

Effect of Nitrate and Ammonium Nitrogen Sources on the Growth, Photorespiration, Dark Respiration and Activities of Related Enzymes in Wheat (*Triticum aestivum* L.)¹

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Abstract: Wheat seedlings were grown hydroponically with 100 ppm of either nitrate or ammonium as the N source. Plant growth, CO₂ compensation concentration, dark respiration rate and activities of several related enzymes were measured and analyzed. The purpose was to study the influence of N forms on the carbon metabolism in plants. Wheat seedlings grew better in solution supplied with nitrate. The detrimental effect of ammonium on the growth could be reverted partially by increasing the pH of culture medium to 7.0. CO₂ compensation concentration was 15 ppm lower for nitrate-grown plant than for ammonium-grown plants. However, the difference was also oriented partly by the pH of culture solution. pH value of tissue extracts was not affected by N treatment despite the marked fluctuation of pH in the surrounding nutrient medium. Ammonium-grown plants had higher total titratable acidity but much lower malate content than nitrate-grown plants, suggesting that a considerable amount of CO₂ was fixed in both the leaf and root tissue. This assumption is further supported by the facts that wheat seedlings supplied with ammonium nutrition had higher RuBP carboxylase activity in the leaves and PEP carboxylase activity in the roots. Values of the latter (up to 210 $\mu\text{mol/gfw. hr}$) is at least 2-3 times higher than those normally found in the leaf blades of most C₃ plants. Wheat seedling supplied with ammonium-N had higher NAD-malate dehydrogenase but similar spartate aminotransferase activity when compared with those supplied with nitrate-N. Ammonium nutrition could increase the CO₂ compensation concentration of wheat seedlings with a temperature range of 21 to 35°C and under 2, 21 and 100% oxygen concentrations. Glycolate oxidase activity was slightly higher but dark respiration rate was significantly higher for plants grown in ammonium than in nitrate. The linear relationship between CO₂ compensation concentration and oxygen concentration and the extrapolated finite compensation concentration at zero oxygen suggest that in addition to photorespiration, 'dark' respiration also contribute to CO₂ efflux in the light and the increased CO₂ compensation concentration observed in ammonium grown plants.

¹Contribution No. 1483 from Taiwan Agricultural Research Institute. Thanks are due to the National Science Council, R. O. C. for granting a fellowship, and to Drs. G. E. Edwards and M. S. B. Ku, Dept. of Botany, Washington State Univ., for providing facilities and advices on this study.

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Photorespiration, the O_2 - and light-dependent release of CO_2 from the leaves, is an enigmatic process which is inseparable from photosynthetic CO_2 fixation, yet performing no generally acknowledged function. Yamada and Ikeda⁽²⁸⁾ suggested that photorespiration plays a role in dissipating surplus reducing energy which is harmful to the plants under low CO_2 concentration. According to this hypothesis, nitrate and sulfate consume reducing energy in the same manner as CO_2 and hence are able to affect carbon metabolism. It has been reported that both C_3 and C_4 plants fed with ammonium N have higher CO_2 compensation concentration than plants fed with nitrate-N^(4,26). Both glycolate oxidase and RuBP carboxylase/oxygenase activities have been suggested to be related to this phenomenon^(9,18) However, 'dark' respiration is also proposed to contribute CO_2 efflux in the light⁽¹⁾.

The sources and availability of N are believed to affect carbon metabolism in the plants^(5,10). Its implication on plant productivity has also been assessed⁽⁴⁾. Many research projects focused on the influences of nitrate and ammonium nutrition on photosynthesis and related reactions have been executed and published in recent years. Photorespiration is usually of major concern because of its relationship with plant productivity. However, factors controlling CO_2 compensation concentration as a result of N influence have not been fully understood. In the present investigation, several variables affecting CO_2 compensation concentration were studied with wheat plants grown in either nitrate or ammonium nutrient solution. The purpose was to further clarify the inter-relationship between the metabolism of N and carbon.

Materials and Methods

Seeds of a spring wheat (*Triticum aestivum* L.) cultivar, Dirkwin, of about the same weight (30–35mg) were soaked in aerated distilled water for 24 hr followed by germinating in petri dish and kept in a growth chamber (Conviron model E-15, Controlled Environments Co.) at day/night (14/10 hr) temperature of 26/18°C under darkness for six days. Uniform seedlings about 8 cm in height were transplanted to plastic pots and grown hydroponically with the basic salts of Hoagland formulation. Four seedling were grown in a pot with 2.5 l of culture solution. Nutrient solution was aerated vigorously using a small compressor connected via perforated Tygon tubings. Plants were kept in the same growth chamber under a daily regime of 14-hr light at 26°C and 10-hr dark at 18°C. Illumination was provided with fluorescent and tungsten lamps giving 700 $\mu E/m^2 \cdot sec$ of irradiation at the top of the plants.

The culture solution contained the following essential elements with the specified concentrations (mg/l): K^+ : 234, Ca^{+} : 200, Mg^{2+} : 48, PO_4^{3+} : 95, SO_4^{2-} : 192, BO_3^{3-} : 2.9, Fe (as Fe-EDTA): 2.2, Mn^{2+} : 0.27, Zn^{2+} : 0.13, Cu^{2+} : 0.13, and MoO_4^{2-} : 0.07. Nitrogen was supplied as either NO_3^- -N or NH_4^+ -N with a concentration of 100 ppm. The pH of the freshly prepared solution was 4.95–5.00.

In experiments 1 and 3, wheat seedlings were grown in the above solution. pH was determined daily and the solution was renewed in an 48-hr interval. At the 11th day after transplanting, part of the plants was transferred from ammonium to nitrate

medium and part from nitrate to ammonium medium in experiment 1. In experiment 2, pH of the culture solution containing either NO_3^- or NH_4^+ was adjusted daily to 3.0 and 7.0, respectively, and the changes of pH were checked immediately before each pH adjustment. Items of measurement and analysis were shown in the tables and figures of the text.

