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Differences in Steady-State Net Ammonium and Nitrate Influx by Cold- and Warm-Adapted Barley Varieties[1]

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ABSTRACT

A flowing nutrient culture system permitted relatively rapid determination of the steady-state net nitrogen influx by an intact barley (*Hardeum vulgare* L. cv Kombar and Olli) plant. Ion-selective electrodes monitored the depletion of ammonium and nitrate from a nutrient solution after a single pass through a root cuvette. Influx at concentrations as low as 4 micromolar was measured. Standard errors for a sample size of three plants were typically less than 10% of the mean.

When grown under identical conditions, a variety of barley bred for cold soils had higher nitrogen influx rates at low concentrations and low temperatures than one bred for warm soils, whereas the one bred for warm soils had higher influx rates at high concentrations and high temperatures. Ammonium was more readily absorbed than nitrate by both varieties at all concentrations and temperatures tested. Ammonium and nitrate influx in both varieties were equally inhibited by low temperatures.

In most plants, low temperatures generally increase reliance upon NH_4^+ as a mineral nitrogen source (12). Ammonification is less temperature-sensitive than nitrification so that the relative availability of NH_4^+ *versus* NO_3^- increases in cold soils (6, 20). In addition, roots absorb NH_4^+ more readily than NO_3^- at low temperatures (2, 8, 15, 19, 24). These previous studies, however, examined temperature effects upon NH_4^+ and NO_3^- absorption only at substrate concentrations (0.5–1.1 mM) which usually saturate influx. The present work used new methods to monitor, in cold- and warm-adapted barley (*Hordeum vulgare* L.) varieties, the influence of low temperatures upon the balance between NH_4^+ and NO_3^- absorption at limiting substrate concentrations.

MATERIALS AND METHODS

Nutrient flow system. The flow system for steady-state net NH_4^+ and NO_3^- influx measurements is depicted in Figure 1. The reservoirs contained nutrient solutions with different concentrations of NH_4NO_3. A 3-way valve (Altex) selected which reservoir would supply nutrient solution to the cuvette. The two 2-way solenoid valves (General Valve) directed to the ion-selective electrodes either the solution leaving the reservoir or the solution leaving the cuvette. The difference in NH_4^+ and NO_3^- concentrations between these solutions was ascribed to plant nutrient uptake according to:

$$Q = \frac{J\Delta C}{m}$$

Where Q is net influx rate; J is flow rate; ΔC is concentration difference; and m is root dry weight.

A constant flow rate through the cuvette and the electrodes was maintained with piston metering pumps (Fluid Metering). The flow rate through the cuvette was varied between 1.7 and 6.0 ml min^{-1} with the first metering pump (Fig. 1) to keep the depletion of the nutrient solution between 5 and 15%; the flow rate to the electrodes was held at 1.0 ml min^{-1} with the second metering pump. To prevent air bubbles from becoming trapped under the electrodes' sensing tips, the solution flowing to the electrodes was degassed (Fig. 1). First, a peristaltic pump (Technicon) periodically injected air into the solution stream, thereby creating large air bubbles which served to absorb small bubbles. The solution stream was then heated to 45 C. Subsequently, the air was collected in the upper chamber of a bubble trap and drawn off by a second channel of the peristaltic pump (9). Ammonium in the solution was converted to ammonia for detection with an ammonia electrode by adding 10 N NaOH to the solution in a 1 to 20 proportion with a third channel of the peristaltic pump. A slotted rubber stopper with an appropriately-sized hole was positioned around the stem of the experimental plant and the stopper fitted into the cuvette to make a gas-tight seal (Fig. 2). The roots of the experimental plant were placed within a screen basket so as to permit thorough mixing of the nutrient solution yet avoid damage to delicate roots. The temperature of the cuvette was controlled by pumping a heat exchange fluid through the coils surrounding the screen basket. The temperature of the solution flowing past the electrodes was set by proportional temperature controllers regulating the heater and water bath.

An Orion 93-07 nitrate electrode and a Radiometer K701 calomel reference electrode were placed in a flow-through holder (Fig. 3). The inner filling solution for the reference electrode was saturated KCl and the outer filling solution was 0.5 M K_2SO_4. The Orion 95-10 ammonia electrode was used with a 95-00-25 flow-through cap.

All tubing and fittings were made of Teflon or stainless steel. The void volumes of the root cuvette, electrode holder, and tubing were 25, 0.3, and 4 ml, respectively.

