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Soil Fertility and the Organic Composition of Plants, Lysine, Arginine and Aspartic Acid Variations[1]

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SYNOPSIS. Lysine and arginine contents of bromegrass varied inversely with concentration of calcium and potassium in growth medium. Aspartic acid accounted for much of increased nitrogen content of bromegrass when heavily fertilized with nitrogen.

THIS investigation was undertaken in an effort to further elucidate the function of some soil fertility factors in plant biosynthesis.

Soil fertility and allied soil chemistry investigations in the past have been concerned primarily with the mechanisms by which nutrients were supplied to plants and the ability of a particular soil to deliver nutrient elements to the plant. In recent years, however, many soil fertility and plant nutrition investigations have considered not only the ability of the soil to deliver nutrient elements and the ability of the plant to take them up, but, in addition, the organic composition of the plant grown in several environments (8, 13, 14, 16, 17, 19).

Amino acid variation in green plant tissues produced under a variable nutritional environment has been demonstrated by Sheldon et al. (20) and Sheldon[5] in forage grasses; by Renner et al. (17) in wheat seed; and by Mertz et al. (13) in alfalfa. Several reviews (14, 20, 24, 25) have reported many other investigations on this subject. Syrett and Fowden (23) demonstrated that chlorella varied widely in basic amino acid content depending on the amount of energy reserves (glucose) available. They proposed that chlorella cells lacking sufficient energy reserves for protein synthesis stored excess nitrogen as basic amino acids and amides. Mertz et al. (13) found that sulfur-deficient alfalfa showed decreased cystine and methionine content, a sharp increase in arginine, and a very large increase in aspartic acid (asparagine?). It was shown that sulfur-deficient alfalfa was higher in total nitrogen and amide nitrogen, but lower in all amino acids except aspartic acid and arginine. Renner et al. (17) showed that sulfur fertilization of wheat significantly increased the leucine, isoleucine, valine, methionine, and threonine content with a concomitant improvement in baking quality. Arnow et al. (3) found that chorella could utilize arginine as a nitrogen source more effectively than either nitrate or ammonia when manganese was present in adequate amounts. In fact all of the amino acids of the ornithine-citrulline-arginine cycle stimulated chlorella growth in the presence of manganese. This suggested that the enzyme arginase, a manganese containing enzyme, was functional in chlorella and that arginine might accumulate in plant cells due to depression of the activity of the element manganese.

McKee (12) found that asparagine accumulated in large amounts in "dying" barley leaves and seedlings with a simultaneous decrease in other nitrogen fractions. This suggested an increased rate of proteolysis or a decreased rate of biosynthesis, However, work by Iljin (9) showed that lime-induced chlorosis caused a decrease in protein nitrogen and an increase in nonprotein nitrogen. Other work with sulfur-deficient clover (2) and alfalfa (13) showed approximately the same decrease in protein nitrogen and increase in nonprotein nitrogen. Possibly lime-induced chlorosis and

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[5] Sheldon, V. L. Biosynthesis of amino acids and soil fertility. Ph.D. Thesis, University of Missouri, 1950.

sulfur deficiency have the same effect on the nitrogen metabolism of plants.

Siegel et al. (19) showed that nitrogen assimilation in soybeans was definitely a function of calcium when ammonia was the nitrogen source. It was also shown that adequate calcium in the soil reduced the requirement for carbohydrate in ammonia assimilation or that calcium promoted the synthesis of additional carbohydrates. It was demonstrated that soybeans grown in soils very high in nitrogen and low in calcium produced plants very high in Kjeldahl nitrogen but were very low in yield. With calcium fertilization, however, they found a more moderate increase in Kjeldahl nitrogen, a pronounced increase in growth and increased calcium uptake on high nitrogen fertilized soils. Other work with chlorella (25) has shown that with adequate energy supplies and a favorable inorganic nutrient solution these plants will not store amides and basic amino acids to very high levels.

The writers interpret from the work of others that a deficiency of inorganic nutritional factors may seriously impair the nitrogen metabolism of the plant.