Carbon dioxide compensation concentration was determined in a closed gas exchange system. Fully-expanded wheat leaf blades were enclosed in a ventilated plexiglass chamber. Illumination was provided by an 800W quartz-tungsten lamp (Sylvania DXW) cooled with an 8-cm layer of running tap water. Temperature inside the chamber ($28 \pm 0.2^\circ\text{C}$) was controlled by circulating water through a jacket and was measured using a copper-constantan thermocouple. The decrease of CO_2 concentration inside the chamber was measured with an Anarad model 500 infrared gas analyzer. The concentration at equilibration was taken as CO_2 compensation concentration. Details of the measurement were illustrated by Clegg *et al.* ⁽²⁾ and Ehleringer and Cook ⁽⁷⁾.

In most cases, the plexiglass chamber was flushed briefly with CO_2 -free air before determining CO_2 compensation concentration. In experiment 3, the chamber was also flushed, individually, with 2, 21 and 100% oxygen (practically CO_2 -free). Temperature inside the chamber was also kept at 21 or 35°C in addition to the 38°C mentioned above. The measurements proceeded from low to high temperature and oxygen concentrations.

After measuring CO_2 compensation concentration, dark respiration was measured in the same system. The chamber was darkened with aluminum foil, briefly flushed with CO_2 -free air, and the leaves allowed to respire until a constant rate of CO_2 evolution was maintained, which occurred in 6 to 10 min. The leaves were removed and the leaf area determined with a Li-Cor LI-3100 leaf area meter. Dark respiration was calculated as $\text{mg CO}_2/\text{dm}^2 \cdot \text{hr}$ ⁽²³⁾.

Activity of RuBP carboxylase (RuBPC) was determined by the method of Lorimer *et al.* ⁽¹⁹⁾, PEP carboxylase (PEPC) was after Spalding and Edwards ⁽²⁴⁾, NAD-malate dehydrogenase (MDH) after Edwards and Gutierrez ⁽⁶⁾, NADP-malate dehydrogenase after Johnson and Hatch ⁽¹⁵⁾, aspartate aminotransferase after Hatch and Mau ⁽¹³⁾, and glycolate oxidase after Fair *et al.* ⁽⁸⁾. Malate was determined according to the method of Hatch ⁽¹²⁾, total titratable acidity by the method of Ku *et al.* ⁽¹⁷⁾, soluble protein by Lowry *et al.* ⁽²⁰⁾ and chlorophyll by Wintermans and De Mots ⁽²⁷⁾. Results shown are the means of two to four replicates.

Results

Vigorous plant growth in the nitrate medium (initial pH 5.0) caused rapid drift towards alkalinity in the culture solution, conversely with the ammonium medium plant growth caused an acid drift. Fig. 1 showed that during a 2-day duration (13th to 15th day after transplanting, switching of solution from nitrate to ammonium or ammonium to nitrate was held at the 11th day), wheat seedlings supplied with nitrate increased pH of the culture medium from 5.0 to 6.5–6.8 and with ammonium from 5.0 to about 3.

The extent of pH drift was correlated with plant age and size. For example, the increase of pH for the nitrate-grown plants between the 15th and 17th days was from 5.0 to as high as 7.4. The drift occurred mainly in the first day after the renewal of culture solution (Fig. 1).

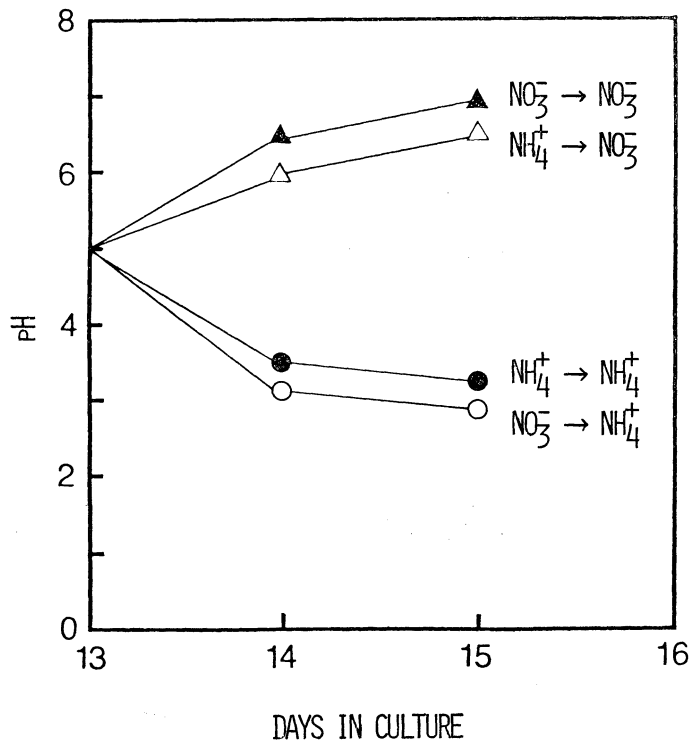


Fig. 1. Changes in pH of culture medium when wheat seedlings were grown hydroponically with different sources of nitrogen. The initial pH of the culture medium was 5.0. (Exp. 1)

Wheat seedlings grew better in the nitrate than in the ammonium culture solution (Fig. 2, experiment 1). Between the 5th day and 21st day after transplanting, nitrate-fed plants showed a 16-fold increase in fresh weight while ammonium-fed plants had only a 3-fold increase. For the plants transferred from nitrate to ammonium or from ammonium to nitrate at the 11th day, the growth pattern afterwards was similar to those grown continuously in ammonium and nitrate solutions, respectively. In other words, the poor growth caused by ammonium nutrition could be reversed by switching the plants to nitrate-containing solution. Conversely, growth of the nitrate-fed plants was retarded if ammonium was provided as the sole N source. However, older seedlings tended to adapt ammonium-N better than younger ones. Between 11th and 21st days, $\text{NO}_3^- \rightarrow \text{NH}_4^+$ plants gained 4.5 g fresh weight per seedling but plants grown continuously in ammonium gained only 1.8 g.

pH value of the culture solution also showed significant effect on the growth of wheat seedlings (Fig. 4, experiment 2). Growth was extremely poor in the NO_3^- -pH 3.0

treatment. The shoots were dark green in color with no visual increase in plant height. The roots became thicker and yellowish in color and were curled in order to avoid contacting the injurious acidic solution. Increasing medium pH was beneficial to the growth of plants fed with ammonium. As shown in Fig. 4, NH_4^+ -pH 7.0 treatment was better than NH_4^+ -pH 3.0 in sustaining plant growth. Similar to the result of experiment 1 (Fig. 2), NO_3^- -pH 7.0 was the best among four treatments in terms of gain of plant fresh weight. It is apparent that an interaction between N sources and pH value exists.

