N-CARRIERS, LIGHT AND TEMPERATURE INFLUENCES ON THE FREE AMINO ACID POOL COMPOSITION OF RICE PLANTS[1] /

*MANLIO S FERNANDES**

Resumo

Foi estudada a composição do "pool" de amino ácidos livres em plantas de arroz, recebendo NO_3^- ou NH_4^+, sob duas combinações de luz e temperatura. A composição em amino ácidos variou em função do teor usado, e da fonte de N. A relação N-amino/N-amido foi menor nas plantas sob NH_4^+ do que nas sob NO_3^-. As plantas sob *NO_3^- mostraram uma redução na relação N-amino/N-amido com o aumento da temperatura. O "pool" total foi sempre maior nas plantas sob NH_4^+ do que nas sob NO_3^-. Sob condições de baixa luz e alta temperatura as plantas sob NH_4^+ mostram o maior acúmulo de amino-N livre, tendo as amidas formado 80% do total. Sob essas condições o peso fresco das plantas foi também drasticamente reduzido, sugerindo uma grande mobilização de esqueletos de carbono para a assimilação do N-NH_4^+ em ligações amino e amido. O significado destas diferenças bioquímicas é discutido.*

Introduction

The factors that are known to affect the amino acid composition of plants are numerous. Among them: the nitrogen source applied to the plant, the level of application of that source and the effect of light and temperature (1, 9, 10). Weismann (10) demonstrated that NH_4^+-fed plants accumulate the highest amount of amides while NO_3^--fed plants accumulate the minimum and that plant supplied with NH_4^+ had more asparagine than glutamine whereas NO_3^- supplied plants had more glutamine that asparagine. In a later work Thenabadu (9) found darkness and heavy nitrogen applications to influence the asparagine content.

The majority of the research dealing with determinations of the N-status of rice plants (7, 8) was done without taking into consideration the energy levels in the environment where the plant grew (4).

The purpose of this investigation was to study the effect of adding two concentrations of NH_4^+ or NO_3^- to on free amino acid composition of rice plants, subjected to different light-temperature regimes.

Two combinations of light and temperature that are expected to occur throughout the growing season in the humid tropics (2) were chosen to study their effect on amino acid composition with added N-carriers and suggest how compositional features of the amino acid pool might be used to evaluate the N-status of rice plants when the energy fluxes in the environment are known.

Materials and methods

Nutrient solutions

A solution containing 3.0 me l^{-1} KH_2PO_4, 1.0 me l^{-1} $CaCl_2$, 6.0 me l^{-1} $MgSO_4$ with a full complement of micronutrients added as 1.0 ml l^{-1} of a solution of 2.86 gl^{-1} H_3BO_3, 1.81 gl^{-1} $MnCl_2.4H_2O$, 0.22 gl^{-1} $ZnSO_4.7H_2O$, 0.08 gl^{-1} $CuSO_4.5H_2O$ and 0.02 gl^{-1} H_2MoO_4 H_2O was used as the basal solution in all treatments. Iron was supplied as Fe-citrate (5 ml from a 1 000 ppm Fe solution).

---

[1] Received for publication on August, 1982.
Contribution from the Department of Soil Science, Universidade Federal Rural do Rio de Janeiro, Km 47 Ant. Rio São Paulo, Seropédica – 23 460 – Rio de Janeiro – RJ – BRASIL

* Associate Professor of Soil Science.

Two different nitrogen sources at two levels of concentration were added to the basal solution: $Ca(NO_3)$ at 20 and 150 ppm N and $(NH_4)_2SO_4$ at 20 and 150 ppm N. The pH of NO_3 solutions was adjusted to 5.5 with dilute HCl. Finely ground $CaCO_3$ was used to avoid sharp drops in the pH of NH_4 solutions (5) keeping the pH around 6.0. Nutrient solutions were renewed every three days.

Growth chamber

The N-supplied plants were submitted to two selected combinations of light and temperature (low light-high temperature and low light-low temperature) in a Sherer programable growth chamber model 511-38.

For the low light-high temperature treatment growth chamber conditions were set at: 35C, 17 280 lux from incandescent and fluorescent lamps and 12 hr photoperiod.

Settings for the low light-low temperature 12 hr photoperiod were: 17 280 lux and 24C.

Cultural methods

Seeds of rice (IR-8) were germinated in deionized water, planted in vermiculite and grown for two weeks in an environmental chamber. Chamber conditions for the 12 hour photoperiod were as follows: temperature 28°C, light 8 640 lux. The plants were then transferred to solution culture containers in a growth chamber to receive their treatments.