EXPERIMENTAL PROCEDURE

Bromegrass (*Bromus inermis*—Var. Southern type) was grown in 2-gallon glazed stoneware jars in a medium consisting of 2% electrodialyzed (less than 0.2 micron) Putnam clay, prepared according to Albrecht (1), and 98% acid-washed white quartz sand. The amounts of calcium, potassium, nitrogen, phosphorus, and sulfur were variable. The basic experimental design included all possible combinations of the 5 elements at 2 levels, high and low, or 32 treatments, with calcium at 100% and 75% of the exchange capacity, and potassium at 8% and 2% of the exchange capacity. The anions were replenished at each harvest by adding nitrogen at 100 pounds and 20 pounds per acre as ammonium nitrate, phosphorus at 100 pounds and 20 pounds of P_2O_5 per acre, and sulfur at 50 pounds and 20 pounds per acre. Magnesium, manganese, iron, zinc, copper, cobalt, molybdenum, and boron were added initially to the clay in constant amounts and were not replenished.[6] Treatments were applied to randomly selected pots and were replicated 3 times.

All pots were planted January 22, and thinned to 50 plants per pot 2 weeks later and harvested 6 weeks later. All pots were harvested at successive 6 to 8 week intervals for a total of 4 harvests. Once each week the pots were randomly rearranged on the greenhouse bench in order to minimize variation. All pots were watered daily or as needed, with distilled water. When plants were harvested, they were placed between two large sheets of filter paper and immediately immersed in liquid air and dried by the "freeze dry" technique. The frozen material was "buried" in fresh 20-mesh "activated alumina" in a high-vacuum desiccator and the desiccator was evacuated in the cold for 1 hour to less than 0.05 mm. Hg. The desiccator was usually evacuated at least 1 more time within the next 6 hours and allowed to remain in the cold for 48 more hours. The dried samples were removed from the desiccator and ground in a micro (Wiley) mill equipped with a 60-mesh screen. The resulting powder was stored over activated alumina until analyzed.

Carbohydrates were determined by the arsenomolybdate method of Nelson (15) as modified by Somogyi (21) and further modified for plant tissue.[6] Nitrogen was determined by the standard Kjeldahl method of the A.O.A.C. (10).

Microbiological methods of analysis for amino acids have been shown to give acceptable quantitative results (7, 12, 18, 20, 22). All amino acids were determined on whole plant tissue which had been hydrolyzed with 10% hydrochloric acid or 4N sodium hydroxide. Arginine was determined according to the procedure of Stokes et al. (22) as further modified;[6] the microorganism used was *Streptococcus faecalis* ATCC #R9790.[7] Lysine and aspartic acid were assayed according to Henderson and Snell (7) and Snell (20) as further modified[6] using Leuconostoc mesenteroides P-60*.

[6] Reed, L. W. Biosynthesis in plants as influenced by the inorganic nutrient balance in the soil. Ph.D. Thesis, Univ. of Missouri, 1953.

[7] Microorganisms were obtained from the American Type Culture Collection, Georgetown University Medical School, Washington 6, D. C.

Table 1—Effect of high cations and variable anions on lysine and arginine contents of bromegrass (second harvest).

Treatment*			pH of growth media	Yield/pot, g. dry matter	% N	Lysine, mg/g.	Arginine, mg/g.	% of total N	
N	P	S						Lysine N	Arginine N
H	H	H	5.89	13.80	2.14	5.31	6.29	2.72	9.40
L	H	H	6.21	6.52	1.19	4.24	3.64	6.81	9.76
H	L	H	6.77	11.20	2.35	4.50	5.49	4.45	8.37
L	L	H	6.87	6.59	1.29	3.65	4.01	5.41	9.95
H	L	L	6.84	6.25	2.72	4.83	7.84	2.76	7.36
L	L	L	7.25	7.04	1.19	3.67	4.45	5.90	11.79
H	H	L	6.42	12.71	2.12	5.03	6.04	4.40	9.17
L	H	L	6.70	7.71	1.28	3.65	4.24	5.47	10.77

* H = high, L = low; Ca and K were high in all treatments.

Table 2—Effect of variable calcium and variable anions on lysine and arginine contents of bromegrass (second harvest).