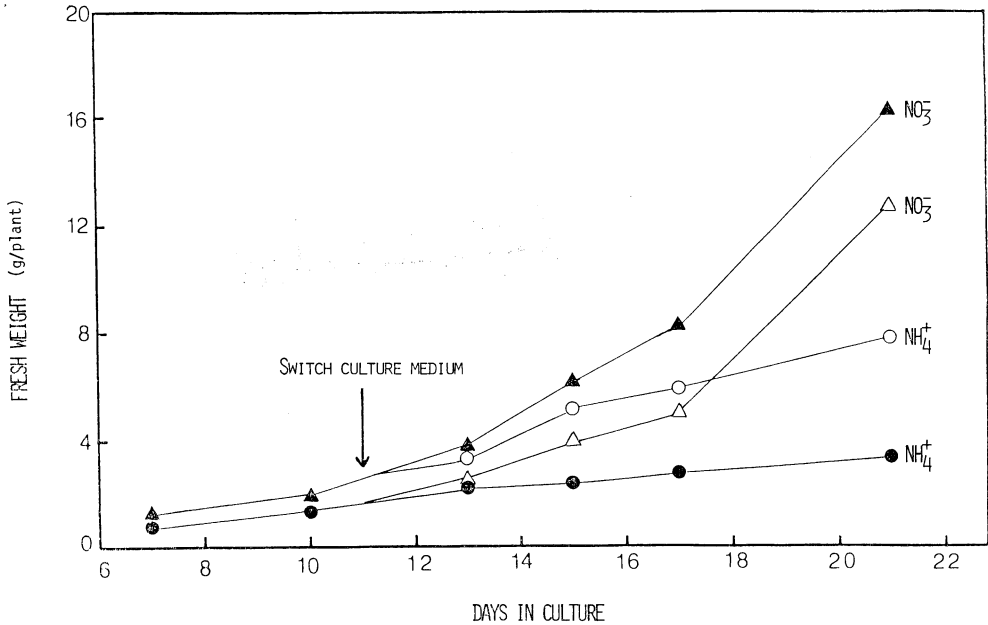


Fig. 2. Influence of nitrogen source on the fresh weight of wheat seedlings. Culture medium was renewed every two days. Switching of culture medium (NO_3^- to NH_4^+ or NH_4^+ to NO_3^-) was performed on the 11th day. (Experiment 1)

Data collected on CO_2 compensation concentrations of leaf blades of nitrate- and ammonium-grown plants were shown in Fig. 3 (experiment 1). The average value of CO_2 compensation concentration was 72 ppm for the ammonium-grown plants and 57 ppm for the nitrate-grown plants. It appears that growth under nitrate may result in CO_2 compensation concentration values about 15 ppm lower than growth under ammonium. No significant fluctuation was observed during the course of the study. When the wheat plants were transferred from a nitrate-N medium to an ammonium-N medium, CO_2 compensation concentration increased from 57 to 69 ppm within one day and decreased gradually thereafter. The value was only 57.5 ppm 9 days after medium switching (Fig. 3). On the other hand, CO_2 compensation concentration decreased sharply immediately after transferring the plants from ammonium to nitrate solution. The decrease, from 72 ppm to 57 ppm, was completed within two days (Fig. 3).

In experiment 2, higher and increasing CO_2 compensation concentration values were

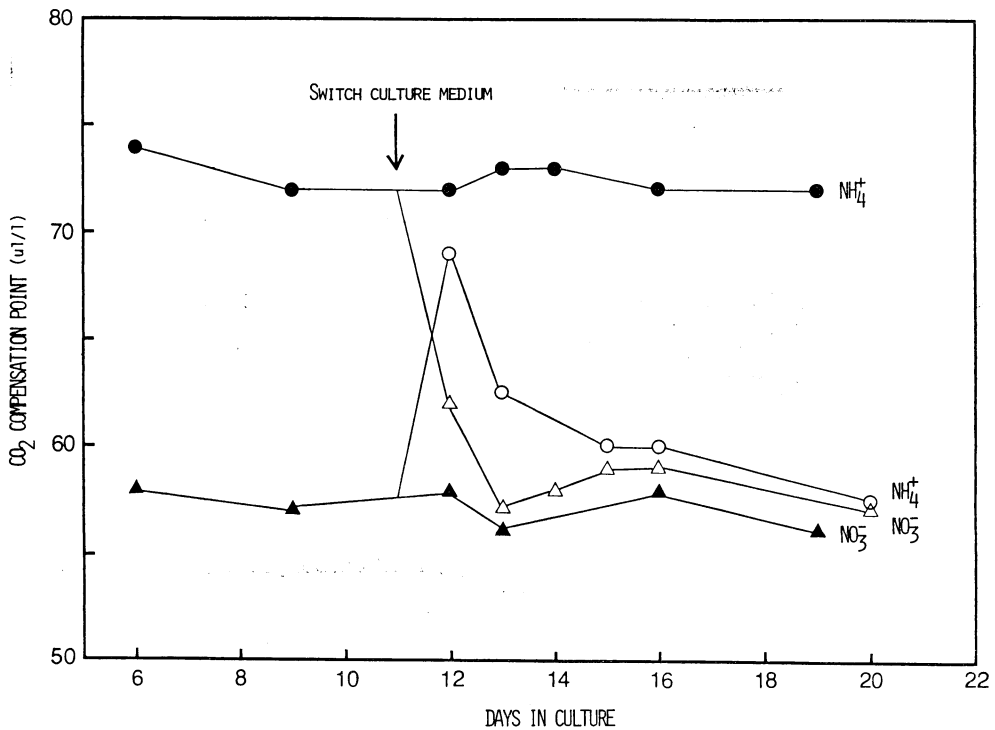


Fig. 3. Effect of nitrogen source on the CO₂ compensation concentration of the leaf blades of wheat seedlings. (Experiment 1)