A total of twenty-four 2-liter polyethylene nutrient solution containers were used in this experiment. Twelve containers were used per each light-temperature treatment and three for each level of N-source. Each container supported four plants held in position by holes in the lids with flexible foam material. Both containers and lids were coated with aluminum foil.

Analytical procedures

Harvesting. Harvesting was performed 10 days after the treatments started. Individual plants were removed from each culture vessel and separated into roots and shoots. Plant tops were weighed and used for further analysis.

Amino-N extraction. The alcohol extraction was performed with a Vir-Tis 45 homogenizer. One gram of plant tissue was homogenized with 20 ml of 80% ethanol. The homogenate was passed through four layers of cheese cloth and the residual cake re-extracted. Combined extracts were then filtered through Whatman number one filter paper. Pigments and proteins remaining in solution were extracted by partition with chloroform and water. The extract was brought to a 50-ml volume with 80% ethanol and stored at -5°C until analysed.

Quantitative amino acid analysis The plant extracts (80% ethanol) were evaporated to dryness with a rotatory evaporator under vacuum and temperatures below 40°C. The residue was resuspended in lithium-citrate buffer, pH 1.9. Amino acids were then determined quantitatively by passing 0.5μliter of sample through an automatic amino acid analyser. Acidic and neutral amino acids were separated with lithium-citrate buffers as outlined by Bergen, Henneman and Magee (3). The peak areas for the amino acids were calculated by multiplying the height of peak by the width at half of the height. Besides the relative quantities of amino acids in the free pool, ratios of amino acid nitrogen (total amino acid content) to amide nitrogen (asparagine + glutamine) were selected for diagnostic use in rice plants.

Results and discussion

Fresh weight

At low light-low temperature, values for fresh matter production were higher than those obtained at low light-high temperature and were affected only slightly by nitrogen concentration (Table 1).

Fresh weight values in plants under low light-high temperature were low and remained relatively constant with increasing NH_4^+ dosage whereas the values decreased as NO_3^- concentration was increased.

The lower values at low light-high temperatures of plants fed NH_4^+ indicates that this nitrogen source together with higher temperature does place a greater stress on plant growth, while favorable growing conditions result from the same temperature when plants were fed 20 ppm NO_3^-.

Amino acid composition of rice plants fed NO_3^-

Aspartic acid, serine, glutamic acid, alanine, cysteine and the amides aparagine and glutamine were the major amino acid constituents in the free pool. The relationship of these amino acids and amides to the concentration of applied NO_3^- were as shown in Table 3. The free amino acid pool size of plants under low light-high temperature increased with increasing NO_3^- concentration. Large differences in the relative proportions represented by the amides and non-

Table 1. Fresh weight of rice plants (IR-8) grown under two combinations of light and temperature: 1) low light-high temperature (LL-HT) and 2) low light-low temperature, (LL-LT), and supplied with NO_3^--N or NH_4^+-N at two levels.

Treatments		Light and temperature combinations	
N-carrier	N-levels	LL-HT	LL-LT
		g/4 plants	
NO_3	20	2.18	1.87
	150	0.99	1.61
NH_4	20	0.72	1.67
	150	0.51	1.77
LSD (0.05)		0.26	
Treatment averages		1.10	1.69*

* Differences significant at 0.05

amides were observed. The relative levels of cysteine, aspartic and glutamic acids decreased while the levels of asparagine and glutamine increased with increasing NO_3^- concentration. The level of both serine and alanine did not change over the concentration range tested. The amino-N/amide-N ratio was 5.17 in plants fed 20 ppm NO_3^- and 2.06 for plants supplied 150 ppm.

Plants under low light-low temperature had a different response to NO_3^- concentration. The amino acid pool size was higher for the 20 ppm concentration and the levels of serine, glutamic acid and aspartic acid increased with increasing concentration. The amino/amide ratio was 1.31 for 20 ppm NO_3 plants and 2.05 for 150 ppm.

Apparently, the effect observed upon fresh weight in plants fed NO_3^- at low light-high temperature (Table 1), was not due to a change in the amino acid composition. Since there was no accumulation of free amino-N in the tissues of these plants the low values for fresh weight of the plants fed 150 ppm NO_3^- might be due to a lack of carbon skeletons in the low light supply, while the higher temperature promotes an increase in the uptake of NO_3^-. Fernandes (4) has shown that the lower assimilation of NO_3^- may be due to its sequestration into the substrate pool without any increase in the NR level or activity. Also, higher temperature affects the uptake of NO_3^- and restricts the availability of carbon skeletons; therefore, the effect of NR in this process must be a secondary one.