The signals from the electrodes were amplified with an electrometer instrumentation amplifier (Fig. 4). The ion-selective electrode was connected to one input and the reference electrode to the other input; the nutrient solution was set at relative ground. Peak to peak signal noise was typically less than 0.03% of a decade in concentration (0.0003 pK units) and was insensitive to movement in room or the operation of other electrical equipment. Electrode drift was usually less than 0.3% of a decade in concentration (0.003 pK units) over an hour.

Growth Conditions. Two varieties of barley, *Hordeum vulgare* L. (cv. Kombar and Olli), were grown in an environmental chamber with 18-h days, 6-h nights, 20 C day and night, and a

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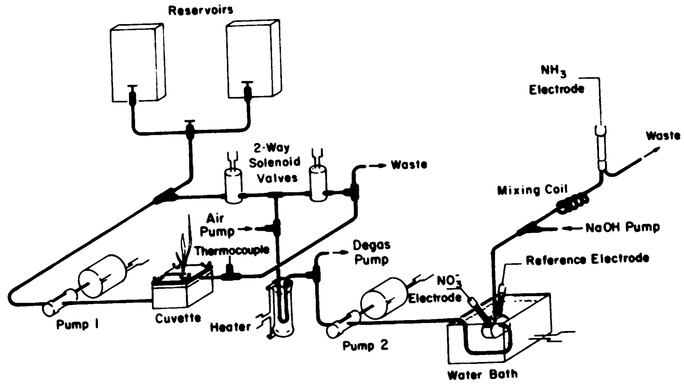

FIG. 1. Diagram of the nutrient flow system for monitoring NH_4^+ and NO_3^- uptake by an intact plant.

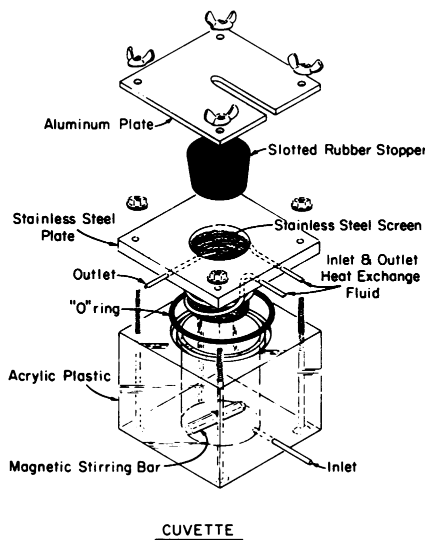

FIG. 2. Detail of the root cuvette.

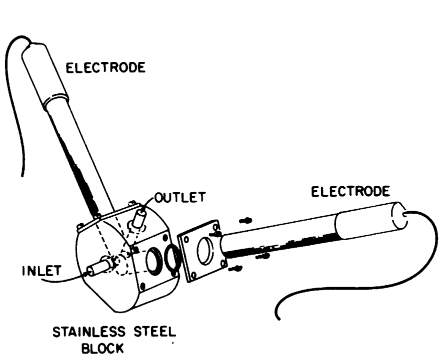

FIG. 3. Detail of the electrode holder.

PPFD[3] of 450 μE m^{-2} s^{-1} PAR at plant height. Approximately 30 plants were suspended on gauze above 20 liters of a complete nutrient solution (13) in which the initial concentration of ammonium was 2.0 mM and nitrate was 3.4 mM. After 2 weeks, when the nutrients in the solution had been depleted to about 4 μM NH_4^+ and 80 μM NO_3^-, a plant was transferred to the cuvette. At least 6 h were allowed for the plant to reach a steady-state in the cuvette before measurements were taken. The shoot was maintained at 25 C. The PPFD at plant height was 600 μE m^{-2} s^{-1}. The roots were in the dark. The solution flowing through the cuvette contained 5 to 200 μM NH_4NO_3, 4 mM Na_2SO_4, and 100 μM $CaSO_4$. Each plant was used for measurements of NH_4^+ and NO_3^- influx rate either at a given temperature and six concentrations of NH_4NO_3 (in the sequence 5, 10, 20, 50, 100, and 200 μM) or at a given concentration of NH_4NO_3 and five temperatures (in the sequence 15, 25, 15, 10, 5, and 2.5 C). Three replicate plants were monitored on consecutive days.

Kombar barley (provided by Northrup, King & Co. Fresno, CA) was bred for the warm soils of central California. Olli barley (provided by Alaskan Agricultural Extension Service, Fairbanks, AK) was bred for the cold soils of Finland.

Apparent K_m and V_{max} values were calculated using the method of Wilkinson (23).

[3] Abbreviations; PPFD, photosynthetic photon flux density.