observed in NH₄⁺-pH 3.0 treatment (Fig. 5). The increase was from 70 to 85 ppm during an 8-day duration from 6 to 14 days after transplanting. Compared with the results of Fig. 3, it appears that lowering pH of the ammonium culture medium tended to give higher CO₂ compensation concentration of wheat seedlings. In contrast, high pH (7.0) showed an effect to lower the compensation point (from 73 to 61 ppm) when ammonium N was supplied to the plants. The poorly-grown plants in NO₃⁻-pH 3.0 treatment also had increased compensation concentration values which were, however, lower than those of the NH₄⁺-pH 3.0 plants. The above results show that in addition to N sources, pH of the culture medium has a definite effect on the status of CO₂ equilibration of wheat plant in a closed system.

Although significant changes in pH of different N media were observed as a result of wheat growth, comparisons on pH of tissue extracts among N treatments did not show marked differences (Table 1). pH values of leaf blade extracts were between 5.89 and 5.99 for the 17-day-old seedlings. Extracts of roots grown in nitrate at the time of assay had pH of 6.14–6.33 which were slightly higher than the extracts from ammonium-grown roots (5.75–6.05). Possibly the low pH of ammonium medium had more pronounced influence on the growth of root than on leaf tissue. The total titratable acidity and malate content of leaf blades and roots of experiment 1 are shown in Tables 1 and 2, respectively. In plants supplied with ammonium N at the time of assay there was a significant higher titratable acidity but lower malate content, especially in the leaf tissue.

Table 1. Effects of nitrate and ammonium nutrition on pH and total titratable acidity in the leaf and root extracts of wheat seedlings (Exp. 1)

N Source of Culture Medium ¹	pH		Titratable Acidity ²	
	Leaf	Root	Leaf	Root
NO ₃ ⁻	5.89	6.33	29.3	14.1
NO ₃ ⁻ →NH ₄ ⁺	5.89	6.05	84.6	26.7
NH ₄ ⁺	5.95	5.75	103.0	31.6
NH ₄ ⁺ →NO ₃	5.99	6.14	36.3	19.5

¹17-day-old seedlings were used for assay. Culture media were renewed every 2 days. Switch of medium was performed at the 11th day.

²In the unit of $\mu\text{eq/gfw}$.

Table 2 also showed the results of some biochemical analyses of experiment 1. In the leaf blades of the 21-day-old wheat seedlings, PEP carboxylase (PEPC) activities of various N treatments ranged from 29 to 37 $\mu\text{mol/mg chl} \cdot \text{hr}$ which are similar to the activities normally found in C₃ plants. Seedlings grown continuously in nitrate or ammonium medium had relatively higher activities than those transferred to a different medium at the 11th day. In the case of RuBP carboxylase (RuBPC), plants of NH₄⁺ and NH₄⁺→NO₃⁻ treatments had significantly higher activities than the plants grown conti-

Table 2. Effect of N nutrition on the activities of various enzymes and soluble protein and malate contents in the leaf and root of wheat seedlings¹ (Exp. 1)

Measurement	NO ₃ ⁻	NO ₃ ⁻ →NH ₄ ⁺	NH ₄ ⁺	NH ₄ ⁺ →NO ₃ ⁻
Leaf				
PEP carboxylase ($\mu\text{mol/mg chl} \cdot \text{hr}$)	34.4	28.9	36.5	29.0
RuBP carboxylase ($\mu\text{mol/mg chl} \cdot \text{hr}$)	873	863	1,115	1,059
Aspartate aminotransferase ($\mu\text{mol/mg chl} \cdot \text{hr}$)	94	99	126	96
NADP-malate dehydrogenase ($\mu\text{mol/mg chl} \cdot \text{hr}$)	48	40	56	48
NAD-malate dehydrogenase ($\mu\text{mol/mg chl} \cdot \text{hr}$)	6,400	6,110	8,810	7,640
Soluble protein (mg/gfw)	38.5	36.9	39.5	40.2
Malate ($\mu\text{eq/gfw}$)	4.43	0.09	0.32	1.22
Root				
PEP carboxylase ($\mu\text{mol/gfw} \cdot \text{hr}$)	48	141	210	47
Soluble protein (mg/gfw)	9.9	13.3	15.5	10.2
Malate ($\mu\text{eq/gfw}$)	3.60	0.15	0.94	2.30

¹21-day-old seedlings were used for assay of RuBPC, aspartate aminotransferase and NADP- and NAD-MDH. All other measurements were made on 17-day-old seedlings. Switch of culture medium was performed at this 11-th day.

nuously or originally in NO_3^- medium (1,059-1,115 vs. 863-878 $\mu\text{mol}/\text{mg chl} \cdot \text{hr}$).

NAD-MDH is the principal enzyme responsible for the conversion of oxalacetate to malate in wheat while NADP-MDH is of negligible importance (Table 2). Wheat seedlings grown continuously or originally in ammonium medium had significantly higher NAD-MDH activities, similar to the results obtained on RuBPC. Activity of aspartate aminotransferase, which catalyzes the conversion of oxalacetate to aspartate, were at least 60 times lower than those of NAD-malate DH (Table 2). These results suggest the preference of wheat seedlings in utilizing oxalacetate during photosynthesis. Aspartate aminotransferase activity was higher for plants continuously fed with ammonium than for plants in the other three treatments.

