Effect of Long Term NH_{4+} Nutrition on Growth and Yield of Wheat Plants[1]

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ABSTRACT

The effect of NH_4 nutrition in the development and growth of plants (cv. Klein dorado) was studied in a pot experiment under glasshouse conditions. Plants were grown in 1 kg sand (three plants per pot) and fertilized with a buffered solution containing NO_3- or NH_4 as N-sources. Nitrogen concentration per pot was maintained at 2.0 mol . m^{-3}. Tillering was retrained by manual removal. Plant samples were taken from 30 d after sowing until ripening. Root growth was severely inhibited and shoot growth was markedly depressed by long term NH_4 nutrition. Nevertheless, the specific absorption rate of nitrogen (SARN) was similar in both N treatments until almost two weeks after anthesis. Ear enlargement was accelerated and spikelet differentiated at a faster rate in NH_4 fed plants. However, at ripening, the total number of spikelets per ear, florets per spikelet and grain yield were significantly lower in NH_4 treatment. Total nitrogen concentration in different plant parts did not differ between N treatments, although larger amounts of free NH_4 and total free amino acids were found in the NH_4 treatment during the whole sampling period. Results are discussed in relation to toxic effects due to accumulation of endogenous free ammonium.

Key words: Ammonium toxicity, ear development, free amino acids, *Triticum aestivum* L.

RESUMEN

Plantas jóvenes de trigo (cv. Klein dorado) fueron cultivadas, en condiciones de invernáculo, en macetas con 1 kg de arena (tres plantas por maceta), las que se fertilizaron periódicamente por medio de un tampón con una solución con NO_3 o NH_4 como fuentes de nitrógeno. Las plantas fueron demacolladas manualmente y la concentración de N por maceta se mantuvo en aproximadamente 2.0 mol . m^{-3}. Se realizaron cosechas periódicas en cada tratamiento desde 30 d después de la siembra hasta la madurez. El tratamiento prolongado con NH_4, como única fuente de N, redujo el crecimiento de las raíces. No obstante, la tasa específica de absorción de N (SARN) fue similar en ambos tratamientos, hasta casi dos semanas después de la antesis. Tanto el alargamiento del ápice como la diferenciación de las espiguillas se aceleraron en las plantas tratadas con NH_4, las cuales, en la madurez, presentaron un menor número de espiguillas por espiga, flores por espiguilla y mayor porcentaje de flores fértiles abortadas en comparación con el tratamiento con NO_3. La concentración de N en las distintas fracciones no difirió significativamente entre ambos tratamientos, aunque las plantas cultivadas con NH_4 acumularon mayores niveles de NH_4 y aminoácidos libres durante el período de muestreo. Los resultados se discuten en relación con los posibles efectos tóxicos provocados por la acumulación de NH_4 libre en los tejidos.

INTRODUCTION

Different forms of N nutrition distinctly affect both growth and chemical composition of plants (4, 15, 24, 25). Nitrate is the most abundant N sources present in the soil, and the usual N source for the plants. However, ammonium nutrition has often been proposed as an optimum N source for plant growth as it avoids energy wastes due to NO_3 reduction (24). Symptoms of water stress and toxicity have been reported by many authors when NH_4 was used as the sole N source (1, 4, 5, 7). Several factors have been thought to be responsible for such symptoms, but, unlike the nitrate ion, the accumulation of free NH_4 in plant tissues exerts "per se" toxical effects (13). Nevertheless, many plant species are adapted to NH_4 as the sole N source (24), and some crops like wheat or rice increased their yield when NH_4 was added to a nutrient solution containing NO_3N (9, 28, 29).

Grain production is the main component of crop yield, and all those factors affecting ear development also affect grain number and quality. Several authors have reported increments of grain yield when nitrogen fertilization was increased (10, 11, 18, 27); however there is little information on the effects of different N sources on spike development and grain setting. In this paper, we studied the effects of NO_3 and NH_4 nutrition on growth, ear development and grain yield of wheat plants grown under greenhouse conditions.