Treatment*				pH of growth media	Yield/pot, g. dry matter	% N	Lysine, mg/g.	Arginine, mg/g.	% of total N	
Ca	N	P	S						Lysine N	Arginine N
H	H	H	L	6.00	8.09	3.13	16.07	8.47	9.97	3.12
L	H	H	L	4.70	6.91	3.53	6.95	10.45	4.22	9.66
H	H	L	L	6.29	8.74	3.16	12.23	6.67	7.41	8.03
L	H	L	L	4.75	3.23	4.27	6.18	10.34	3.59	9.88
H	H	L	H	6.00	5.92	3.60	11.91	8.11	6.41	7.22
L	H	L	H	4.51	1.00	3.44	5.48	13.22	3.26	11.03
H	H	H	H	5.50	10.25	2.87	12.32	8.00	8.84	8.55
L	H	H	H	4.50	9.05	3.11	6.90	9.70	4.26	10.11

* H = high, L = low; K was low in all treatments.

RESULTS AND DISCUSSION

In this investigation of the array of amino acids in bromegrass under a variable nutritional environment it became evident that lysine, arginine, and aspartic acid were in some way related to the balance of the basic elements in the soil. The operating mechanism or mechanisms were obscure, but in a general way the arginine and lysine levels seemed to be related to the concentration of calcium in the root medium.

It was shown that with cations high the quantity of lysine and arginine in plant tissue appeared to follow in a general way the percentage of nitrogen (table 1). It was found that the lysine concentration decreased approximately 22% while arginine decreased approximately 35.5% with a 46.9% decrease in nitrogen content between high and low nitrogen treatments. The pattern changed dramatically, however, when calcium was variable, potassium uniformly low, and nitrogen high (table 2). With nitrogen uniformly high and potassium low, a decrease in calcium treatment caused a 50% decrease in the amount of lysine, regardless of the sulfur or phosphorus levels. Arginine, however, showed exactly the opposite tendency with a 100% increase in arginine content with low calcium treatment. An even more interesting relation was noted between the lysine and arginine contents of each sample: that is, with potassium low and nitrogen high the lysine-arginine ratio was approximately 2:1 when calcium was high, while the reverse ratio, 1:2, was noted when calcium was low. Lysine nitrogen as percent of total nitrogen tended to increase from 30 to 100% when the low nitrogen treatment was compared with high nitrogen treatment with calcium and potassium high as shown in table 1. However, when nitrogen was uniformly high, lysine nitrogen as percent of the total nitrogen decreased more than 50% when the calcium level was low. Arginine nitrogen as percentage of the total nitrogen tended to remain more or less constant with change in the level of available basic elements. The arginine content,

Table 3—Effect of low cations and variable anions on lysine and arginine contents of bromegrass (second harvest).

Treatments*			pH of growth media	Yield in g. dry matter	% N	Lysine mg/g.	Arginine, mg/g.	% of total N	
N	P	S						Lysine N	Arginine N
H	L	L	4.75	7.30	4.27	6.18	10.34	3.59	9.88
L	L	L	4.81	5.76	1.91	4.77	5.07	4.78	8.51
H	L	H	4.51	4.45	3.44	5.48	13.22	3.26	11.03
L	L	H	4.66	4.68	2.39	5.43	6.23	4.37	9.45
H	H	H	4.50	12.10	3.11	6.90	9.70	4.26	10.11
L	H	H	4.45	4.32	2.17	5.41	7.21	4.77	10.64
H	H	L	4.70	9.40	3.53	6.95	10.45	4.22	9.66
L	H	L	4.70	6.49	2.17	4.90	6.54	4.39	9.66

* H = high, L = low; Ca and K were low in all treatments

however, with bases low showed approximately a 50% increase with high nitrogen treatment over low nitrogen treatment as shown in table 3. Similar observations have been made by other writers as reported by Mertz et al. (13). Their investigations have shown that sulfur-deficient alfalfa tends to accumulate nonsulfur amino acids. It was reported that arginine, an amino acid containing 34% nitrogen, and asparagine, an amide containing 21.2% nitrogen, tended to accumulate in sulfur-deficient plants with a concomitant decrease in all other amino acids. Sheldon[5] observed that Korean lespedeza grown on low-calcium soil tended to be higher in arginine than plants grown on calcium-sufficient soils, while the lysine content remained constant. Syrett and Fowden (23) for chlorella and Mertz et al. (13) for alfalfa also found very high accumulations of basic amino acids or amides.