The high PEPC activity, up to 210 $\mu\text{mol}/\text{gfw} \cdot \text{hr}$, found in the roots of ammonium-grown plants was at least 2-3 times higher than those exhibited by leaf blades when the same unit is used for comparison (84.0 and 60.1 $\mu\text{mol}/\text{gfw} \cdot \text{hr}$ for NH_4 and $\text{NO}_3^- \rightarrow \text{NH}_4^+$ treatments, respectively). The high PEPC activities were associated with high soluble protein contents in the roots (Table 2).

Table 3 indicated that maintaining the pH of nitrate or ammonium medium at 3.0 or 7.0 did not affect consistently PEPC activity in the leaf blades. In spite of the higher activities of RuBPC and glycolate oxidase in ammonium than in nitrate treatment, changes in pH did not show significant effect on the activities of the two enzymes within individual N source treatment. Similar to the results shown in Table 2, ammonium-grown plants had very high PEPC activity in the roots. Increasing pH from 3.0 to 7.0 in ammonium medium was able to enhance malate content in both leaf blades and roots and decreasing pH from 7.0 to 3.0 in nitrate medium gave higher malate content in the leaf blades. One remarkable fact is that the poorly-developed roots in NO_3^- -pH

Table 3. Effect of N form and pH of the culture medium on the activities of various enzymes and soluble protein and malate contents in the leaf and root of wheat¹ (Exp. 2)

Measurement	NO_3^-		NH_4^+	
	pH 7.0	pH 3.0	pH 7.0	pH 3.0
Leaf				
PEP carboxylase ($\mu\text{mol}/\text{mg chl} \cdot \text{hr}$)	36.0	33.7	34.8	35.8
Glycolate oxidase ($\mu\text{mol}/\text{mg chl} \cdot \text{hr}$)	121	107	136	133
RuBP carboxylase ($\mu\text{mol}/\text{mg chl} \cdot \text{hr}$)	616	713	846	853
Soluble protein (mg/gfw)	33.8	41.8	33.7	40.5
Malate ($\mu\text{eq}/\text{gfw}$)	7.70	9.40	0.32	1.99
Root				
PEP carboxylase ($\mu\text{mol}/\text{mg chl} \cdot \text{hr}$)	63.5	49.6	149.2	136.0
Soluble protein (mg/gfw)	9.5	31.8	12.1	11.8
Malat ($\mu\text{eq}/\text{gfw}$)	3.77	2.64	0.63	1.64

¹13-day-old seedlings were used for all analyses.

3.0 treatment contained soluble protein almost 3 times higher than those in the other treatment (Table 3). Actually soluble protein content as high as 60 mg/gfw was recorded in the roots of this treatment prior to 10 days after transplanting (data not shown).

The response of CO₂ compensation concentration to oxygen concentration was determined to study the contribution of photorespiration to the increased compensation concentration caused by ammonium nutrition. Fig. 6 showed that at any given oxygen concentration or temperature, leaf blades had a higher CO₂ compensation concentration when plants were grown in ammonium than in nitrate medium. The difference increased with increasing temperature or oxygen concentration. CO₂ compensation concentration increased linearly with oxygen concentration from 2 to 21 to 100% at each temperature studied which indicated possible contribution of 'dark' respiration to CO₂ efflux by either photosynthesizing or non-photosynthesizing cells, or both.

The leaf blade dark respiration rates of plants supplied with different N sources in relation to oxygen concentration were then determined and the results are shown in Fig. 7. It is obvious that dark respiration rates were higher for ammonium-grown plants than for nitrate-grown plants at each oxygen concentration studied. There was a marked increase in dark respiration when oxygen concentration increased from 2% to 21%. The increase from 21% to 100% oxygen was less significant. The non-linearity between dark respiration and oxygen concentration was not parallel to the relationship between CO₂ compensation concentration and oxygen concentration as shown in Fig. 6.

Discussion

The culture medium containing anionic nitrate became more alkaline and those with cationic ammonium more acidic with the advance of wheat growth (Fig. 1). The changes in external pH are due to excretion of hydrogen ions by the plants upon ammonium uptake, and hydroxyl ions upon nitrate uptake⁽²⁵⁾. The lower pH exhibited by switching plants from nitrate to ammonium than by growing continuously in ammonium is probably the result of larger root volume and hence higher absorption capability of the former.

Weight gains by adding ammonium to an all nitrate system have been observed in many plant species⁽¹⁴⁾. Cox and Reisenauer⁽³⁾ reported that the growth rate and yield of wheat were enhanced under similar condition. The enhancement was suggested to result from the reduced energy requirement in using ammonium in protein synthesis. However, ammonium alone usually possesses a detrimental effect on plant growth. Results of this experiment showed that the growth of wheat seedlings was inferior when grown under ammonium nutrition (Fig. 2). Possible causes are the toxicity of ammonium ion to plant metabolism and the lowering of pH in culture medium. Fig. 4 indicated that the growth of wheat seedlings was increased when the pH of the ammonium-containing medium was adjusted daily to 7.0.

Despite the significant changes of pH in culture media, the pH values of tissue ex-

tracts of leaf blades and roots showed insignificant difference among N treatments (Table 1). Only the roots of wheat seedlings grown continuously in ammonium showed a lower pH of 5.75. In general, when ammonium ion is assimilated into organic-N in the roots, the excess hydrogen ions generated are excreted to the surrounding solution. On the other hand, hydroxyl ions produced in the cell cytoplasm when plants are grown in nitrate are partly excreted from the roots and partly neutralized by the production of organic acids⁽²²⁾. Furthermore, ionic balance within the plant is achieved by the action of organic acid anions such as malate and citrate⁽¹⁴⁾.

Total titratable acidity in the leaf blades and roots were higher in the ammonium than in the nitrate treatment which were in accordance with the pH values of plant tissue (Table 1). However, the differences in titratable acidity were not in agreement with those in malate concentration (Table 2). These results suggest that a considerable amount of CO₂ was fixed (acidification) by the ammonium-plants⁽¹⁷⁾. Further evidences were that wheat seedlings supplied with ammonium nutrition had higher RuBPC activity in the leaves and PEPC activity in the roots than plants supplied with nitrate nutrition (Table 2).

