Nitrate reductase activity and yield of dry mass and protein content in cucumber seedlings supplied with nitrates and ammonium*

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Abstract

The presence of NH_4^+ ions in a nutrient solution containing NH_4NO_3 with the K^+ ions removed, caused an inhibition of nitrate reductase (NR) activity in cucumber leaves. The lack of K^+ in a NaNO_3 medium also decreased the NR activity. Addition of K^+ to these media suppressed the inhibitory effect of NH_4^+ and enhanced the NR activity in the leaves of plants growing in NaNO_3 nutrient solution. The results suggest that K^+ is essential for NO_3^- reduction, Na^+ is less effective in this process, whereas NH_4^+ ions markedly inhibit NO_3^- reduction. The protein content and increment of dry mass of cucumber plants grown 10 days with $\text{NH}_4\text{-N}$ as the sole source of nitrogen was significantly lower as compared with $\text{NO}_3\text{-N}$ supplied plants or with plants fed with both forms of mineral nitrogen (NH_4NO_3). The results show that cucumber prefers the nitrates, although it can utilize the ammonium form of nitrogen.

INTRODUCTION

Plants which absorb nitrogen as the ammonium form only, generally do not grow as rapidly as these supplied with the nitrate form of nitrogen (Kirby, 1968; Blair et al., 1970; Kubik-Dobosz, 1975; Suder-Moraw and Buczek, 1977). The influence of NH_4^+ on growth and yield of plants is determined both by the rate of absorption of ammonium and the resulting effect of NH_4^+ uptake on the pH of the ambient solution. Ammonium absorption increase as pH increase, whereas NO_3^- uptake tended to decrease with

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increasing pH (Jungk, 1970). When both NH_4^+ and NO_3^- were present in the same nutrient media, NH_4^+ was reported to inhibit NO_3^- uptake in some species (Minotti et al., 1969a and b; Buczek, 1979), whereas NO_3^- generally did not influence the uptake of NH_4^+ . It might, however, have been expected that NO_3^- and NH_4^+ would influence the uptake and metabolism of each other, because both are sources of N and both are metabolised when NO_3^- is reduced to NH_4^+ , along the same pathways. Moreover, some reports have demonstrated that ammonium inhibits NR activity (Smith and Thompson, 1971; Frith, 1972; Radin, 1973; and Buczek, 1979).

The present aim was to study the effect of NH_4^+ , Na^+ and K^+ ions on the level of nitrate reductase activity (NR) in the course of 10-day growth of 12-day-old cucumber seedlings. The effect of nitrates and ammonium and of both forms of mineral nitrogen present in the same nutrient solution on the yield of dry mass and protein content was also examined.

METHODS

The plant material used in this investigation were 12-day-old cucumber (*Cucumis sativus* L. cultivar. *Monastyrski*) seedlings. After 4 days of germination, 4 uniform seedlings were placed in 1.5-l. glass jars containing a continuously aerated pretreatment nutrient solution containing $\text{NH}_4\text{-N}$ as the sole source of nitrogen. The nutrient solution used in the pretreatment period included the following concentrations of salts in mM: $(\text{NH}_4)_2\text{SO}_4$ — 1.87; K_2SO_4 — 0.096; $\text{Ca}(\text{H}_2\text{PO}_4)_2$ — 0.22; MgSO_4 — 0.37 and microelements as described in the preceding paper (Buczek, 1979). The pH of the solution was adjusted to 5.8. When the first leaf had well sprouted (approximately after 12 days) one set of seedlings were dried, weighed (dry mass) and analyzed for protein content. The remaining group of plants were transferred to another nutrient solution (induction medium) containing 7.1 mM NaNO_3 or 7.1 mM NH_4NO_3 as source of nitrogen with increasing doses of K_2SO_4 . For comparison one group of seedlings were grown in medium containing equivalent amount of nitrogen (3.55 mM) in the form of $(\text{NH}_4)_2\text{SO}_4$. The remaining macro- and microelements were the same as in the pretreatment solution. The pH (5.8) of induction media was corrected every day to maintain the pH level as constant as possible. The induction media were changed after 24 hr and 5 days of growth of the plants. The experiments were conducted in a greenhouse in June and repeated in July.