Stress conditions resulting from higher NO_3^- concentration and higher temperatures seemed to promote an increase in the amide content of the free pool, while favorable growing conditions were associated with a more balanced distribution (higher amino/amide ratios). We should note however, that

Table 2. Free amino acids in rice plants (IR-8) grown under low light-high temperature and low light-low temperature conditions, with N supplied as nitrate or ammonium.

Amino Acid	LL-HT								LL-LT							
	Nitrogen supplied (ppm)								Nitrogen supplied (ppm)							
	NO_3^-				NH_4^-				NO_3^-				NH_4^-			
	20		150		20		150		20		150		20		150	
	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%	µMoles	%
Asp	1.34	10.5	0.92	5.1	1.70	1.4	3.89	2.3	0.55	3.3	0.79	6.3	0.58	2.7	0.47	1.3
Thre	0.34	2.6	0.62	3.5	1.76	1.4	2.74	1.6	0.51	3.1	0.20	1.6	0.42	2.0	0.58	1.6
Ser	1.75	13.6	2.62	14.6	2.99	2.4	2.95	1.7	0.99	6.0	1.08	8.6	0.89	4.2	0.96	2.6
Asp. NH_2	0.51	3.9	2.00	11.2	33.31	26.7	21.65	12.5	0.92	5.6	0.41	3.3	5.23	24.4	8.17	22.2
Glut	3.21	25.1	2.92	16.3	6.9	5.5	8.66	5.0	3.80	23.0	3.37	26.9	2.58	12.0	3.57	9.7
Glut NH_2	1.57	12.3	3.85	21.5	68.14	54.7	122.02	70.5	6.21	37.6	3.69	29.4	9.01	42.0	19.11	51.9
Pro.	nd	nd	1.33	7.4	1.69	1.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Gly	0.20	1.7	0.22	1.2	0.85	0.7	nd	nd	0.27	1.6	0.19	1.5	0.25	1.2	0.38	1.0
Ala.	1.71	13.4	1.91	10.7	3.96	3.2	3.94	2.3	2.37	14.5	1.94	15.5	1.73	8.0	2.44	6.6
Cys	1.75	13.8	0.66	3.7	1.73	1.4	3.64	2.1	0.46	2.8	0.40	3.2	0.39	1.8	0.57	1.5
Ile	0.07	0.5	0.20	1.1	0.41	0.3	0.98	0.6	0.09	0.6	0.08	0.6	0.08	0.4	0.16	0.4
Leu.	0.10	0.8	0.26	1.5	0.40	0.3	1.03	0.6	0.13	0.8	0.15	1.2	0.10	0.5	0.19	0.5
Tyr	0.13	1.0	0.15	0.8	0.23	0.2	0.33	0.2	0.10	0.6	0.12	1.0	0.08	0.4	0.14	0.4
Phen.	0.12	0.9	0.27	1.5	0.42	0.3	1.17	0.7	0.10	0.6	0.12	1.0	0.10	0.5	0.10	0.3
Total	12.80	100	17.93	100	124.50	100	173.00	100	16.50	100	12.54	100	21.45	100	36.84	100
Amino/ amide	5.17		2.06		0.23		0.20		1.31		2.05		0.51		0.35	

nd = none detected

the contents of alanine and serine, making up a significant amount of the pool, did not show interactions with NO_3^- concentration. It is interesting to note that when the amino acid pool has a more favorable composition (amino/amide) it has a smaller content of total free amino-N indicating a better utilization of the amino acids by plants.

Amino acid composition of rice plants fed NH_4

At low light-high temperature an increase in the pool size of free amino acids with increasing NH_4^+ concentration was observed. The levels of asparagine, glutamate, alanine and serine tend to decrease while the level of glutamine increased as the NH_4^+ concentration was increased. Cysteine was only present at high amounts at 150 ppm whereas alanine was present at high amounts at both concentrations. The amino/amide ratio was 0.23 for 20 ppm NH_4^+-N and 0.20 for 150 ppm.