De Benedetti *et al.*⁽⁵⁾ reported that ammonium-grown chloroplasts had higher RuBPC activity resulted from the activation of the enzymatic reaction by ammonium ion, or the faster utilization of RuBP in the presence of ammonium. Golvano *et al.*⁽¹⁰⁾ suggested that ammonium nutrition seemed to stimulate the enzyme activity in parallel to the stimulation of protein synthesis. Table 2 showed that soluble protein concentration was relatively the same between N treatments for the 21-day-old seedlings. It is probable that higher RuBPC activity of ammonium-grown plants is the result of higher activity rather than amount of RuBPC protein.

Nitrogen sources showed little influence, if any, on the PEPC activity of leaf blades (Table 2). In nitrate-grown plants, the ratio of activities of RuBPC/PEPC was lower than in ammonium-grown plants. similar result has been reported by Winter *et al.*⁽²⁶⁾ on *Moricandia arvensis*, a C₃-C₄ intermediate. Little information is available on the influence of N forms on PEPC activity in the roots. This investigation revealed that ammonium had a consistent and significant effect in stimulating PEPC activity in the wheat roots (Tables 2 and 3, and unrepresented data). The higher activity was accompanied by a higher content of soluble protein (Table 2). The extraordinarily high activity is not commonly found in the root tissue of C₃ plant. Its implication, in addition to the acidification reaction proposed earlier, has yet to be determined.

Oxalacetate formed in the cells can be reduced to malate catalyzed by NAD-MDH or converted to aspartate by aspartate aminotransferase. Wheat seedlings possessed high activity of NAD-MDH (up to 8,800 $\mu\text{mol}/\text{mg chl} \cdot \text{hr}$, see Table 2) which was at least 5 times higher than the combined activity of NAD- and NADP-MDH in a succulent C₄ dicot, *Portulaca grandiflora*, reported by Ku *et al.*⁽¹⁷⁾. On the contrary, a spartate aminotransferase activity was low in the tested plants (ca. 100 $\mu\text{mol}/\text{mg chl} \cdot \text{hr}$). Generally, activities of both enzymes were higher in plants supplied with ammonium than with nitrate medium. This is not in agreement with the result of malate content (Table 2).

The role of NAD-malate DH in wheat seedlings grown with ammonium N remained to be clarified.

Irrespective of N sources, pH value of the culture medium could affect some physiological characteristics of the hydroponically cultured wheat. Growth of wheat seedlings was extremely poor in nitrate medium with pH value adjusted daily to 3.0. On the other hand, ammonium medium at pH 7.0 was beneficial to the plants compared with ammonium-pH 3.0 treatment (Fig. 4). The experimental results on tomato of Tiedjens and Robins, as reported by Street and Sheat⁽²⁵⁾, showed that although ammonium was absorbed at pH 4.0 it was not assimilated and had a toxic effect; at pH 7.0-8.0 ammonium was effectively assimilated and supported a high level of growth. The low pH (3.0) used in this experiment is considered injuriously acidic to root growth. A significant amount of undissociated nitric acid might be present and hence inhibit the development of root system⁽²⁵⁾. A very high soluble protein content (31.8 mg/gfw) was observed in the roots, but not leaves, of wheat seedlings grown in nitrate at pH 3.0 (Table 3). Possibly soluble protein could counteract the toxic influence caused by the low pH value through some unexplained mechanisms.

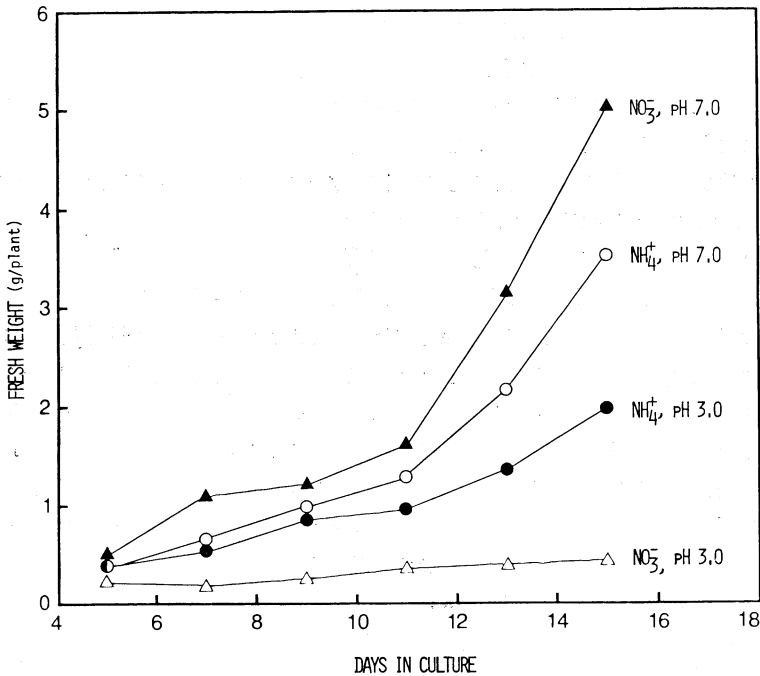


Fig. 4. Influence of nitrogen source and pH of culture medium on the fresh weight of wheat seedlings. Nutrient solution was renewed every 2 days and pH adjusted daily. (Experiment 2)

The high CO₂ compensation concentration associated with ammonium nutrition (Fig. 3) has been reported on a variety of plant species. Cresswell⁽⁴⁾ found both C₃ and C₄ plants to exhibit a lower CO₂ compensation concentration with nitrate than with ammonium as the N source. Fig. 3 of this study also found the CO₂ compensation con-

centration of wheat seedlings was 15 ppm lower with nitrate than with ammonium as the N source. However, such a difference seemed to be pH-oriented as there was a marked decrease in compensation concentration when wheat seedlings were grown in ammonium medium maintained at pH 7.0 (Fig. 5). Photorespiration has been considered to play a role in dissipating surplus reducing energy which might be toxic to photosynthetic tissue. Yamada and Ikeda⁽²⁸⁾ proposed that materials which consume reducing energy, such as nitrate and sulfate, regulate photorespiration and thus plants with nitrate as the sole N source tend to have a lower CO₂ compensation concentration.