RESULTS

Net influx rates of NH_4^+ were higher than those of NO_3^- at all concentrations and temperatures which were tested (Figs. 5 and 6). The NH_4^+ to NO_3^- influx ratio was usually much lower ($P < 0.001$) for Kombar (2.1 ± 0.2) than for Olli (3.6 ± 0.3). Kombar had higher NH_4^+ and NO_3^- influx rates than Olli at the higher concentrations and temperatures whereas Olli had higher rates than Kombar at the lower concentrations and temperatures.

The two cultivars had similar apparent K_m values for NH_4^+ influx (Table I). Kombar, in comparison to Olli, had lower apparent V_{max} values for NH_4^+ and NO_3^- influx at 5 C yet higher values at 15 C. The temperature increase from 5 to 15 C resulted in higher K_m and V_{max} values for all but NO_3^- influx by Olli.

Kombar appeared to have an optimum for NH_4^+ influx at a higher temperature than Olli under both the 10 and 50 μM NH_4NO_3 conditions (Fig. 6). The Q_{10}'s between 2.5 and 5.0 C for NH_4^+ and NO_3^- influx were all approximately equal to 4.

DISCUSSION

Methods. The technique described for monitoring net nitrogen influx has several advantages over previous ones. Net influx rates are commonly estimated by the rate at which a plant depletes NH_4^+ or NO_3^- from a nutrient solution (3–5, 15–17, 19, 22). Most researchers have relied upon colorimetric assays to determine periodically NH_4^+ and NO_3^- levels in the nutrient solution (4, 5, 15, 17, 19). These assays are relatively slow, are prone to dilution errors, and have adequate sensitivity only over a narrow concentration range. Recently, a few workers have used ion-selective electrodes to monitor either NH_4^+ (16) or NO_3^- (3, 17, 22) influx. Ion-selective electrodes require little sample preparation, respond rapidly, and are sensitive over a broad concentration range. However, in each of the previous studies which used ion-selective electrodes (3, 16, 17, 22), the nutrient solution was recycled so that ambient nutrient levels around the root were continuously changing. With the method presented here, ion-selective electrodes were sufficiently stable to permit accurate measurement of both NH_4^+ and NO_3^- depletion from a nutrient solution after a single pass through a root cuvette. Changes in net NH_4^+ and NO_3^- influx rates were detected within 5 min although 45 min were usually allotted per measurement to assure that the plant had reached a steady-state. Experimental errors were small: the coefficient of variation for three replicate plants averaged 20%. Errors generally increased at the high substrate concentrations probably because the relative depletions of NH_4^+ and NO_3^- were small at flow rates consistent with a reasonable system response time. Discrepancies in the response data (*e.g.* Olli NH_4^+ influx at 15 C and 10 and 50 μM) arose only between plants which had both been grown at different times—thus, under slightly different nutrient conditions—and received different experimental pretreatments. This indicates that net nitrogen influx in barley, as in other species (14), is sensitive to the nutritional history of the experimental plant.

