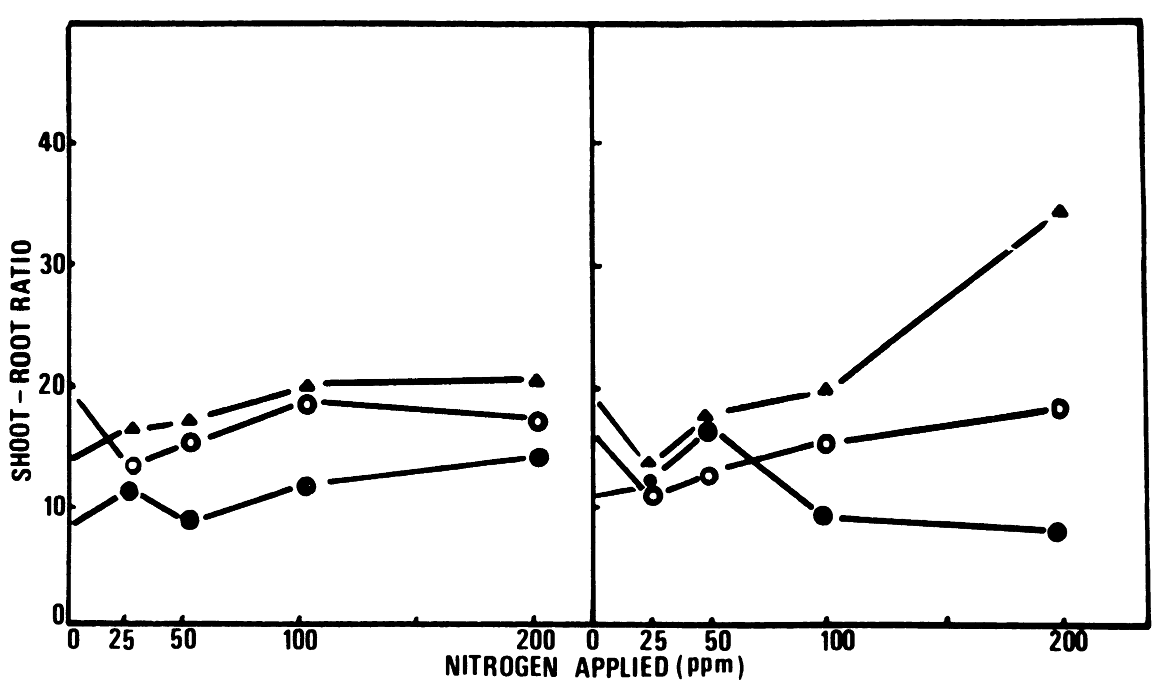

Fig. 1. Shoot/root dry weight of CAM plants as influenced by N form and N rate. *S. telephoides* (●), *K. laxiflora* (▲) and *K. verticillata* (○). $Ca(NO_3)_2$ (left) and $(NH_4)_2SO_4$ (right).