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MATERIALS AND METHODS

Plant culture

Wheat caryopses (*Triticum aestivum* L. cv. Klein Dorado) were germinated on wet tissue paper in the dark. After 72 h, six seedlings were transplanted to plastic pots containing 1 kg of washed sand and periodically irrigated with demineralized water. After a week, plants were reduced to three per pot and fertilization was started. Pots were randomly divided into two groups and the treatments were initiated by fertilizing each pot with 50 ml of a basal solution containing 10.0 mol • m^{-3} KNO_3 or 5.0 mol • m^{-3} $SO_4(NH_4)_2$ as N sources (Table 1).

Table 1. Nutrient composition of basal fertilization solution.

Macro nutrients	mol m^{-3}	Micro nutrients	mmol m^{-3}
KCl	4	KCl	50
$CaCl_2$	4	H_3BO_3	25
KH_2PO_4	2	$MnSO_4.H_2O$	2
FeEDTA 50%	2	$ZnSO_4.7H_2O$	2
$MgSO_4.7H_2O$	1	$CuSO_4.5H_2O$	0.5
		H_2MoO_4	0.5

Final nutrient concentration on each pot was 1/5 of the fertilizing solution. Plants were fertilized twice a week during the first 45 days and thrice a week during the following two weeks. Then, both thee volume and frequency of fertilization were modified according to plant requirements. Samples of solution percolates from randomly selected pots were periodically analyzed for N content, and their pH recorded during the whole experimental period. When necessary, pots were washed with demineralized water in order to avoid excessive ion accumulation. The pH was periodically adjusted to 6.5 ± 1.0 by addition of H_2SO_4 or KOH to the treatment solutions.

Tillering was restrained by manual removal so that only the main shoot was allowed to grow. Three pots (i.e., nine plants) per treatment were sampled from 30 d after sowing (d.a.s.) until ripening, at the time intervals shown in the figures. After sampling, roots were carefully washed and rinsed with demineralized water. Plants were divided into roots, leaves plus stems and ears (if present), fresh weights recorded and then samples were freeze dried for further analysis. At ripening, grains were also analyzed as a distinct fraction. Before frying, shoot apexes were microdissected and kept in a mixture of ethanol (96%)-water– formaldehyde- acetic acid (gl.) (5.0:3.5:1.0:0.5) for further observation. The whole experiment was conducted in a glasshouse with daily temperatures of 10 °C - 15 °C at night and 25 °C - 32 °C during the day.

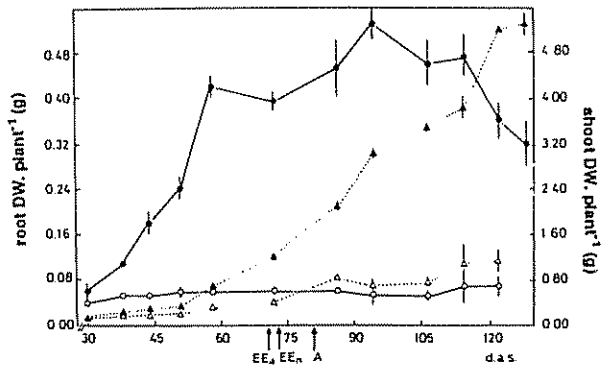
Plant analysis

Total nitrogen was determined by micro Kjeldahl analysis. Total free amino acids and free ammonium analysis: 0.1 g of freeze dried plant material were extracted with 10 ml of a mixture of ethanol-chloroform-water (12:5:3) overnight at 0 °C. After filtration, 1.5 ml chloroform was added and, after shaking and addition of 2.25 ml of distilled water, the aqueous fraction was extracted. Samples were kept at -18 °C until total partitioning and the aqueous fraction was then isolated for free amino acids and ammonium assays.

