

N-CARRIERS, LIGHT AND TEMPERATURE INFLUENCES ON UPTAKE AND ASSIMILATION OF NITROGEN BY RICE SEEDLINGS¹ /

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Resumo

Foi estudada a absorção e o metabolismo de nitrogênio com arroz (Oriza sativa L.) submetido a diversas combinações de luz, temperatura, formas e níveis de nitrogênio (N).

O experimento foi feito em câmaras de crescimento e em solução nutritiva. Foram determinados o peso fresco, amino-N livre, o $N-NO_3^-$ e $N-NH_4^+$, açúcares não estruturais, e N-Kjeldahl do resíduo após extração alcoólica.

A variedade IR-8 usou N mais eficientemente que Come-Cru (C-C). Nitrato foi melhor fonte de N para plantas sob condições ambientais desfavoráveis do que NH_4^+ . Sob condições desfavoráveis, plantas sob NO_3^- parecem exercer controle metabólico sob os níveis internos de N-reduzido, através do deslocamento de NO_3^- do "pool" indutor para o "pool" substrato, enquanto que, em plantas sob NH_4^+ , ocorre um bloqueio do excesso de NH_4^+ sob a forma de amidas.

Os resultados mostram que pode ocorrer acúmulo de excesso de NO_3^- em plantas devido a fatores externos, como fluxos de energia e disponibilidade de N, enquanto que a redução e assimilação são regulados por fluxos internos de energia, relacionados com processos de crescimento. Estas observações são importantes do ponto de vista de manejo de fertilizantes e seleção de variedades, em áreas onde ocorrem fluxos estacionais de NO_3^- no solo.

Introduction

Tropical areas of South America, where upland rice (*Oryza sativa* L.) is grown, have a rainy growing season and a dry season each year. Along the equator in northeastern Brazil, rainfall during the growing season averages 1 950 mm, and the average temperature (26°C) is favorable for rice. Radiation is frequently limiting (seasonal average: 0.5 cal/cm² min), but periods of high light intensity

do occur (1.43 cal/cm² min). Periods of high light intensity are usually accompanied by unfavorably high (34°C) temperatures (1).

At the beginning of the rainy season, tropical soils are characteristically high in nitrate (10). Concentrations ranging up to 200 ppm NO_3^- -N or more are frequently reported. The seasonal "nitrate flush" has been attributed, in specific situations, to upward capillary migration during the dry season (22) and to resurgent microbial activity associated with the first rains after a drying cycle (13). With continuing rains, NO_3^- is leached into the profile, so that NO_3^- in the root zone is low throughout much of the growing season.

The fact that adapted varieties do grow and are moderately productive in the "wet-dry" tropics under conditions of stress caused by unfavorable combinations of light, temperature and N supply suggests that mechanisms exist which enable rice

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plants to take up, and store for later use, the NO_3^- available to them as seedlings during the "nitrate flush".

Rice can take up N either as NH_4^+ or as NO_3^- (8). In the presence of NO_3^- , nitrate reductase activity (NRA) is induced (21). The induction of NRA in rice seedlings has been observed as early as 24 hours after germination (18).

In other plant species, it has been shown that nitrate reductase activity (NRA) is sustained by continual *de novo* synthesis of the enzyme (since it is rapidly degraded) (24). NRA is sensitive to light intensity and temperature, through their effects on photosynthesis, respiratory balance and uptake of NO_3^- (2, 9).

Heimer and Filner (11) used tungstate to create a non-functional nitrate reductase in cultured tobacco cells and were able to separate the NO_3^- uptake system from the nitrate reducing system. Nitrate reductase activity was reduced when NO_3^- was removed from the external medium, even when the level of NO_3^- within the cells was high. The authors proposed the existence of two NO_3^- pools: (i) a short-lived inducing pool and (ii) a long-lived substrate pool. The cells could use the substrate pool for growth but not to replenish the inducing pool.

Glutamine and glutamate are the initial intermediates along the route through which reduced inorganic N is assimilated (15) involving the use of enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT). It should be pointed out also, that glutamate dehydrogenase (GDH) was shown to be induced in rice roots fed NH_4^+ , but not in those fed NO_3^- (12). Both routes (GS/GOGAT, GDH) may be involved in the assimilation of mineral N, the second, most probably, when NH_4^+ levels in the root environment increase (6).

