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# Wild and cultivated barleys show similar affinities for mineral nitrogen

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**Summary.** The kinetics of net ammonium influx were very similar among several cultivars of barley (*Hordeum vulgare*) and several accessions of the wild taxa *H.v. spontaneum* and *H. jubatum*. For net nitrate influx, variation was greater among accessions than among species; accessions from warmer climates had faster rates than those from colder climates. These data indicate that domestication of barley has not reduced its affinity for mineral nitrogen.

Most plants obtain nitrogen – the mineral element they require in greatest quantity – from the soil solution in the form of ammonium or nitrate ions. The few studies which have examined ammonium and nitrate absorption under controlled conditions have focused on a single variety of cultivated species which has been bred for high productivity under heavy nitrogen fertilization (Haynes and Goh 1978, Saric 1981, Runge 1983). Wild plant species which are native to relatively infertile soils may be more efficient in acquiring mineral nitrogen, but the evidence is sparse and indirect (Epstein 1972, Brown 1979, Chapin 1980). In the following study, the kinetics ammonium and nitrate absorption were determined for a cultivated species (*Hordeum vulgare*), its wild subspecies (*H.v. spontaneum*), and a related wild species (*H. jubatum*) which were grown and measured under similar conditions.

## Materials and methods

Three cultivars of barley, *Hordeum vulgare*, were compared with two accessions of *H.v. spontaneum* and two accessions of *H. jubatum*. Olli barley, a cultivar which originates from Scandinavian landraces, is grown in northern latitudes such as Alaska; Kombar barley, which originates from a mixture of Scandinavian, Manchurian, and Mediterranean landraces, is grown in Oregon and Washington; Prato barley, which originates from Mediterranean landraces, is grown in central California (Schaller and Matchett, personal communication). Wild races of *H. vulgare* have been designated *H. spontaneum* but they are biologically the same species (Harlan 1976). *H.v. spontaneum* generally occurs in the Middle East; I examined accessions from an upland region and a coastal region of Israel. *H. jubatum* L. (foxtail barley), a tufted perennial ($2n=28$), can hybridize with *H. vulgare* ($2n=14$) but the resulting progeny are sterile (Morrison 1959). *H. jubatum* occupies many diverse habitats throughout North America (Bowden 1962, Best et al. 1978); I examined accessions from Fairbanks, Alaska and Davis, California.

Seeds of all taxa were germinated on wet toweling and then suspended above light-tight root boxes containing 1/20 strength of a modified Hoagland's solution (Epstein 1972) replenished every 4 days. The seedlings were grown in an environmental chamber at 25 C for a 16 h day and at 15 C for a 8 h night with a relative humidity of 70% day and night. Photon flux at plant height was 450 $\mu$mol $m^{-2}$ $s^{-1}$.

When the third leaf emerged, two to four weeks after germination, a plant was transferred to a measurement system more than 8 hours before experimental data were taken. In this measurement system, the roots and shoots were enclosed by separate, but contiguous, cuvettes (Bloom and Epstein 1984, Schulze and Bloom 1984). Ion selective electrodes monitored simultaneously the depletion of ammonium and nitrate ions from the nutrient solution flowing through the root cuvette (Bloom and Chapin 1981). This solution contained 1 mM $Na_2SO_4$ to adjust ionic strength, 0.1 mM $CaSO_4$ to maintain membrane integrity, 0.5 $\mu$M $K_2PO_4$ to avoid phosphate deficiencies, and equimolar amounts of $NH_4^+$, $K^+$, $NO_3^-$, and $Cl^-$. The pH of this unbuffered solution fluctuated only 0.1 of an unit from about pH 5.4 to 5.3 in passing through the root cuvette. The roots were kept at 15 C in the dark while the shoot was kept at 25 C under a photosynthetically active radiation of 1000 $\mu$mol $m^{-2}$ $s^{-1}$ and a vapor pressure deficit of 5 mbar. Each plant was used for a series of measurements at sequentially increasing concentrations (5, 10, 20, 50, 100 and 200 $\mu$M) of $NH_4Cl$ and $KNO_3$ and was held at a given concentration for 2 to 3 hours until the net influx of both ammonium and nitrate reached a steady rate. This protocol – in particular, the use of plants grown at low nutrients and the sequence of measurements from low to high concentrations – minimizes the influence of internal storage pools so that net influx more strongly reflects the nature of the transport system (Epstein 1972).