Total free amino acids were analyzed through the ninhydrin method (32). Free ammonium was determined by distillation of 0.5 ml aliquots plus 0.5 ml buffer pH 11 (NaOH 1 N: $Na_2B_4O_7$ • 10 H_2O 0.5 M, 1:1). Free NH_{4+} was collected on 2 ml of 2% boric acid and titrated with H_2SO_4.

RESULTS

No differences were observed between plant dry weight in both N treatment at the first sampling. However, NO_3- fed plants grew at a faster rate than the NH_{4+}-treated plants so that, at ripening, they had accumulated a 500% higher total dry weight (Fig. 1).

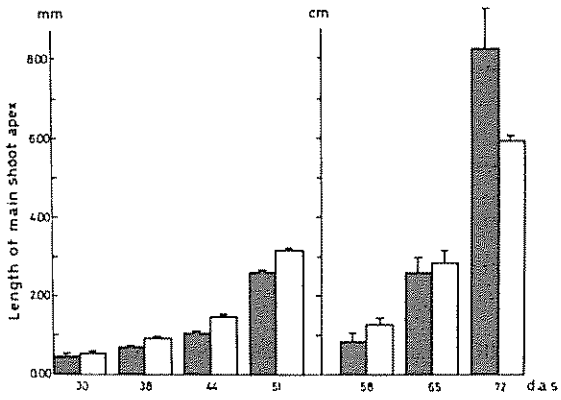


EE = Ear emergence (a= NH_{4+} treatment; n= NO_3- treatment). A = Anthesis. Vertical bars represent SE.

Fig. 1. Root dry weight (o) and shoot dry weight ( ) of plants supplied with NH_4-N (open symbols) or NO_3-N (closed symbols).

Root dry weight in the NH_{4+} treatment remained almost constant during the whole sampling period, while it largely increased with time until two weeks after anthesis in the NO_3 treated plants (Fig. 1). Shoot growth was also several times faster in the NO_3 fed plants than in the NH_{4+} treatment.

The ammonium-fed plants showed a faster shoot apex elongation (Fig. 2) accompanied by an earlier spikelet initials differentiation (data not shown). Nevertheless, anthesis occurred at almost the same date in both treatments.

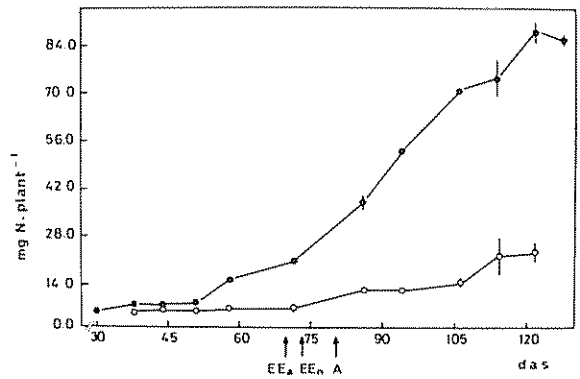


Vertical bars represent SE.

Fig. 2. Length of the main shoot apex from 30 d.a.s. until spike emergence in plants treated with NH_4-N (open bars) or No_3-N (closed bars).

Ear maturation occurred at a faster rate under NH_{4+} nutrition, so that NH_{4+}-fed plants had completely matured 10 d before NO_3 treatment. At ripening, the number of spikelets per spike and grains per spikelet were significantly lower in NH_{4+} fed plants (Table 2). From the average number of florets per spikelet recorded at ear emergence (Table 3), 66.7%, 57.1% and 50.0% of the basal, central and terminal spikelets respectively had aborted in NH_{4+}-treated plants, while the corresponding values for control treatment were 33.3%, 37.5% and 33.3%. On the other hand, 40% (basal spikelets) to 60% (central and terminal spikelets) of the remaining florets set grain in the NO_3 fed plants, while no basal florets and only a 33% of the central and terminal spikelets did the same under NH_{4+} nutrition (Table 2). Thousand-grain weight was also lower in this treatment with respect to that with NO_3 (Table 4).