The amination of glutamate or aspartate may serve also to detoxify excess NH_4^+ by converting it into the corresponding amides, which can accumulate without detriment to the cell (14). A similar protective role was proposed for nitrate reductase in NO_3^- -fed plants (2).

An understanding of the metabolic responses of rice to energy fluxes and nitrogen supply can be useful in breeding and selection for varietal improvement as well as in determining a fertilizer source of N and method of application. Pertinent findings from a nutrient culture study are reported here.

Materials and methods

Light and temperature combinations

Three combinations of light and temperature were used, when N was applied as NH_4^+ or as NO_3^- at three concentrations. Table 1 shows the levels of light, temperature and N used, as well as the light-temperature combinations. These light-temperature combinations are the most probable to be found during the growing season in the humid tropics.

Varieties

Two rice varieties were used: Come-Cru (C-C) a variety from the northeastern areas of Brazil (5°S , 45°W), grown in rather poor soils without fertilizers, and resistant to lodging and shedding. It represents the type of the "old", tropical varieties. IR-8, the second variety, is short statured and of the "new" type, responsive to N-fertilizers (20).

Plants were grown in controlled environment chambers (Sherer, 511-38 Model). Rice seeds were germinated in distilled water, transferred to 2-liter polyethylene containers filled with vermiculite, and grown under 8 640 lux with 12 hour photoperiods at 28°C . At this stage, seedlings received as basic nutrient solution: KH_2PO_4 , 3 meq/l; CaCl_2 , 1 meq/l; MgSO_4 , 6 meq/l; H_3BO_3 , 2.86 g/l; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 1.81 g/l; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.22 g/l; CuSO_4 .

Table 1. Levels of light, temperature, and nitrogen supply used in this investigation.

1. Light levels	a. high light supply (HL) – 40 000 Lux
	b. low light supply (LL) – 17 280 Lux
2. Ambient temperature levels	a. high temperature (HT) – 35°C
	b. low temperature (LT) – 24°C
3. Light and temperature combinations	a. high light – high temperature (HL-HT)
	b. low light – high temperature (LL-HT)
	c. low light – low temperature (LL-LT)
4. Nitrogen levels in the nutrient solutions	a. ammonium nitrogen (NH_4^+)
	5 ppm, 20 ppm, and 150 ppm N as ammonium sulfate
	b. nitrate nitrogen (NO_3^-)
	5 ppm, 20 ppm, and 150 ppm N as calcium nitrate

5H₂O, 0.08 g/l; H₂MoO₄ · H₂O, 0.02 g/l. Nitrogen was applied at 20 ppm as NH₄NO₃. Iron citrate was added (5 ml/l) from a 1 000 ppm Fe stock solution.

After two weeks, plants were removed from the vermiculite, washed, and placed in the experimental nutrient solutions with NH₄⁺ or NO₃⁻ at the levels shown in Table 1. Five rice seedlings were held in the holes of the lids of 2-liter polyethylene containers. To avoid heating and light penetration, the containers and lids were covered with aluminum foil. Finely ground calcium carbonate was added to the NH₄⁺ solution to avoid sharp drops in pH (13). The pH of the NH₄⁺ nutrient solution was adjusted to 6.0, and that of NO₃⁻ solution adjusted to 5.5. The nutrient solutions were changed every three days.

After a 10-day experimental period, the plants were harvested, between 5 and 6 p.m. Roots and shoots were weighed. One gram or less of plant tissue (shoots) was homogenized with 20 ml of 80% ethanol. The homogenate was passed through four layers of cheesecloth and then reextracted. Combined extracts were filtered through Whatman no 1 filter paper by gravity flow. Pigments and proteins remaining in solution were extracted by partition with chloroform and water. The extract volume was adjusted to 50 ml with ethanol and stored at -5°C until further analysis.

Free amino-N was determined colorimetrically by the method of Moore and Stein (16). Ammonium and NO₃⁻-N were determined in 5 ml of the extract by the fractional distillation methods of Bremner and Keeney (4). Soluble sugars were determined by the anthrone method of Yemm and Willis (23). The data for varieties, N sources and N levels for the three combinations of light and temperature were submitted to statistical analysis in accordance with a completely random factorial design, with three replications.