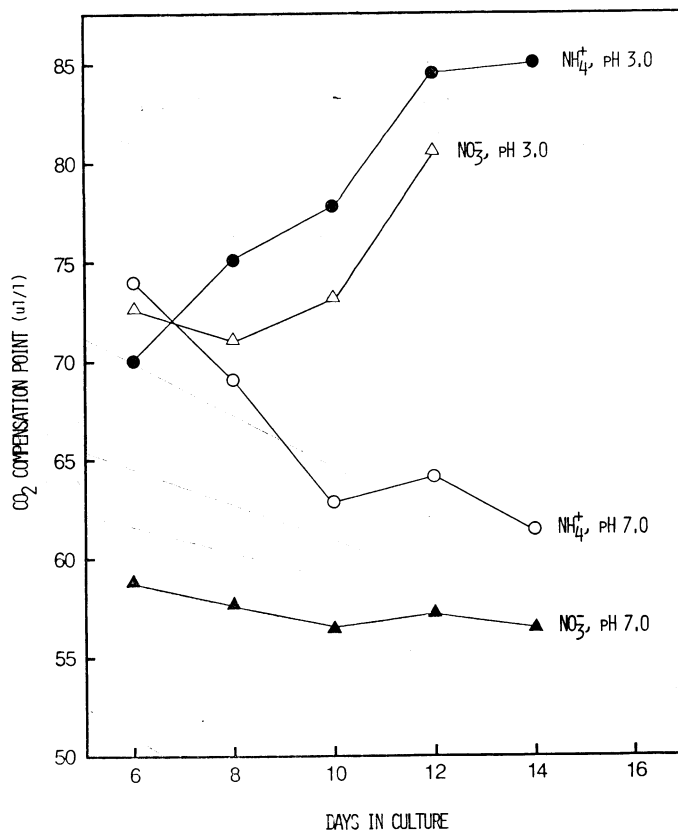


Fig. 5. Effect of nitrogen source and pH of culture medium on the CO₂ compensation point of wheat seedlings. (Experiment 2)

The mechanism of how N nutrition affects photorespiration has not been fully understood. Fair *et al.*⁽⁹⁾ reported that the increase in glycolate oxidase activity in the ammonium-grown plants was greater than the corresponding increase in RuBPC activity and the difference was reflected to the higher CO₂ compensation concentration. From Table 3 of this experiment, glycolate oxidase activity was slightly higher for ammonium- than for nitrate-grown plants. However, such difference is not in accordance with the trends of changing CO₂ compensation concentrations as shown in Fig. 5. Kestler *et al.*⁽¹⁶⁾ suggested that the ratio of RuBP oxygenase (RuBPO)/RuBPC determined the photosynthetic CO₂ compensation concentration in leaves of higher plants. Nitrogen

nutrition did not show any effect on the PEPC activity (Tables 2 and 3). While RuBPC activity increased in ammonium-grown plants, RuBPO activity was not verified in this experiment or in other related reports. Winter *et al* ⁽²⁶⁾, in studying *Moricandia arvensis*, found 2-fold higher PEPC activity and low ratio of activities of RuBPC/PEPC in the nitrate-grown than ammonium-grown plants. They concluded that PEPC may have a relatively greater role in carbon assimilation in plants supplied with nitrate. Although the present study obtained similar results on the ratio of activities of RuBPC/PEPC in response to N nutrition, the difference in ratios was caused completely by RuBPC because both nitrate and ammonium treatment failed to show any significant difference in PEPC activity (Table 2). In other words, enzymatic reactions controlling photosynthetic CO₂ compensation concentration in relation to N status need to be studied in the future.

In an attempt to elucidate whether the increase in CO₂ compensation concentration caused by ammonium is due to an increase in the photorespiratory rate, the influence

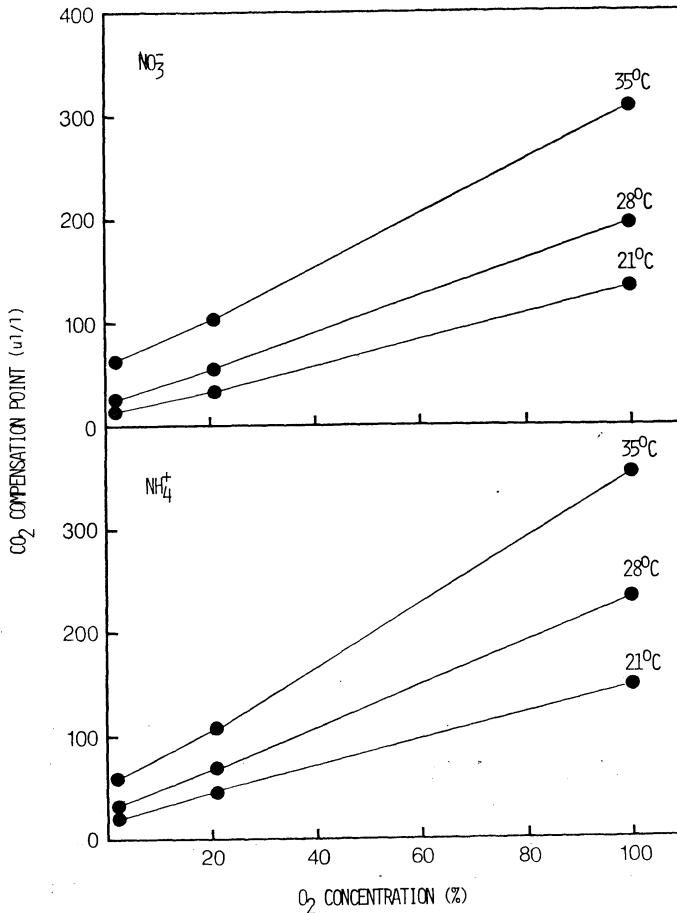


Fig. 6. Influence of temperature and O₂ concentration on the CO₂ compensation point of wheat seedlings supplied with different forms of nitrogen. 14-day-old seedlings were used. (Experiment 3)