Total N content per plant was slightly higher in the NO_3 treatment than in the NH_{4+}-fed plants during the first 20 d of sampling (Fig. 3). However the NO_3 fed plants accumulated nitrogen at a faster rate after this period. As this increment can be related to either a higher plant growth or to a higher N absorption per unit root weight, the specific absorption rate of N (SARN) was estimated in both N treatments (8). SARN did not differ between NO_3 and NH_{4+}-fed plants until anthesis, although root growth was almost totally inhibited in the latter. The values obtained between 30 and 86 d.a.s. (5 d after anthesis) were 1.64 and 1.69 mg N. root $DW^{-1}.d^{-1}$ respectively. At 94 d.a.s., root growth of NO_3 fed plants began to decline until maturity; however, their SARN almost doubled that of NH_{4+} treatment during the same period (2.31 vs. 1.19 mg N.root DW^{-1} . d^{-1}).



EE = Ear eemergence (a = NH_4 treatment; n= NO_3 treatment).
A = Anthesis Vertical bars represent SE.

Fig. 3. Total nitrogen content in plants supplied with NH_4-N (o) or NO_3-N ().

Nitrogen percentage (N%) in shoot and spikes was almost the same under both NH_{4+} or NO_3 nutrition, and only the roots of NH_{4+}– treated plants showed a significantly higher N concentration than the NO_3 treatment during the sampling period (Fig. 4).

Table 2. Number of spikelets, florets and grain yield in the mature ear of wheat plants supplied with NO_3-N (data rounded to the nearest digit).

	Florets • Spikelet^{-1}		
	Basal spikelets (2 and 3)*	Central spikelets (8, 9, 10 and 11)	Penultimate and terminal spikelets
NO_3-	5	6	5
	SE:0.12	SE:0.10	SE:0.15
NH_4+	2	3	3
	SE:0.05	SE:0.20	SE:0.36

	Florets which set grain				
	Basal spikelets (2 and 3)*	Central spikelets (8, 9, 10 and 11)	Penultimate and terminal spikelets	Spikelets spike	Grains spike
NO_3-	2	4	3	19	54
	SE:0.11	SE:0.04	SE:0.10	SE:0.15	SE:0.88
NH_4+	0	1	1	16	8
	SE:0.00	SE:0.05	SE:0.09	SE:0.15	SE:0.70

SE: Standard error.
(*): Numbers between brackets stand for those spikelets, in ascending order, which were sampled.

Table 3. Number of florets per spikelet in the ears of wheat plants supplied with NO_3-N or NH_4-N and sampled at ear emergence (data rounded to the nearest digit).

	Basal spikelets (2 and 3)*	Central spikelets (8, 9, 10 and 11)	Penultimate and terminal spikelets
NO_3-	8	9	8
	SE:0.13	SE:0.08	SE:0.13
NH_4+	6	7	6
	SE:0.13	SE:0.11	SE:0.27

SE: Standard error
(*): Numbers betwen brackets stand for those spikelets, in ascending order, which were sampled.

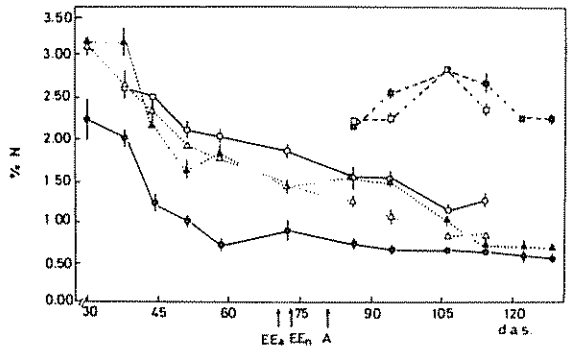
At the first sampling, roots of the NH_4+-treated plants showed 100% more free ammonium per g DW than roots of the NO_3- fed plants (Fig. 5). On the other hand, while ammonium concentration in shoots remained constant in the NO_3- treatment, it largely increased in the NH_4+-fed plants, reaching a peak of 60 µmol • g DW^{-1} at seven days before ripening. At ripening, free ammonium in shoots had diminished, but was very high in the mature ears of NH_4+-fed plants in contrast to the levels found in the NO_3- treatment (Table 4). In both treatments, the concentration of free NH_4+ in the grain fraction accounted for less than 0.5% of that present in the whole spike.