Results and discussion

Ammonium and nitrate were used with about equal effectiveness by both varieties under the low stress conditions of LL-HT (Table 2). The effectiveness of NH₄⁺ was sharply reduced by high temperature. Utilization of NO₃⁻ was unaffected, except at high concentration (150 ppm NO₃⁻-N) and under conditions of unfavourable balance associated with limiting light at high temperature (LL-HT). Apparently, even low levels of NH₄⁺ in the root environment can be toxic to rice under unfavourable combinations of light and temperature. High levels of NO₃⁻ were needed to hinder growth of plants under

similar stress. When under the stress condition of LL-HT, IR-8 used NO₃⁻-N better than C-C at the two lowest levels (5, 20 ppm), but both varieties behaved similarly in regard to NH₄⁺-N use. Under conditions of low stress, IR-8 behaved better than C-C at 20 and 150 ppm N when NH₄⁺ was used, while both varieties used NO₃⁻ equally well. Overall, IR-8 was more able to use N than C-C. NO₃⁻ was shown to be a better source of N for rice plants than NH₄⁺, under a range of stress conditions. The optimum level of N for both varieties and each carrier was 20 ppm, as has already been observed for rice in nutrient culture (20, 17).

Nitrate in tissues

Nitrate was encountered only in tissues of plants fed NO₃⁻. Accumulation in tissues increased with the level of input (Table 3), and was greatest under conditions of limiting light at high temperature (LL-HT). Nitrate accumulation in the two varieties was similar, except for C-C at the highest input (150 ppm), under LL-HT. The high NO₃⁻ content at the 150 ppm level of input coincided with the lowest fresh weight (Table 2).

Free Ammonium and Amino-N

Free NH₄⁺ reached a high concentration only in plants fed NH₄⁺ at high temperature, with the highest level of input (Table 4). Growth of these plants was severely curtailed (Table 2). Unbalanced uptake of other nutrients may have been involved (5); however, the severity of growth impairment suggests specific toxic effects of NH₄⁺ itself.

Higher levels of free amino-N accumulated in NH₄⁺-fed seedlings. Very large accumulations were associated with high levels of NH₄⁺ input (150 ppm) and high temperatures (Table 5). The two varieties behaved differently. IR-8 accumulated higher levels of amino-N at the 20 and 150 ppm input levels of NH₄⁺ at the HL-HT condition and also had higher amino-N levels at 5 and 20 ppm levels of input under the higher stress condition (LL-HT). The higher levels of amino-N accumulation in NH₄⁺-fed IR-8 than C-C plants under higher stress (LL-HT), without significant difference in growth rates (Tables 2, 4 and 5), indicate fundamental differences in nitrogen metabolism. IR-8 appears to be more tolerant to potentially toxic concentrations of NH₄⁺.

In NO₃⁻-fed plants, reduced N did not accumulate either as free NH₄⁺ or as free amino-N, despite substantial accumulations of NO₃⁻ at higher levels of input (Table 3). More NO₃⁻ appears to have been taken up at the higher temperature, as has been sug-

Table 2. Fresh weight of two rice varieties as related to three light and temperature combinations, two N-carriers and three levels of application.

Light-Temp. combination	Carrier	Level	Varieties		Mean (LT)
			IR-8	C-C	
			g/four plants		
HL-HT	NO ₃ ⁻	5	1.63	1.35	1.16
		20	1.63	1.42	
		150	1.21	1.15	
	NH ₄ ⁺	5	0.74	0.67	
		20	1.48	1.52	
		150	0.70	0.38	
LL-HT	NO ₃ ⁻	5	1.91	1.42	1.07
		20	2.18	1.40	
		150	0.99	0.82	
	NH ₄ ⁺	5	0.75	0.81	
		20	0.72	0.77	
		150	0.51	0.62	
LL-LT	NO ₃ ⁻	5	1.41	1.24	1.49
		20	1.87	1.76	
		150	1.61	1.39	
	NH ₄ ⁺	5	1.28	1.15	
		20	1.67	1.35	
		150	1.77	1.39	
LSD _(0.05)			0.28		0.08
Mean (Varieties)			1.34*	1.15	
Mean (Carriers)			NO ₃ ⁻ 1.47*	NH ₄ ⁺ 1.01	

* Differences significant at P (0.05)

The following interactions were significant: LT x Carrier, LT x Carrier X Variety, LT x Level, Carrier x Level, Lt x Carrier x Level, Variety x Carrier.

gested (2), but accumulated NO₃⁻ did not induce greater N-reducing activity than was needed to support assimilation and growth. This result is consistent with the concept of two discrete nitrate pools (7).