of different oxygen concentrations on CO_2 compensation concentration was studied in this experiment. The positive and linear relationship between the two characters (Fig. 6) strongly suggests that the increased CO_2 compensation concentration exhibited by the wheat seedlings grown in ammonium N nutrient medium is due to an increase in the output of CO_2 by the plant rather than to a decrease in the uptake of CO_2 . Grossman and Cresswell⁽¹¹⁾ found that sugarcane and corn plants receiving ammonium N responded to oxygen in a similar manner to that usually found in C_3 plants. The linear increase of the compensation concentration with oxygen concentration can be explained by considering only the kinetic of the enzyme RuBPC/RuBPO. However, the observed positive value of CO_2 compensation concentration when oxygen concentration is extrapolated to zero (Fig. 6) suggest the possibility of the occurrence of some 'dark' respiration in photosynthesizing cells under illumination (or day respiration), although other cells may also contribute to CO_2 efflux⁽¹⁾. The tricarboxylic acid cycle is likely to be the most important contributor to dark respiration and it occurs in the light.⁽²¹⁾ Results of Fig. 7 showed dark respiration rate was significantly higher for plants grown in ammonium than in nitrate medium under three different oxygen concentrations, which can be used as an indirect evidence to show the possible contraction of dark respiration to the high compensation concentration of ammonium-grown plants.

Carbon dioxide compensation concentration generally increase with increasing temperature (Fig. 6). Laing et al.⁽¹⁸⁾ explained this phenomenon in terms of the kinetic properties RuBPC/RuBPO, i. e., by the temperature dependence of compensation concentration in the absence of any other process. Azcon-Bieto *et al.*⁽¹⁾, on the contrary, showed that simulation of these effects appeared insufficient to account for rapid increase of CO_2 compensation concentration at high temperature and concluded that inclusion of 'dark' respiration yielded more realistic predictions. Parallel relationship between

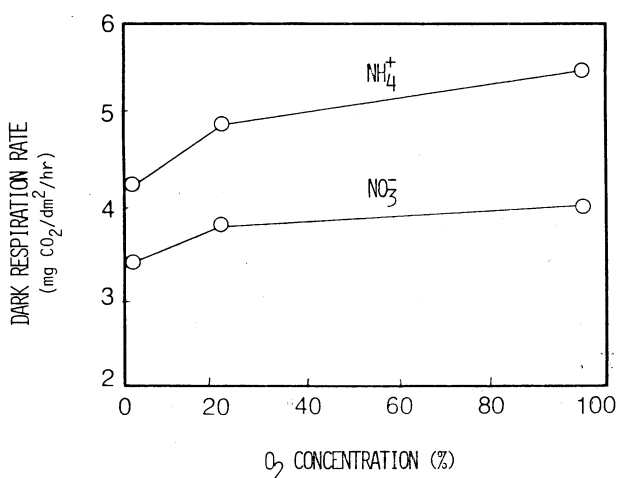


Fig. 7. Influence of O_2 concentration on the rate of dark respiration of wheat seedlings supplied with different forms of nitrogen. Results of 5 measurements on 11- to 21-day-old seedlings were averaged and presented. (Experiment 3)

CO₂ compensation concentration and dark respiration rate at 28 and 35°C under different oxygen concentrations (data not shown) seems in favor of the hypothesis made by Azcon-Bieto *et al.*⁽¹⁾. However, the non-linear response of dark respiration rate to oxygen concentrations (Fig. 7) suggested that although dark respiration might be involved in CO₂ efflux in the light, increased photorespiratory rate is still of importance in contributing the high CO₂ compensation concentration caused by ammonium nutrition.

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硝酸態與銨態氮源對小麥生長、光呼吸、 暗呼吸及相關酵素活性的影響¹

劉 大 江²

摘 要

本試驗以小麥幼苗為材料，以 100 ppm 之硝酸態或銨態氮為氮源，測定小麥生長、二氧化碳補償濃度、暗呼吸速率及一些酵素活性的變化，目的在瞭解氮素代謝對碳素代謝作用的影響。小麥在含硝酸態氮源的水耕液內生長較佳，唯銨態氮源對生長的不利作用可經由維持水耕液 pH 值於 7.0 而獲得部分改善。小麥生長於銨態氮源下的二氧化碳補償濃度較生長於硝酸態氮源下高 15 ppm 左右，但此項差異亦可經由提高水耕液 pH 值而減小。雖然小麥生長於不同氮源可導致水耕液 pH 值的明顯變化，植株萃取液的 pH 值却大致相近。銨態氮源條件下生長的小麥含有極高的可滴定酸度 (total titratable acidity) 和極低的 malate 含量，顯示必需固定較多的二氧化碳以維持植株內的離子平衡 (ionic balance)，此項假設可由葉片內較高的 RuBP carboxylase 活性與根部較高的 PEP carboxylase 活性得到證明，後一酵素於根部的活性可高達 $210 \mu\text{mol/gfw} \cdot \text{hr}$ ，為多數 C_3 型植物葉片同一酵素活性的二至三倍。銨態氮源下生長的小麥同時含有較高的 NAD-malate dehydrogenase 活性，但 aspartate aminotransferase 活性則與硝酸態氮源處理者相近。在 21-35°C 的溫度與 2-100% 氧氣濃度下，銨態氮源處理植株的二氧化碳補償濃度均顯著高於硝酸態氮處理的結果；前者的 glycolate oxidase 活性略高，但暗呼吸速率却顯著高於後者。二氧化碳補償濃度與氧氣濃度間有直線關係，但 0% 氧氣濃度下的二氧化碳補償濃度仍為正值，表示除了光呼吸作用外，暗呼吸與光照情況下的二氧化碳釋放與銨態氮營養導致之高二氧化碳補償濃度有關。

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