The nutrient medium which was used during the influx mea-

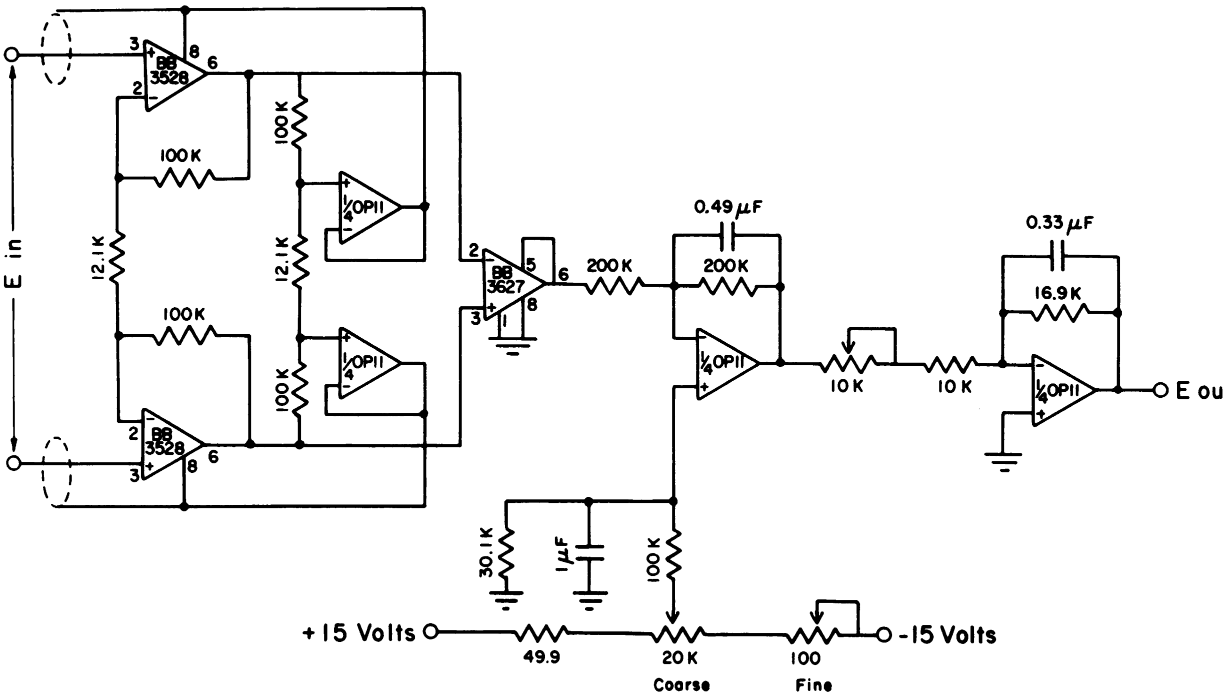

FIG. 4. Schematic of the electrometer instrumentation amplifier. (BB) Burr-Brown; (OP) Precision Monolithics.

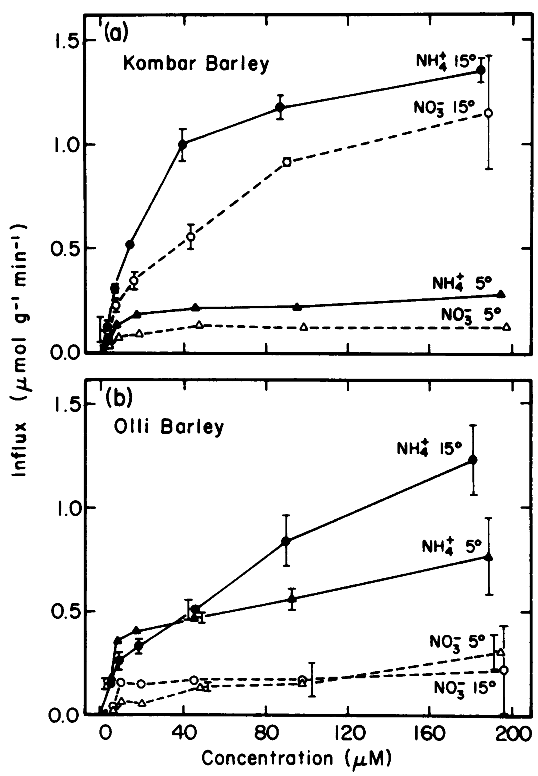

FIG. 5. Net influx of NH_4^+ and NO_3^- as a function of concentration when Kombar barley (a) or Olli barley (b) was offered NH_4NO_3. Net influx is expressed as μmol of NH_4^+ or NO_3^-/g dry weight of root per min. Mean ± SE (n = 3) is given for all data except the 5 C Kombar where mean (n = 2) is given. Error bars are included in the symbol for small SE.

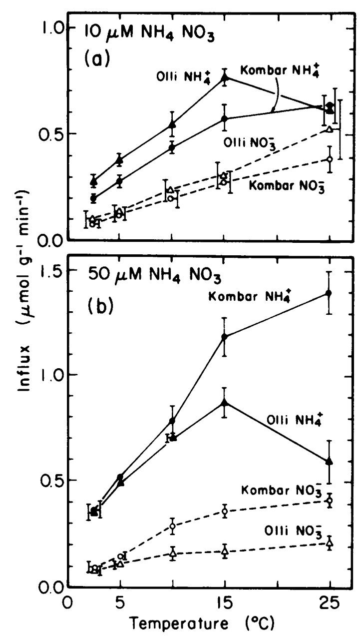

FIG. 6. Net influx of NH_4^+ and NO_3^- as a function of temperature when Kombar and Olli barley were offered 10 μM NH_4NO_3 (a) or 50 μM NH_4NO_3 (b). Net influx is expressed as μmol NH_4^+ or NO_3^-/g dry weight of root per min. Mean ± SE (n = 3) is given for all data. Error bars are included in the symbol for small SE.

surements contained 5–200 μM NH_4NO_3, 4 mM Na_2SO_4 to provide a background of constant ionic strength, and 100 μM $CaSO_4$ to maintain membrane integrity. Other ions were left out of this medium because they might influence NH_4^+ or NO_3^- influx. In particular, we avoided K^+ which may compete directly with NH_4^+ for a common transport mechanism (12) and Cl^- and $H_2PO_4^-$ which, as anions more mobile than SO_4^{2-}, tend to increase NH_4^+ influx (15). There was no indication that K^+, Cl^-, or $H_2PO_4^-$ deficiencies developed during the 12 h of influx measurements.