At the lowest level of stress (LL-LT), rice seedlings fed NH₄⁺ behaved almost as well as rice seedlings fed NO₃⁻, as far as amino-N accumulation is concerned (Table 2). The same is true for the growth patterns.

A negative correlation was found ($r = 0.96^{**}$) between amino-N levels and fresh weight of seedlings of both varieties when fed NH₄⁺. No significant correlations were found between fresh weight and either NO₃⁻ or amino-N levels of NO₃⁻-fed seedlings.

Kjeldahl-N

Residual Kjeldahl-N, after alcohol extraction, may be considered to represent N assimilated into proteins.

Ammonium-fed IR-8 seedlings accumulated more Kjeldahl-N than C-C plants at the 20 ppm input level, under all three light and temperature combinations (Table 6). When both light and temperature were high (HL-HT), IR-8 assimilated NH₄⁺ more efficiently than C-C at all levels of input. This result also points to differences in N-metabolism between the two varieties as stated above. Rice seedlings at the lowest apparent stress (LL-LT) had lower N-content, a contrast with fresh weight data (Table 2)

Table 3. Nitrate accumulation in two rice varieties as related to three light and temperature combinations, two N-carriers and three rates of application.

Light-Temp. combination	Carrier	Level	Varieties		Mean (L.T)
			IR-8	C-C	
			$\mu\text{moles/g fresh weight}$		
HL-HT	NO ₃ ⁻	5	5.6	15.1	16.2
		20	19.9	15.3	
		150	77.0	60.9	
LL-HT	NO ₃ ⁻	5	8.2	9.7	28.6
		20	33.3	22.9	
		150	106.5	163.0	
LL-LT	NO ₃ ⁻	5	1.8	1.7	13.2
		20	27.4	25.7	
		150	59.6	41.8	
LSD (0.05)			26.7		7.7
Mean (Varieties)			18.9	19.8	

The following interactions were significant: LT x Carrier, LT x Level, Carrier x Level, LT x Carrier x Level

This indicates a shortage of energy for protein synthesis and/or nutrient uptake under the LL-LT condition, while under high light and temperature (HL-HT), higher levels of energy for nutrient uptake and protein synthesis should be available.

Soluble sugars

Ammonium-fed seedlings had lower free-sugar content than NO₃⁻-fed seedlings except under conditions of minimum stress (LL-LT) (Table 7). At the highest level of NH₄⁺ (150 ppm), C-C seedlings had a higher free sugar content than IR-8. No explanation was found for the exceptionally high sugar content of C-C plants under HL-HT at this level of NH₄⁺ (all three replications had high sugar percentage). In the case of IR-8, the highest free-sugar content was encountered with low N-input under conditions of low environmental energy (LL-LT). Table 7 also shows that the HL-HT condition resulted in the highest sugar content. As for N-carriers, NO₃⁻-fed seedlings had significantly higher soluble sugars than NH₄⁺-fed seedlings. These results parallel the data for growth (Table 2).

Discussion

Comparison of Tables 5 and 7 indicates that large quantities of carbon were transferred from the free

sugar pool into the free amino-N pool in NH₄⁺-fed seedlings under high temperature conditions (HL-HT, LL-HT). Under these conditions, up to 80% of the amino-N pool was made up of glutamine (6). Thus, it appears that high respiration rates associated with high temperature resulted in enhanced uptake of in protein synthesis and growth. Conversely, the nitrate reductase system competes with other processes for reducing power and energy, and exercises thereby a degree of regulatory control NH₄⁺. Excess NH₄⁺ was then "locked up" as amides, principally as glutamine. Amino acid imbalances resulting from this abnormal drain on a key intermediate (glutamate) would have interfered with protein synthesis and would account for drastic reductions in seedling growth at all levels of input NH₄⁺ under (LL-HT) conditions (Table 2). Specific toxic effects of free NH₄⁺ may have been expressed also at the highest level of input (Table 4).