Under low light-low temperature NH_4^+-fed plants had a more balanced distribution of amino acids and amides than plants under the previous condition, although the levels of asparagine and glutamine, making the major fraction of the pool, increased with increasing concentration, while the level of glutamate decreased. The amino/amide ratio was 0.51 for the 20 ppm plants and 0.35 for the 150 ppm NH_4^+-fed plants.

Oji and Izawa (6) suggest that glutamine synthesis is a primary reaction of NH_3 assimilation in plants. The presence of glutamine as the amino compound found in NH_4^+ plants supports this contention. The higher levels of glutamine in plants exposed to higher temperatures and higher NH_4^+ concentration suggest that this might be an adaptive plant feature which accumulates glutamine in order to mantain the proper levels of free-NH_3 in the tissues.

As in NO_3^--fed plants at low light-high temperature, NH_4^+-fed plants demonstrated an increase in the amide content promoted by stressing conditions resulting from higher nitrogen concentrations and higher temperatures, while under lower temperatures and lower nitrogen concentrations plants had a more balanced distribution of the amino acids. The imbalance in the amino/amide ratio promoted by the accumulation of amides that become the dominant fraction of the pool could be attributed to a low supply of carbon skeletons plus higher N-uptake as a result of unfavorable light and temperature combinations. Both the total free content of amino acids and the accumulation of amides were higher in NH_4^+ plants than in NO_3^-. The lowest amino/amide ratio (0.20) obtained when higher NH_4^+ concentration and higher temperatures were used is an indication that this nitrogen source, at unfavorable conditions, does place the greater stress on plant growth.

Conclusions

Experiments on the effect of two concentrations of nitrogen carriers on the amino acid composition of rice plants subjected to two combinations of light and temperature, provide evidence that the amino-N to amide-N ratio varied predictably with N-carriers (N-NH_4^+ x N-NO_3^-) and levels (20 x 150 ppm).

An imbalance in the amino/amide ratio promoted by the accumulation of amides that become the dominant fraction of the pool, was probably due to unfavorable light and temperature combinations, leading to lower supply of carbon skeletons plus higher N-uptake. Under lower temperatures and lower nitrogen concentrations plants showed a better balance in the distribution of amino acids in the free pool.

These results suggest that the composition of the free amino acid pool of rice plants is a sensitive indicator of stressing conditions on plant growth and might be used to evaluate the N-status and general health of plants.

Summary

The composition of the amino acid pool of rice plants receiving either NO_3^- or NH_4^+ at two levels, under two combinations of light and temperature expected to occur in the humid tropics was examined.

Amino acid composition of N-supplied plants varied predictably with the relative extents to which the nitrogen source was used and with growing conditions. The ratios of amino nitrogen to amide nitrogen in the free pool decreased with increasing levels of NO_3^- and with higher temperatures. All plants showed higher proportions of amino acids and amino acids plus amides when relying on NH_4^+ than when utilizing NO_3^-. NH_4^+-fed plants under low light and high temperature, had the highest amount of total free amino acids of which amides made up 80% of the total pool. The biochemical significances of the above differences in the amino acid composition was discussed.

Literature cited

1 BEKMUKHAMEDOVA, N. B. Synthetic activity of the root system of corn during ammonia

and nitrate nutrition. Fiziologiya Rastenii 8:51-54. 1961.

2. BASTOS, T. X. e SA, T. D. A. Boletim Agrometeorológico. Brasil, IPEAN, Ministério da Agricultura, 1971. 45 p.

3. BERGEN, W. G., HENNEMAN, H. A. e MAGEE, W. T. Effect of dietary protein level and protein source on plasma and tissue free amino acids of growing sheep. Journal of Nutrition 103:575-585. 1973.

4. FERNANDES, M. S. Effects of light and temperature on the nitrogen metabolism of tropical rice. Ph.D. Thesis. Michigan State University, 1974. 80 p.

5. KARIM, A. Q. M. B. e VLAMIS, I. Comparative study of the effects of ammonium and nitrate nitrogen in the nutrition of rice. Plant and Soil 16:32-41. 1962.

6. OJI, Y. e IZAWA, G. Quantitative changes of free amino acids and amides in barley plants during ammonia and nitrate and assimilation. Plant and Cell Physiology 13:249-259. 1972.

7. ORITANI, T. e YOSHIDA, R. Studies on N-metabolism in crop plants. VII. The nitrogenous compounds in the bleeding sap and various organs of the crop plants. Proceedings of the Crop Science Society of Japan 39:355-362. 1970.