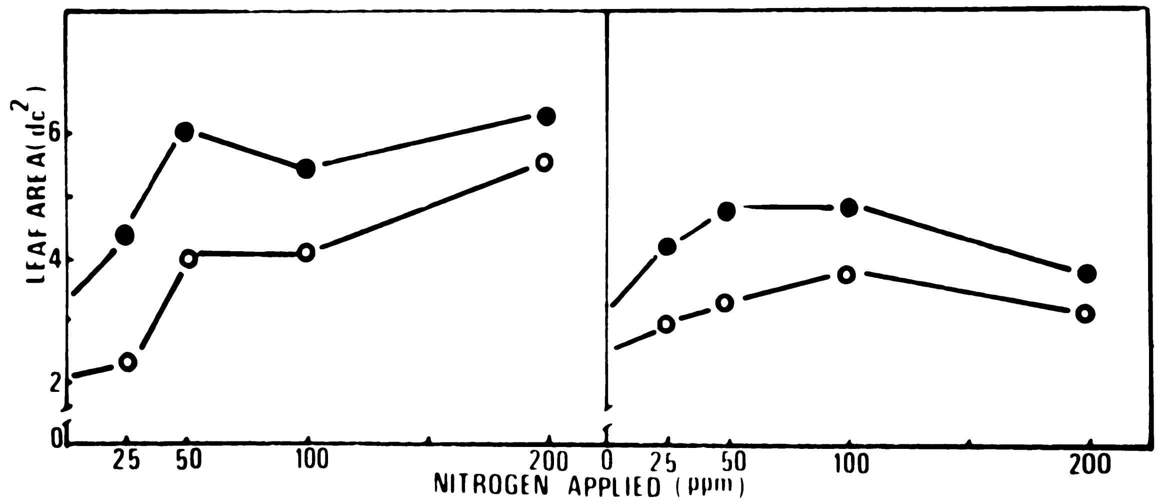

Fig. 2. Leaf area of CAM plants as influenced by N form and N rate. *S. telephoides* (●) and *K. laxiflora* (○). $Ca(NO_3)_2$ (left) and $(NH_4)_2SO_4$ (right).

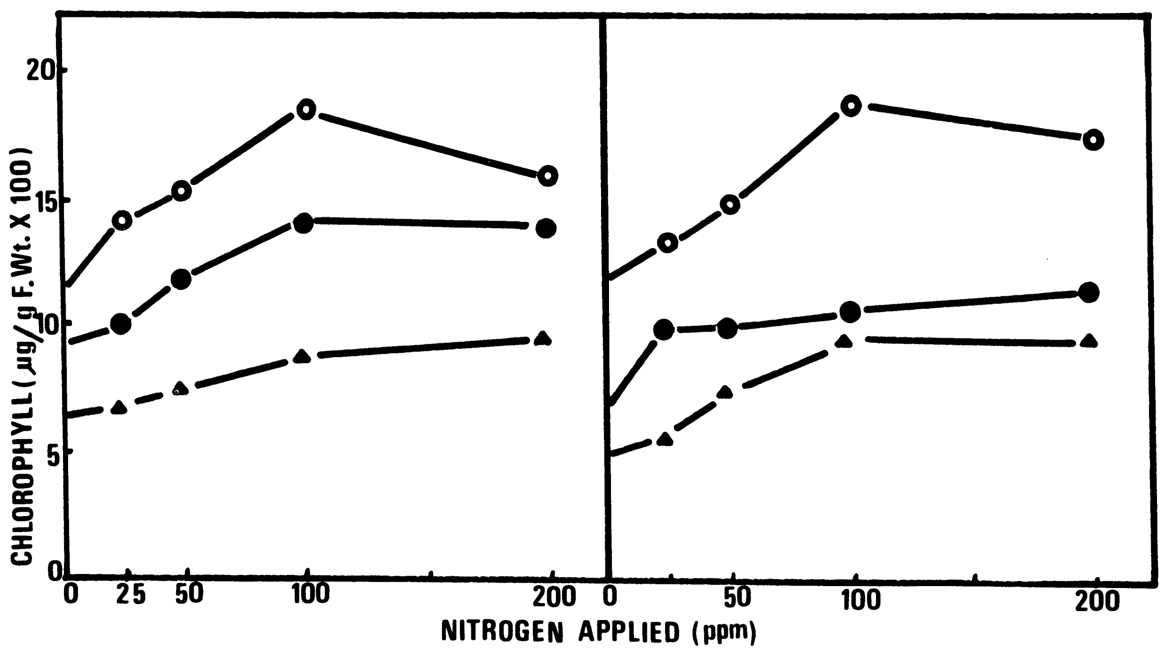

Fig. 3. Leaf chlorophyll concentration of CAM plants as influenced by N form and N rate. *S. telephoides* (●), *K. laxiflora* (▲), and *K. verticillata* (○). $Ca(NO_3)_2$ (left) and $(NH_4)_2SO_4$ (right).