The NR activity was measured in extracts of homogenates from leaves after 24 hr, 5 days and 10 days growth of plants in the induc-

tion media, according to the method of Hageman et al. (1960), described in detail in the preceding paper (Buczek, 1976). Enzyme activity was expressed in nmoles of NO_2^- released per 100 mg of fresh weight of leaf tissue per hour. The results are averages of 3 replications.

After 10 days growth of cucumber in induction media, the plants were harvested and dried at 105°C , weighed and the increment of dry mass was determined from the differences of the final and initial (after 12 days growth of seedlings in pretreatment solution) dry weight. Protein nitrogen was determined by the routine micro-Kjeldahl method described previously (Buczek et al., 1971). The protein yield was calculated by multiplying the amount of protein N by 6.25. The results are means of 6 replications.

RESULTS AND DISCUSSION

Nitrate reductase activity (Table 1), in leaves of 12-day-old cucumber seedlings after 24-hr growth of plants in NH_4NO_3 induction medium lacking K^+ ions, was lowered about twofold as compared with the level in leaves of plants growing in induction medium containing NaNO_3 . In the following days of the experiment the NR activity was similar both with NH_4NO_3 and with NaNO_3 supplied plants, however, an inhibitory affect of NH_4^+ was observed in comparison with plants absorbing NO_3^- in the presence of K^+ ions.

The above results are in agreement with earlier findings (Buczek, 1979), that the presence in the induction medium of the equivalent

Table 1

The effect of K^+ concentration in the presence of 7.1 mM NaNO_3 or NH_4NO_3 in induction medium on nitrate reductase activity in cucumber leaves

Potassium was added as K_2SO_4 . The differences between 2 values denoted with different letters within the same column are significant at 5% level

Induction medium	Concentration of K ⁺ added mM	Activity of nitrate reductase		
		day of measurement after induction		
		1	5	10
		nmoles NO ₂ ⁻ · 100 mg ⁻¹ fresh wt. hr ⁻¹		
NaNO ₃	0.0	212.5a	575.0a	583.3a
	0.19	245.8a	687.5a	629.2a
	1.90	254.2a	779.2b	920.8b
	3.80	420.7b	764.5b	1095.8b
	0.0	125.0c	562.5a	502.5a
NH ₄ NO ₃	0.19	180.0a	791.7b	791.7b
	1.90	308.3d	891.7b	1295.8c
	3.80	421.2b	733.3b	1062.5b

amounts of NH_4^+ and NO_3^- inhibits by about 50 per cent the NR activity in cucumber cotyledons growing for 34 hr in induction medium without K^+ ions. Addition of K^+ to this medium suppressed completely the inhibitory effect of NH_4^+ ions. We suggested that the influence of NH_4^+ on NR activity is indirect and consists in its effect on translocation of NO_3^- within the plants.

The present study showed that the level of NR activity in leaves of plants supplied with NH_4NO_3 in the absence of K^+ was always lower than that in leaves of plants fed with NaNO_3 or $\text{NaNO}_3 + \text{K}_2\text{SO}_4$, and that the inhibitory effect of NH_4^+ ions was more or less constant after 5 days of growth of the plants. Moreover, these results confirm the previous data (Buczek, 1979) that K^+ ions suppress the inhibitory effect of NH_4^+ on NR activity. According to the hypothesis of the Ben Zioni et al. (1971), K^+ plays an essential role by transporting NO_3^- from roots to shoots. As has been shown in a recent paper (Buczek, 1979) and in the present investigations, the function of K^+ in NO_3^- assimilation cannot be replaced by Na^+ or NH_4^+ ions, not only in short-term (24-hr) (Buczek, 1979) but also in long-term (10-day) experiments with cucumber plants. Moreover, it appears that NH_4^+ ions inhibit indirectly NO_3^- assimilation by lowering the NR activity.