Table 4. Dry weight, NH_4 concentration and total free amino acids concentration in the mature ear and grain fractions of wheat plants supplied with NO_3-N or NH_4N.

	Whole ear		
	Dry weight (g)	µmol NH_4 . g DW^{-1}	µmol aa g DW^{-1}
NO_3	3.76 SE:0 05	2.02 SE:0.78	31.58 SE:1.85
NH_4	0.88 SE:0 12	82.54 SE:2.04	96.37 SE:2 17

	Grains			
	Dry weight of I 000 grains (g)	µmol NH4± g DW	µmol aa g Dw	N % grain
NO3-	30 30	0 00 Se: 0.00	0.00 SE: 0 00	3.24 SE: 0 04
NH4+	20.84	0.70 SE: 0.70	8.87 SE: 2 88	3.1 SE: 0.08

SE: standard error



EE = Ear emergence (a= NH_4 treatment; n= NO_3 treatment).
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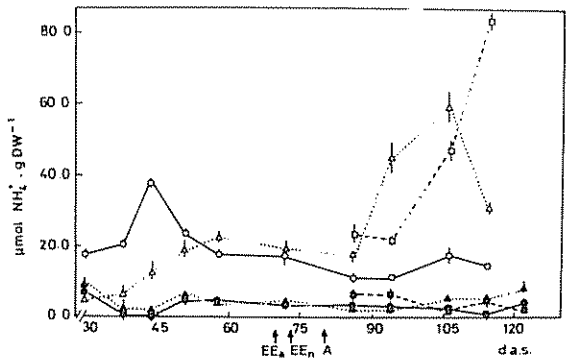
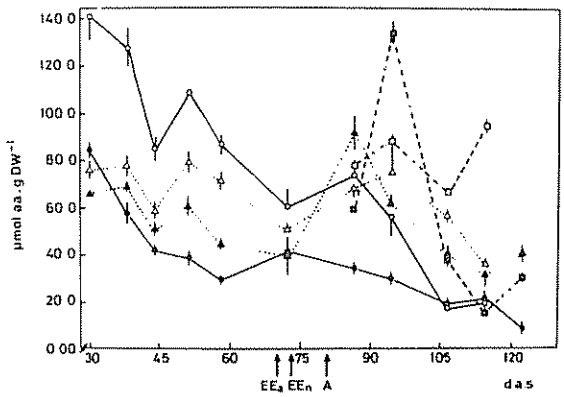
Fig. 4. Nitrogen percent in root (o), shoot ( ) and ear ( ) of plants supplied with NH_4-N (open symbols) or NO_3-N (closed symbols).



EE = Ear emergence (a= NH_4 treatment; n= NO_3 treatment).
A = Anthesis. Vertical bars represent SE.

Fig. 5. Free ammonium concentration in root (o), shoot ( ) and ear ( ) of plants supplied with NH_4-N (open symbols) or NO_3-N (closed symbols).

The concentration of free amino acids in roots was initially 60% higher in NH_4-treated plants, and in both N treatments it decreased with time (Fig. 6). The concentration of free amino acids in shoots was initially lower than that present in the roots, and only slight differences were found between N treatments until ear emergence. In the spike, it increased during the first week after ear emergence, especially in those plants fed with NO_3-N. However, while in this case it rapidly decreased after that time, it remained constant in NH_4 treatment. From the content of total free amino acids present in mature ears, only 6% (NH_4 treatment) and 0% (NO_3 treatment) were in the grain fraction (Table 4).