By contrast, in NO₃⁻-fed plants, reduced N levels appear to have been under metabolic control. Free NH₄⁺ did not accumulate (Table 4), and relatively low and stable levels of free amino-N (Table 5) were maintained in the presence of high levels of substrate carbon in the form of free sugars (Table 7). Near optimal growth occurred under environmental conditions (LL-HT) that were severely damaging to both varieties when N was supplied as NH₄⁺ (Table 2). With both N-sources at the 150 ppm level of input,

Table 4. Free NH_4^+ in two varieties of rice as related to three light and temperature combinations, two N-carriers and three levels of application.

Light-Temp. combination	Carrier	Level	Varieties		Mean (L.I.)
			IR-8	C-C	
			$\mu\text{moles/g fresh weight}$		
HL-HT	NO_3^-	4	2.6	1.6	12.3
		20	0.1	0.1	
		150	1.6	1.1	
	NH_4^+	5	1.7	5.2	
		20	1.3	3.1	
		150	56.7	72.3	
LL-HT	NO_3^-	5	2.2	0.9	14.0
		20	4.4	7.0	
		150	1.4	4.5	
	NH_4^+	5	5.2	10.5	
		20	12.0	12.0	
		150	81.5	26.2	
LL-LT	NO_3^-	5	tr.	0.4	2.8
		20	3.9	5.5	
		150	2.9	3.4	
	NH_4^+	5	1.0	2.4	
		20	3.3	0.3	
		150	7.1	4.1	
LSD (0.05)				11.8	3.4
Mean (Varieties)			10.5	8.9	
Mean (Carrier)			NO_3^- 2.5	NH_4^+ 17.0*	

* Differences significant at $P(0.05)$.

The following interactions were significant: LT x Carrier, LT x Variety, LT x Variety x Carrier, Variety x Level, LT x Level, LT x Carrier x Level.

imbalanced uptake of other nutrients may have contributed also to curtailment of growth.

The need for continuing *de novo* synthesis of the enzyme is a mechanism whereby nitrate reductase activity is, in fact, regulated by processes involved over the general metabolism of the plant. Because of these linked interdependencies between nitrate reductase activity, respiration and growth, NO_3^- should be a better N-source for rice seedlings than NH_4^+ under unfavorable combinations of light and temperature.

Nitrate accumulations in rice tissues (Table 4) were less damaging to growth (Table 2) than were

diversions of key intermediates in protein metabolism from synthetic pathways into the free amino-N pool (Table 5) by mechanisms for detoxifying excess accumulations of NH_4^+ . This would appear to be an important feature in adaptation to environmental stress in areas characterized by a " NO_3^- -flush" early in the growing season.

Accumulations of mineral N in either oxidized or reduced forms were less damaging to IR-8 than to the older standard variety. Greater tolerance to high levels of mineral N in seedling stages likely derives from the larger leaf surface and greater photosynthetic efficiency of the newer short-strawed varieties and contributes to their greater responsiveness to fertilizer-N.

Table 5. Free amino-N in two varieties of rice as related to three combinations of light and temperature, two N-carriers and three levels of application.

Light-Temp. combination	Carrier	Level	Varieties		Mean (LT)
			IR-8	C-C	
			μmoles/g fresh weight		
HL-HT	NO ₃ ⁻	5	14.9	25.5	77.2
		20	22.3	29.5	
		150	27.1	31.8	
	NH ₄ ⁺	5	94.5	86.4	
		20	77.1	51.2	
		150	254.8	210.7	
LL-HT	NO ₃ ⁻	5	21.0	17.2	103.7
		20	23.0	22.4	
		150	25.9	29.5	
	NH ₄ ⁺	5	107.7	79.5	
		20	158.7	99.9	
		150	327.6	332.2	
LL-LT	NO ₃ ⁻	5	11.4	18.0	30.9
		20	28.1	28.0	
		150	26.8	26.3	
	NH ₄ ⁺	5	19.2	18.3	
		20	29.8	37.4	
		150	60.0	67.8	
LSD (0.05)			23.2		6.7
Mean (Varieties)			73.9*	67.3	
Mean (Carriers)			NO ₃ ⁻ 23.8	NH ₄ ⁺ 117.4*	

* Differences significant at P(0.05).

The following interactions were significant: LT x Varieties, LT x Varieties x Carrier, LT x Carrier, LT x Levels, Carrier x Levels, LT x Carrier x Levels.

Summary

The uptake and assimilation of nitrogen by tropical rice (*Oryza sativa* L.) under environmental stress were studied using controlled environments with different combinations of light, temperature, N-carriers, and N-concentrations.