Table 2

Increment of dry mass and protein content of 12 day-old cucumber seedlings after 10 day growth in induction media

Potassium was added as K_2SO_4

Induction medium		Dry weight	Protein content		Dry weight
N form (mM)	K^+ added (mM)	$\text{mg} \cdot \text{plant}^{-1}$	$\text{mg} \cdot \text{plant}^{-1}$	$\text{mg} \cdot \text{g}^{-1}$ dry wt.	protein
NaNO_3 (7.1)	0.0	88.87a	10.80a	77.60a	8.2
	0.19	171.67b	21.13b	95.17a	8.1
	1.90	171.84b	30.93c	139.24b	5.6
	3.80	202.48c	31.73c	125.77b	6.4
	0.0	61.59d	9.47a	84.64a	6.4
NH_4NO_3 (7.1)	0.19	124.27e	20.00b	117.99b	6.2
	1.90	164.47b	33.23c	162.77c	4.9
	3.80	171.17b	32.39c	146.27b	5.3
	0.0	44.43f	4.32d	65.27a	10.3
	0.19	55.23d	5.71d	75.32a	9.7
$(\text{NH}_4)_2\text{SO}_4$ (3.55)	1.90	91.33a	9.80a	89.76a	9.3
	3.80	84.43a	8.83a	81.33a	9.6

The experiments of Kubik-Dobosz (1975 and Suder-Moraw and Buczek (1977) have demonstrated a lower yield of dry mass in tomato seedlings supplied with $\text{NH}_4\text{-N}$ as compared with those fed

with $\text{NO}_3\text{-N}$ alone. Under our culture conditions a similar relationship appeared in cucumber seedlings. The fact that equivalent amounts of NH_4^+ and NO_3^- in the medium depressed the increment of dry mass (Table 2) of cucumber seedlings may suggest that this species prefers the nitrate form of mineral nitrogen and that cucumber plants are sensitive to ammonium (Matsumoto, 1968).

The yield of protein per plant was also reduced in seedlings supplied with $\text{NH}_4\text{-N}$ alone in comparison with the protein content in plants growing in media containing $\text{NO}_3\text{-N}$ or both forms of mineral nitrogen (NH_4NO_3). However, the lower dry weight/protein ratio (Table 2) in NH_4NO_3 -supplied plants as compared with $\text{NO}_3\text{-N}$ alone fed seedlings may indicate utilization of the ammonium form of mineral nitrogen for protein synthesis in cucumber. This would agree with the findings of Ingestad (1972) in cucumber and Warncke and Barber (1973) in maize. The above cited authors believed that when both NH_4^+ and NO_3^- were present in the same nutrient medium, the yield of dry weight and production of protein were dependent on the concentration of nitrogen and the $\text{NH}_4^+/\text{NO}_3^-$ ratio. Thus, our results suggest that cucumber prefers nitrate, although it can utilize simultaneously the ammonium form of nitrogen.

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Aktywność reduktazy azotanowej oraz plon suchej masy i zawartość białka w siewkach ogórka pobierających azotany i sole amonowe

Streszczenie

Badano wpływ azotanów i jonów amonowych oraz obydwu form azotu mineralnego podanych roślinom równocześnie, na plon suchej masy, zawartość sumy białka oraz aktywność reduktazy azotanowej (NR) w trakcie 10-dniowego wzrostu 12-dniowych roślin ogórka.

Stwierdzono, że aktywność NR w liściach po 24-godzinym wzroście roślin w pożywce zawierającej NH_4NO_3 i pozbawionej potasu była prawie dwukrotnie niższa w porównaniu do roślin rosnących w pożywce zawierającej NaNO_3 . Brak K^+ w pożywce NaNO_3 obniżał również aktywność NR. Dodanie jonów potasu do obydwu pożywek w odpowiednim stężeniu zniosło hamujący wpływ jonów NH_4^+ na aktywność NR oraz wzmogło aktywność enzymu w liściach roślin rosnących w pożywce NaNO_3 . Wyniki sugerują, że jony K^+ są niezbędne dla

redukcji NO_3^- , jony Na^+ są mniej efektywne w tym procesie, a jony NH_4^+ wyraźnie hamują redukcję NO_3^- . Zawartość białka i przyrost suchej masy roślin ogórka, które rosły 10 dni w pożywce zawierającej $\text{NH}_4\text{-N}$ jako jedyne źródło azotu, była istotnie niższa w porównaniu do roślin pobierających wyłącznie $\text{NO}_3\text{-N}$ lub obydwie formy azotu (NH_4NO_3). Wyniki wskazują, że azotany są korzystniejszą formą azotu dla ogórków, aczkolwiek rośliny te mogą wykorzystywać azot amonowy.