EE = Ear emergence (a= NH_4 treatment; n= NO_3 treatment).
A = Anthesis. Vertical bars represent SEE.

Fig. 6. Total free amino acids concentration in root (o), shoot ( ) and ear ( ) of plants supplied with NH_4-N (open symbols) or NO_3-N (closed symbols).

DISCUSSION

The causes of the toxic effects of the ammonium ion on higher plants have been widely reviewed (13, 24), but not aspects are completely clear. Impaired plant growth, high shoot: root ratios and premature senescence have been reported for young wheat plants (9, 12) and other species (1, 6, 31) when NH_4 was supplied at increasing external concentrations or during a prolonged period. Mehrer and Mohr (22) have recently described these symptoms as the "ammonium toxicity syndrome".

While in several cases the observed effects could be explained as a consequence of different metabolic changes due to NH_4 uptake and assimilation, it is known that the endogenous accumulation of free ammonium is "per se" deleterious to plant growth (5, 13, 17, 30).

In the present experiment, plant growth was severely inhibited by ammonium nutrition in both shoots and roots, (Fig. 1) and later the ear (Tables 2 and 3). However, the organ most severely affected was the root, since it slightly grew only slightly during the whole sampling period under NH_4 nutrition (Fig. 1), with a consequent increase in the shoot:root ratio. This impairment of root growth may be a consequence of the high free ammonium concentration (Fig. 5). However, the absorption capacity of the root did not seem to have been affected, as the SARN from 30 d.a.s. until almost two weeks after anthesis were similar in both N treatments.

An estimation of the N translocation during 30 d to 86 d after sowing showed that the ammonium-fed plants had transported almost 100% more mg N per day and per mg of root DW than the NO_3 fed plants (data not shown). This would indicate that shoot growth under NH_4 nutrition was not initially impaired because of a deficient N translocation. The high levels of free NH_4 present in both root and shoot tissues of plants supplied with NH_4 -N suggest that toxic effects due to an excessive accumulation of free NH_4, rather than N deficiency, were responsible for the observed symptoms.

Most of the NH_4 absorbed by the roots is assimilated within the roots via thee glutamine synthetase-glutamate synthase pathway, so that glutamine and other amino acids are the N derivatives transported to the shoot (2, 3, 19). As the root plays a key role in the process of NH_4 detoxification, the high levels of NH_4 found under our experimental conditions suggest that the capacity for ammonium assimilation of the root system has been largely overcame (Fig. 5). The fact that NH_4 assimilation was occurring is suggested by the high concentration of free amino acids present both in root and shoot tissues (Fig. 6), although NH_4 accumulation could also have affected protein synthesis (17).

Reduced shoot growth, accelerated apex development and leaf senescence are symptoms observed under water stress or nitrogen stress (8, 21, 23, 27), so that they are not specific responses to NH_4, but general responses of plants to stress. However, root growth was severely diminished under NH_4 nutrition rather than stimulated, as it is often observed under water or N stress (14, 16).

On the other hand, while N% in the grain fraction did not differ between treatments (Table 4), both grain setting and grain filling were markedly diminished in those plants with high endogenous concentrations of free NH_4 (Tables 2 and 4).

Apex development and ear emergence were initially accelerated by ammonium feeding (Fig. 2). Similar results have been previously reported for wheat plants (20), though the physiological underlying mechanism is still unknown.

It can be concluded that, under NH_{4+} nutrition, while the plant becomes older and the shoot:root ratio changes as a consequence of a highly impaired root growth, the NH_{4+} detoxification capacity of the plant decreases, thus increasing the ion concentration in the leaves and ears to toxic levels.

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