The electrode holder (Fig. 3) is made of conductive material and has the ion-selective and reference electrodes facing the same solution. This design reduces the effects of flow rate, solution conductivity, and temperature upon the potential difference be-

Table I. *Influence of Temperature upon K_m and V_{max} Values for NH_4^+ and NO_3^- Influx by the Kombar and Olli Barley*

Cultivar	Ion	Root Temperature	K_m	V_{max}
		C	*µM ± SE*	*µmol/g dry weight·min ± SE*
Kombar	NH_4^+	5	12 ± 3	0.28 ± 0.02
		15	28 ± 4	1.58 ± 0.07
	NO_3^-	5	12 ± 10	0.14 ± 0.03
		15	62 ± 25	1.53 ± 0.26
Olli	NH_4^+	5	10 ± 4	0.69 ± 0.07
		15	37 ± 16	1.10 ± 0.20
	NO_3^-	5	29 ± 21	0.22 ± 0.06
		15	5 ± 4	0.19 ± 0.03

tween the electrodes (21).

The electrode amplifier (Fig. 4) has several advantages over commercial pH meters. Commercial pH meters, with one or two exceptions, are single-ended amplifiers which set the reference electrode to ground (1). Double-junction reference electrodes, which are usually used with ion-selective electrodes, have impedances of about 5,000 to 20,000 ohms. Noise generated across this impedance will be seen as signal. Moreover, ion-selective electrodes with different references will mutually interfere if used simultaneously. The instrumentation amplifier used here will reject noise common to the ion-selective and reference electrode with a better than 10^6 to 1 ratio. Any number of electrodes, ion-selective or reference, can be used simultaneously without mutual interference because the potential of each electrode is measured with respect to the nutrient solution. In particular, this makes possible simultaneous use of NH_3 and NO_3^- selective electrodes which require separate reference electrodes.

The technique described here estimates net influx—the difference between gross influx and efflux. Since amines interfere with the NH_3-selective electrode and several monovalent anions interfere with the NO_3^--selective electrode, root exudation of these substances (18) would cause errors in net influx estimates. The experimental plants had the appearance of low salt-high carbohydrate plants; therefore, it is unlikely that root exudation of these substances or NH_4^+ or NO_3^- was a significant fraction of influx. When the roots were treated with NH_4NO_3-free solution, the ion-selective electrode could not detect concentration differences in the solution before and after it flowed through the root cuvette.

Plant Response. Differences in nitrogen absorption by the two barley varieties reflect differences in the environments for which they were bred. Cold soils usually have less NH_4^+ but far less NO_3^- available in the soil solution than warm soils (6, 12, 20). Olli, the cold-soil variety, had a stronger preference for NH_4^+ and had larger NH_4^+ and NO_3^- influx than Kombar at low temperatures and concentrations; whereas Kombar, the warm-soil variety, had larger influx than Olli at high temperatures and concentrations.

Low temperatures reduced net NH_4^+ and NO_3^- influx in both varieties by similar proportions. Consistent with this result, Lycklama (15) reported that net NH_4^+ and NO_3^- influx by perennial ryegrass were equally sensitive to low temperatures. By contrast, Clarkson and Warner (2) showed for Italian ryegrass that the differential between gross influx of NH_4^+ and NO_3^- increased as root temperature was lowered. They also found gross nitrogen influx by a winter-dormant variety of perennial ryegrass to be more low temperature-inhibited than that by a winter-active variety (2). Differences in the low temperature response of NH_4^+ and NO_3^- efflux might account for these conflicting results.

Both Olli and Kombar barley absorbed NH_4^+ more readily than NO_3^- at all NH_4NO_3 concentrations below saturation levels and throughout the temperature range likely to be encountered in nature (11). A similar preference for NH_4^+ over NO_3^- has been found in other species (2, 7, 15) and is caused, at least in part, by NH_4^+ inhibition of NO_3^- uptake and reduction. In Arivat barley, NO_3^- influx from 500 µM KNO_3 was reduced 25% by the addition of 500 µM NH_4Cl (17).

The results presented here for Olli and Kombar barley suggest short-term influx measurements may predict long-term performance of cultivars under different nutrient regimes. Varietal differences in barley K^+ uptake rates were correlated with yield under K^+-limited conditions (10). Experiments are now in progress to compare yields of Olli and Kombar barley at high and low NH_4NO_3 conditions.

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