either forms had a significant effect only on root growth of *K. laxiflora*. However, when averaged over all N rates, N-treated plants produced significantly higher top dry weight than untreated seedlings (Table 1). In contrast, root, stem and leaf dry yields were not significantly affected by N form except in *S. telephoides* where higher leaf dry weight was obtained from NO_3^--N treated plants (Table 1). The suppressing effect of NH_4^+-N nutrition on plant growth is well documented (1, 13, 14, 17, 19, 20). The NH_4^+-N ion has been reported to accelerate respiratory breakdown of carbohydrates (2), uncouple photosynthetic phosphorylation (9) and result in disruption of chloroplast membranes (21). Pill and Lambeth (19) observed that ammonium nutrition reduced tomato (*Lycopersicon esculentum* Mill.) growth because of increased water stress and impaired ion uptake. Barker et al. (3) suggested that rapid assimilation of NH_4^+-N tends to lower the rhizosphere pH which may adversely affect plant growth. In the present study, N application had a significant effect on the growth of CAM plants.

It is evident that the shoot-root ratio of *K. verticillata* and *K. laxiflora* plants increased with increasing NH_4^+-N over 25 ppm, whereas in *S. telephoides*, the ratio increased up to 50 ppm N (NH_4^+-N) and declined with a higher concentration (Fig. 1). There was a general trend of increased shoot-root ratio in *K. laxiflora* and *S. telephoides* as NO_3^--N level was increased.

Leaf area. Leaf area was not measured in *K. verticillata* since this species has long, round rod-like leaves. Leaf area

Table 2. Effect of N form and N rate on the N concentration of root, stem and leaf of CAM plants.

N rate (ppm)	*S. telephoides*			*K. laxiflora*			*K. verticillata*		
	NO_3^--N	NH_4^+-N	Mean	NO_3^--N	NH_4^+-N	Mean	NO_3^--N	NH_4^+-N	Mean
				Root N concn (% dry wt)					
0	1.50	1.21	1.35	1.37	1.16	1.26	1.40	1.53	1.46
25	1.56	1.33	1.44	1.53	1.22	1.37	1.47	1.66	1.56
50	1.60	2.34	1.97	1.43	1.39	1.41	1.56	1.60	1.58
100	2.34	2.11	2.22	1.88	1.90	1.89	1.77	1.65	1.71
200	2.64	2.69	2.66	1.90	2.46	2.18	1.92	2.31	2.11
Mean	1.92	1.93		1.62	1.62		1.62	1.75	
LSD 5%									
N rate	0.23			0.26			0.12		
N form	NS			NS			0.07		
Rate × form	NS			0.38			0.18		
				Stem N concn (% dry wt)					
0	0.82	0.94	0.88	0.97	0.87	0.92	0.84	0.76	0.80
25	1.01	0.97	0.99	0.86	0.88	0.87	1.09	0.79	0.94
50	1.05	1.10	1.07	1.23	1.49	1.36	1.25	1.14	1.19
100	1.70	1.33	1.51	1.23	1.15	1.19	1.72	1.35	1.53
200	2.26	2.37	2.31	1.84	1.71	1.77	1.75	2.22	1.98
Mean	1.36	1.34		1.22	1.22		1.33	1.25	
LSD 5%									
N rate	0.19			0.32			0.20		
N form	NS			NS			NS		
Rate × form	NS			NS			0.28		
				Leaf N concn (% dry wt)					
0	1.03	1.17	1.10	1.15	1.10	1.12	1.06	1.18	1.12
25	1.42	1.34	1.38	1.15	1.23	1.19	1.32	1.22	1.27
50	1.58	2.04	1.81	1.36	1.30	1.33	1.56	1.66	1.61
100	2.24	2.29	2.26	1.65	1.65	1.65	1.92	2.24	2.08
200	2.74	2.91	2.82	1.89	2.22	2.05	2.12	2.30	2.21
Mean	1.80	1.95		1.44	1.50		1.59	1.72	
LSD 5%									
N rate	0.22			0.12			0.15		
N form	0.01			NS			0.09		
Rate × form	NS			0.18			NS		