Some explanation of these results may be offered by noting that arginine takes part in the ornithine-citrulline-arginine cycle. In the event of a low base supply and a high nitrogen supply, ammonia concentration in the plant may increase enough to be toxic to plant tissues. In this cycle, ornithine adds 1 molecule of carbon dioxide and 1 molecule of ammonia to become citrulline, then citrulline adds 1 molecule of ammonia to become arginine. It was shown that when bases were high, particularly calcium, and uptake of bases high less chance existed for ammonia uptake from ammonium nitrate and then arginine did not accumulate. Arginine probably did not accumulate for several other reasons as well. Bear (4) concluded that the total milliequavilents of cations and of anions in a plant tended to be constant. This conclusion was confirmed by Lundegardh. The investigation reported here shows that Bear's observations may be further interpreted by suggesting that organic bases may actually substitute for metallic cations in maintaining the proper electrical condition of the protoplasm, where acidic constituents were not assimilated. It has been shown (23) that lack of glucose and several elements, particularly manganese, could retard assimilation of basic amino acids into protein in chlorella and that sulfur (13) could impair assimilation in alfalfa. Other work (19) has shown calcium to be intimately associated with protein synthesis in the soybean. The data presented here indicate that calcium deficiency may impair nitrogen assimilation and basic amino acids, particularly arginine, may accumulate to quite high levels.

Other mechanisms, however, may be available to explain these results. Greenberg (5) reports that arginase, an arginine splitting enzyme, is most active in the neutral to basic pH range. In plant tissues with an abundant supply of bases, arginine could not accumulate because of the increased arginase activity while the lack of bases could retard arginase activity and result in accumulation of arginine.

Aspartic acid and asparagine have been reported to vary in plant tissues between very wide limits (12, 13, 16).

Table 4—Effect of variable inorganic nutrition of bromegrass on aspartic acid content (second harvest).

Treatments*					pH of growth media	Yield/pot, g. dry matter	% N	Aspartic acid	
Ca	K	N	P	S				mg/g.	% of total N
H	H	H	H	H	5.89	13.95	2.14	17.46	7.23
H	H	H	L	H	6.77	13.84	2.35	16.03	10.09
H	H	H	L	L	6.84	11.36	2.72	18.02	6.97
H	H	H	H	L	6.42	14.34	2.12	17.45	8.12
H	L	H	H	H	5.50	11.20	2.87	16.32	6.30
H	L	H	L	L	6.29	11.17	3.16	22.69	7.54
H	L	H	H	L	6.00	12.01	3.12	16.96	5.72
H	L	H	L	H	6.00	10.42	3.60	30.62	8.96
L	H	H	H	H	4.85	13.34	2.21	11.24	5.34
L	H	H	L	H	4.97	8.55	2.89	23.18	6.90
L	H	H	L	L	5.40	9.44	2.89	23.06	8.46
L	L	H	L	L	4.75	7.30	4.27	62.89	15.47
L	L	H	H	H	4.50	12.10	3.11	40.48	13.92
L	L	H	H	L	4.70	9.40	3.53	36.76	11.68
L	L	H	L	H	4.51	4.45	3.44	73.47	14.19

* H = high, L = low.

Table 5—Effect of variable inorganic nutrition of bromegrass on carbohydrate content (second harvest).

Treatments*					pH of growth media	Yield/pot, g. dry matter	Reducing sugar, %	Non-reducing sugar, %	Starch %	Hemicellulose, %
Ca	K	N	P	S						
H	L	H	L	L	6.38	5.69	4.60	17.50	0.51	9.93
H	L	L	L	L	6.29	11.19	1.95	19.30	0.50	8.93
H	L	H	H	H	5.50	11.20	5.11	15.56	0.16	8.67
H	L	L	H	H	5.29	4.57	2.30	16.00	0.51	9.78
L	H	H	L	L	5.40	9.44	3.80	16.50	0.50	8.62
L	H	L	L	L	5.47	5.62	1.20	15.90	0.81	9.70
L	H	H	H	L	5.20	12.88	7.00	18.05	0.74	-
L	H	L	H	L	5.36	5.28	1.97	19.30	0.85	9.40
H	L	H	H	L	6.00	12.01	5.70	16.50	0.01	8.40
H	L	L	H	L	6.10	5.71	3.87	15.50	trace	9.90

* H = high, L = low.