Two week old rice seedlings were put under experimental conditions in nutrient solutions for

a 10-day period. Fresh weight, free-amino-N, NO₄⁻-N, NH₄⁺-N, nonstructural sugars and Kjeldahl-N of the plant residues after alcohol extraction were determined.

Variety IR-8 was found to be superior to variety Come-Cru in N-utilization. Nitrate was shown to be a better source of N than NH₄⁺ for rice plants grown under environmental stress. The data point to the existence of two mechanisms for metabolic regulation

Table 6. Kjeldahl-N in two varieties of rice as related to three combinations of light and temperature, two N-carriers and three levels of application.

Light-Temp. combination	Carrier	Level	Varieties		Mean (L.T)
			IR-8	C-C	
			% dry weight		
HL-HI	NO ₃ ⁻	5	2.94	3.77	3.44
		20	3.49	3.68	
		150	3.14	3.22	
	NH ₄ ⁺	5	3.67	2.95	
		20	4.09	3.09	
		150	4.30	2.98	
LL-HI	NO ₃ ⁻	5	2.37	3.08	3.07
		20	3.39	2.91	
		150	2.93	2.98	
	NH ₄ ⁺	5	3.33	2.86	
		20	3.49	2.71	
		150	3.12	3.43	
LL-LT	NO ₃ ⁻	5	2.05	1.72	2.53
		20	2.49	2.42	
		150	2.69	2.50	
	NH ₄ ⁺	5	2.27	1.95	
		20	3.41	2.82	
		150	3.16	2.91	
LSD (0.05)			0.55		0.16
Mean (Varieties)			3.15*	2.88	
Mean (Carrier)			NO ₃ ⁻ 2.89	NH ₄ ⁺ 3.14*	

* Differences significant at P(0.05)

The following interactions were significant: Variety x Carrier, LT x Variety x Carrier, LT x Level, LT x Variety x Level

of the reduced-N in plants depending on N-source. In plants fed NH₄⁺, the mechanism is one of tying up excess NH₄⁺ in organic forms. In the NO₃⁻-fed plants, NO₃⁻ appeared to be sequestered from the nitrate reductase inducing system, as well as from general metabolism. This phenomenon of NO₃⁻ sequestration permitted the seedlings to maintain normal metabolism, in spite of large accumulations of NO₃⁻ which were favored by high temperature and by high levels of NO₃⁻ in the root environment. The evidence

shows that NO₃⁻ can accumulate in rice seedlings in response to factors of external energy flux and available N supply, whereas its reduction and assimilation are regulated internally by energy fluxes associated with growth. These findings have important implications for fertilizer management and for selection of upland rice varieties for use under tropical conditions, where the beginning of the growing season is frequently characterized by a "NO₃⁻ flush" in the soil.

Table 7. Sugar accumulation in two varieties of rice as related to three light and temperature combinations, two N-carriers and three levels of applications.

Light-Temp. combination	Carrier	Level	Varieties		Mean (LT)
			IR-8	C-C	
			% fresh weight		
HL-HT	NO ₃ ⁻	5	2.9	2.3	2.4
		20	2.6	2.4	
		150	2.5	1.7	
	NH ₄ ⁺	5	1.5	1.3	
		20	1.7	1.7	
		150	1.2	7.3	
LL-HT	NO ₃ ⁻	5	2.9	2.1	1.8
		20	2.9	2.4	
		150	2.2	2.3	
	NH ₄ ⁺	5	1.3	1.1	
		20	1.1	0.7	
		150	0.7	1.6	
LL-LT	NO ₃ ⁻	5	2.8	1.8	2.0
		20	2.4	1.4	
		150	1.2	2.2	
	NH ₄ ⁺	5	3.5	2.1	
		20	2.5	1.9	
		150	0.9	1.5	
LSD (0.05)			0.89		0.26
Mean (Varieties)			2.0	2.1	
Mean (Carrier)			NO ₃ ⁻ 2.3*	NH ₄ ⁺ 1.9	

* Differences significant at P(0.05).

The following interactions were significant: LT x Variety, Variety x Carrier, LT x Carrier, LT x Variety x Carrier, Variety x Level, LT x Level, LT x Variety x Level, Carrier x Level, Variety x Carrier x Level, LT x Variety x Carrier x Level.

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