Table 3. Elemental composition of CAM plant stems and leaves.[z]

Species	Plat part	Ca (% dry wt)	Mg (% dry wt)	K (% dry wt)	Na (ppm)	P (% dry wt)
S. telephoides	Leaf	5.58±0.114	0.42±0.012	2.94±0.067	864±34	0.26±0.007
	Stem	4.29±0.311	0.24±0.014	2.82±0.231	623±47	0.21±0.007
K. laxiflora	Leaf	4.82±0.136	0.27±0.015	2.94±0.051	890±41	0.16±0.007
	Stem	2.55±0.257	0.47±0.038	2.36±0.146	469±13	0.22±0.007
K. verticillata	Leaf	4.93±0.107	0.28±0.090	2.32±0.093	822±29	0.17±0.006
	Stem	3.92±0.280	0.32±0.014	1.34±0.201	599±31	0.12±0.007

[z]Mean ± SE.

was generally higher in NO_3^--N treated plants than those supplied with NH_4^+-N (Fig. 2). For instance, 82 and 95% increases in the leaf area of *S. telephoides* and *K. laxiflora* respectively were obtained when 50 ppm N as NO_3^--N was applied, whereas those increases for 50 ppm N as NH_4^+-N were 60 and 32%, respectively. Nitrate-N addition up to 200 ppm N and NH_4^+-N application up to 100 ppm increased leaf area.

Chlorophyll. The chlorophyll concentration in *K. laxiflora* was lower than in the other 2 species (Fig. 3). Furthermore, the chlorophyll concentration in *K. laxiflora* and *S. telephoides* supplied with N as NO_3^--N was higher than in those provided with NH_4^+-N. Nitrogen application in either form increased the chlorophyll concentration of *K. laxiflora* and *S. telephoides,* while in *K. verticillata,* the chlorophyll concentration was increased up to 100 ppm N as NO_3^--N or NH_4^+-N.

Total N concentration. The total N concentration in roots and leaves of *K. verticillata* and in leaves of *S. telephoides* supplied with NH_4^+-N was higher than in plants provided with NO_3^--N (Table 2). The somewhat higher N concentration in the leaves of NH_4^+-N treated CAM plants as compared with

NO_3^--N treated plants may be caused to some extent by a concentration effect due to the relatively lower yields in this treatment. The findings that roots of CAM plants grown with NH_4^+-N were higher in total N than those on nitrate treated plants suggest that NH_4^+-N was detoxified in the root through direct incorporation into organic N, whereas NO_3^--N was transported to tops for enzymic reduction by nitrate reductase (24). The N concentration in *S. telephoides* grown on N-amended soil was higher than in the other species. Moreover, N application significantly increased N concentration in 3 CAM plant species.

Mineral composition. The leaf and stem elemental composition of 3 CAM plant species showed no significant difference between N form, N rate or their interaction (Table 3). Thus, the data were averaged over N form and rate within each species and plant part. The fact that mineral composition was not significantly affected by N form is in disagreement with results reported by others (1, 5, 7, 13, 16, 17). Out data indicate that the Ca and Na concentrations in leaves were higher than those in stems in all species. A similar relationship was observed for K in *K. laxiflora* and *K. verticillata.* Moreover, *S. telephoides* leaves had higher Mg and P concentrations than stems, whereas the reverse was observed in *K. laxiflora.* The leaves of *S. telephoides* had higher Ca, Mg and P concentrations than those of the other CAM plants.

In conclusion, it appears that N requirement of CAM plants is relatively low in calcareous arid soils in Iran. Furthermore, the growth of CAM plants fertilized with NH_4^+-N generally was reduced as compared with those fertilized with NO_3^--N, with *K. laxiflora* apparently being less affected than the other species.

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