Mertz et al. (13) showed that aspartic acid and asparagine increased in sulfur-deficient alfalfa plants with a very large concomitant increase in total nitrogen. The data presented in table IV show substantial variations in the total aspartic acid content of bromegrass. With nitrogen high and bases high, the aspartic acid content did not increase appreciably. With bases low and nitrogen high, however, aspartic acid increased greatly. Virtanen (24) believed that aspartic acid was the key compound in transamination reactions. If this key metabolite accumulates in plant tissues in excess amounts when the basic elements, particularly calcium, are low, then it should be permissible to suggest that protein synthesis has been reduced in intensity or even stopped and that excess metabolite is allowed to accumulate. A common technique in enzymology is to add some metabolite in excess quantities or exclude some key metabolite at selected points in a chain of enzymatic reactions. By this technique metabolites leading up to a selected point often accumulate in excess quantities. It may be interpreted from these data that the withdrawal of calcium and potassium may, in effect, block some enzyme or enzymes functioning in amino acid or protein synthesis or that withdrawal hastens proteolysis. These data show that the simple agronomic practice of liming as suggested by Bear (4) may drastically change not only the relative concentration of the total amino acid or nitrogen in the plant, but may change the synthesis rate of protein as well. Mertz et al. (13) reported indications that the relative amounts of cytoplasmic protein from sulfur-deficient alfalfa plants vary from the normal plant protein, and that electrophoretic studies indicate possible changes in the cytoplasmic proteins.

The carbohydrate content of plants as it is influenced by the inorganic nutrition of the plant has not been investigated to any appreciable extent. In bromegrass considerable variation was noted between concentration of carbohydrate fractions in the plant and treatments as shown in table 5. It was apparent that high nitrogen treatments resulted in

considerable increase in reducing sugars. This observation does not imply that nitrogen is most important for carbohydrate synthesis, but to point out that all elements are required for carbohydrate synthesis and that nitrogen is particularly important. It is not justified, however, to view the mere accumulation of carbohydrates as an indication of balanced metabolism, but to propose that carbohydrates serve as the energy source for metabolic reactions and that the synthesis of nitrogen compounds (amino acids and protein) will require metabolizable carbohydrates. Gregory and Sen (6) showed that variations in connection with concentration of nitrogen, potassium, and phosphorus caused significant change in the protein metabolism of barley leaves. Reed[6] found that when large variations in the concentration of several carbohydrates in bromegrass, due to variable nutrient treatment, when large variations in the concentration of nitrogen compounds in the plant occurred. These data suggest that nitrogen, carbohydrate, and salt metabolism are all interrelated in the total metabolism of the plant.

SUMMARY

The studies reported here indicate that nitrogen fertilization of a soil will not guarantee constant percentages of amino acids in plants unless other elements required for plant growth are present in adequate amounts and balance to dispose of nitrogen by assimilation. These data also indicate that the assay of protein in a forage plant cannot be reliably made by percentage nitrogen data alone.

Agricultural scientists are aware of the fact that the soil has long been recognized as the base of agricultural production. In the elucidation of the chemodynamics of the soil-plant complex, insufficient information has been collected for even the most rudimentary integration. The facts revealed by this study showed that the inorganic elements play a decided but not well-understood role in amino acid and carbohydrate synthesis.

The concentration of any one constituent of the plant is not constant under a variable environment. This has long been recognized as regards the concentration of inorganic elements. Variation in organic constituents of the plant due to variation in the inorganic environment should be expected.

It was found that the concentration of several amino acids and carbohydrate fractions of bromegrass varied widely when grown in a variable root environment. Lysine and arginine concentrations in the plant appeared to be related to the concentration of calcium and potassium in the root medium. When calcium and potassium were high the lysine:arginine ratio was 2:1; however, low calcium and potassium resulted in a 1:2 ratio of lysine:arginine.

Aspartic acid, one of the key compounds in nitrogen metabolism of plants, varied between wide limits in bromegrass. Total nitrogen is an unsatisfactory criterion for assaying protein quality. Samples very high in nitrogen did not vary appreciably in essential amino acids when compared to lower nitrogen plants. The difference in nitrogen content was due to aspartic acid and/or asparagine.

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