Three to six replicate plants were monitored on consecutive days. The apparent $K_m$'s and $V_{max}$'s were estimated according to the approach of Wilkinson (1961). Statistical comparisons were made using a 3-way ANOVA with one repeated measure: species were one classification, accessions were the second, and concentrations of ammonium or nitrate were the third repeated measure because one individual received several sequential treatments.

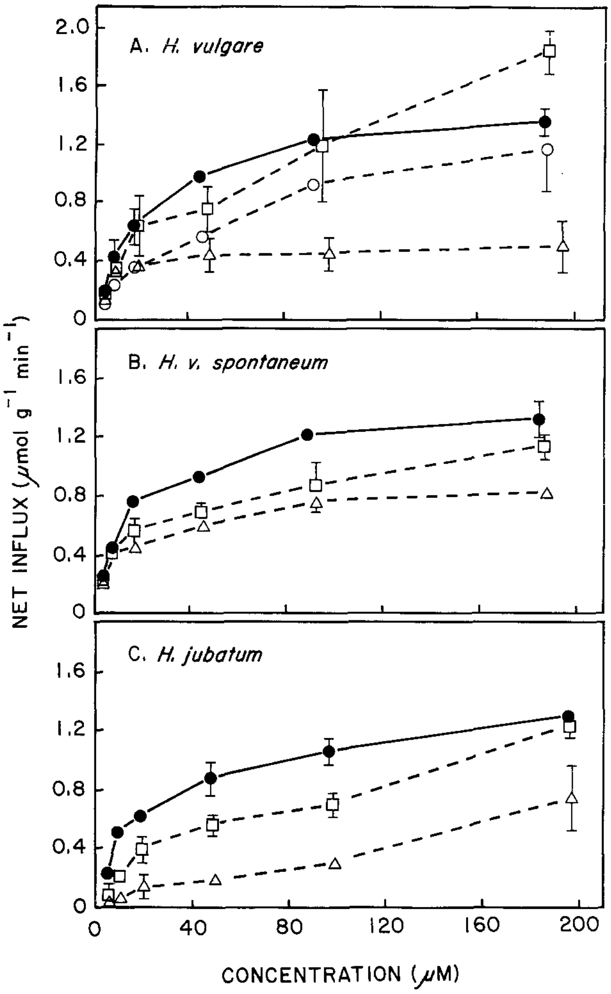

**Fig. 1 A–C.** Concentration dependence of net $NH_4^+$ and $NO_3^-$ influx per dry weight root when plants were offered equal concentrations of $NH_4Cl$ and $KNO_3$. Small ranges or standard errors are incorporated into the symbols. **A** Shown for *Hordeum vulgare* are the composite mean and range in the means of three cultivars for net $NH_4^+$ influx (●) and the means and standard errors for net $NO_3^-$ influx of the individual cultivars: Olli (△), Kombar (○), and Prato (□). **B** Shown for *H.v. spontaneum* are the composite mean and range in the means of two accessions for net $NH_4^+$ influx (●) and the means and standard errors for net $NO_3^-$ influx of the individual accessions: one collected from an upland area of Israel (△) and the other collected from the coast of Israel (□). **C** Shown for *H. jubatum* are the composite mean and range in the means of two accessions for net $NH_4^+$ influx (●) and the means and standard errors for net $NO_3^-$ influx of the individual accessions: one collected in Fairbanks (△) and the other collected in Davis (□)

## Results

The kinetics of net ammonium influx were not significantly different ($P > 0.05$) among all the cultivars and accessions tested (Table 1); therefore, Fig. 1 presents only the species' averages for the ammonium kinetics. For net nitrate influx, the differences among *H. vulgare, H.v. spontaneum,* and *H. jubatum* were less than the differences among the cultivars or accessions of each: the F-test ratio was 9.9 ($df = 2$) for the variation over species and 20.3 ($df = 1$) for the variation over cultivars or accessions. Net nitrate influx was slower for cultivars or accessions from colder soils than for those from warmer soils (Fig. 1, Table 1).