8. OZAKI, K. Determination of top dressing for rice in the field by the detection of asparagine. Soil and Plant Food 1:88-90. 1955.

9. THENABADU, M. W. Evaluation of the nitrogen nutrition status of the rice plant by plant analysis. Plant and Soil 37:41-48. 1972.

10. WEISSMAN, G. S. Influence of ammonium and nitrate on the protein and free amino acids in shoots of wheat seedlings. American Journal of Botany 46:339-346. 1959.

Notas y comentarios

Rodrigo Gámez, Premio Bernardo Houssay 1983

Rodrigo Gámez, científico costarricense, actualmente Director del Centro de Investigaciones en Biología Celular y Molecular de la Universidad de Costa Rica, fue seleccionado para recibir el premio "Bernardo Houssay", otorgado por la Organización de Estados Americanos (OEA), a comienzos de agosto de 1983, por sus investigaciones sobre enfermedades viróticas de cultivos alimenticios importantes para la América Central, sus agentes causantes (entre los que descubrió nuevos tipos de virus), sus insectos transmisores (entre los que halló algunos de familias no señaladas anteriormente como propagadores de la enfermedad).

El Dr. Gámez realizó sus estudios en la Facultad de Agronomía de la Universidad de Costa Rica, los que continuó con estudios de posgrado en las Universidades de Florida e Illinois. En el intervalo entre estas dos universidades de los Estados Unidos, el British Council le otorgó una beca de dos años para recibir entrenamiento y realizar investigaciones, en la Estación Experimental de Rothamsted, Inglaterra, sobre la transmisión de virus por insectos.

En su discurso en la ceremonia de entrega del premio, durante las sesiones que el Consejo Interamericano para la Educación, Ciencias y Cultura (CIECC) realizó este año en Kingston, Jamaica, el Dr. Gámez destacó que, poco tiempo después de regresar a Costa Rica, después de sus estudios en Inglaterra y Estados Unidos, fue invitado por el Programa Centroamericano de Mejoramiento de Cultivos y Alimenticios (PCMCA), que funcionaba en el IICA, Turrialba, a trabajar en dos de los cultivos más autóctonos de la región, el maíz y el frijol. Expresó, en este discurso, que América Central no sólo brindó la materia prima para sus investigaciones, sino una motivación social para contribuir a elevar la calidad de la vida del pueblo. En este ambiente tropical, halló cosas como virus de características hasta ese momento desconocidas; mecanismos pecualiares de transmisión; reacción de la planta a la infección, y, un hallazgo inesperado, como el que los cultivos asociados, que realiza el campesino centroamericano desde hace mucho tiempo, constituyen un mecanismo valioso para el combate de las enfermedades viróticas.

Para **Turrialba**, es particularmente satisfactorio resaltar el desarrollo de la labor del Dr. Gámez. En estas páginas aparecieron, a partir de 1968, algunas de sus primeras contribuciones originales al conocimiento de los virus del maíz y del frijol en América Latina, colaboración que nos es muy grato reconocer, ha continuado hasta la fecha. Otra actividad iniciada por Rodrigo Gámez fue la de investigaciones en equipo con científicos separados por largas distancias, algo que en América Latina era casi desconocido. Aquí también **Turrialba**, como órgano de la comunidad latinoamericana de científicos en ciencias agronómicas, fue escogida para la publicación de algunos de los primeros resultados de esta cooperación. Así, ya en 1974, publicamos el resultado de estudios ultraestructurales de tejidos de hojas de frijol infectados por dos "strains" del virus del mosaico rugoso del frijol, realizados desde Brasil, Costa Rica y Colombia, por Gámez, Kitajima, Gálvez y Tascon. Esta labor conjunta de virólogos latinoamericanos, iniciada por el Dr. Gámez, ha continuado, incorporándose hasta ahora especialistas de Venezuela, México, Perú y Panamá, creándose así una red, verdadera y funcional, de la que el Dr. Gámez se muestra muy satisfecho, como lo destacó en su discurso en Kingston.

El premio Houssay fue establecido por el CIECC en su tercera reunión, celebrada en Panamá en 1972, y honra la memoria del científico argentino, Dr. Bernardo Houssay, el primer latinoamericano que ganó un Premio Nobel en ciencias. Es otorgado cada año en ciencias biológicas, exactas o agrícolas, y en investigación técnica de importancia para el desarrollo. Está limitado a personas de América Latina. Adalberto Gorbitz.