**Table 1.** Kinetics of ammonium and nitrate influx for different barleys. Apparent $K_m$ and $V_{max}$ values and the relevant standard errors were calculated by the method of Wilkinson (1961) for the data presented in Fig. 1 A–C

| Species | Variety | Ion | $K_m$ (μM) | $V_{max}$ (μmol/g-min) |
|---|---|---|---|---|
| *Hordeum vulgare* | Olli | $NH_4^+$ | 17±7 | 1.51±0.17 |
| | | $NO_3^-$ | 7±2 | 0.51±0.03 |
| | Kombar | $NH_4^+$ | 28±5 | 1.58±0.08 |
| | | $NO_3^-$ | 62±25 | 1.53±0.26 |
| | Prato | $NH_4^+$ | 20±6 | 1.41±0.12 |
| | | $NO_3^-$ | 86±45 | 2.52±0.61 |
| *H.v. spontaneum* | upland | $NH_4^+$ | 15±5 | 1.33±0.11 |
| | | $NO_3^-$ | 12±2 | 0.84±0.03 |
| | coastal | $NH_4^+$ | 15±5 | 1.45±0.13 |
| | | $NO_3^-$ | 17±5 | 1.11±0.09 |
| *H. jubatum* | Fairbanks | $NH_4^+$ | 28±6 | 1.42±0.10 |
| | | $NO_3^-$ | 187±141 | 0.92±0.43 |
| | Davis | $NH_4^+$ | 18±6 | 1.35±0.15 |
| | | $NO_3^-$ | 84±30 | 1.52±0.24 |

## Discussion

The uniformity in the kinetics of net ammonium influx which was observed for diverse *Hordeum* taxa suggests that the mechanism of ammonium absorption is a conserved physiological trait. Similarities in ammonium transport among lower organisms (Kleiner 1981) support this contention. The apparent $K_m$'s measured in the present study were 20±2 μM, higher than those found for lower organisms (0.25 to 9 μM) but in the middle of the range for higher plants (7 to 40 μM) (Van den Honert and Hooymans 1955, Tromp 1962, Lycklama 1963, Fried et al. 1965, Cox and Reisenauer 1973, McRoy and Alexander 1975).

Slower net nitrate influx in cultivars or accessions from colder soils seems reasonable in view of their natural habitat. Relative availability of nitrate versus ammonium in soils decreases rapidly with decreasing temperature because nitrification is much more sensitive to low temperatures than ammonification (Thaigalingham and Kanehiro 1973, Flint and Gersper 1974). Nitrate availability, rather than the kinetics of influx, probably limits nitrate acquisition from cold soils. Net nitrate influx, particularly for *H. jubatum,* did not strictly follow single substrate kinetics; this may reflect the multiphasic nature of nitrate absorption (Rao and Rains 1976) or the complex interactions between ammonium and nitrate (Bloom and Finazzo, unpublished data).

Although I have no data on the heritability of these responses, the plants were grown and measured under uniform conditions to reduce phenotypic variation. The observed trends among taxa are consistent with Vavilov's Law of Homologous Series: "Species and genera that are genetically closely related are characterized by similar series of heritable variations with such regularity that knowing the series of forms within the limits of one species, we can predict the occurrence of parallel forms in other species and genera (Vavilov 1949)." Net ammonium influx showed little variation in all three taxa whereas net nitrate influx varied in a similar manner for each taxa.

No clear relationship has been established between the nutrient absorption characteristics of a species and its distribution (Chapin 1980). The present study is the first direct comparison of ammonium and nitrate influx among closely related plants native to different habitats. The results indicate that domestication has not significantly reduced the ability of barley to absorb mineral nitrogen under infertile conditions.

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