Using stable isotope natural abundances (δ¹⁵N and δ¹³C) to integrate the stress responses of wild barley (*Hordeum spontaneum* C. Koch.) genotypes

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Abstract

To integrate the complex physiological responses of plants to stress, natural abundances (δ) of the stable isotope pairs ¹⁵N/¹⁴N and ¹³C/¹²C were measured in 30 genotypes of wild barley (*Hordeum spontaneum* C. Koch.). These accessions, originating from ecologically diverse sites, were grown in a controlled environment and subjected to mild, short-term drought or N-starvation. Increases in total dry weight were paralleled by less negative δ¹³C in shoots and, in unstressed and droughted plants, by less negative whole-plant δ¹⁵N. Root δ¹⁵N was correlated negatively with total dry weight, whereas shoot and whole-plant δ¹⁵N were not correlated with dry weight. The difference in δ¹⁵N between shoot and root varied with stress in all genotypes. Shoot–root δ¹⁸N may be a more sensitive indicator of stress response than shoot, root or whole-plant δ¹⁵N alone. Among the potentially most productive genotypes, the most stress-tolerant had the most negative whole-plant δ¹⁵N, whether the stress was drought or N-starvation. In common, controlled experiments, genotypic differences in whole-plant δ¹⁵N may reflect the extent to which N can be retained within plants when stressed.

Key words: *Hordeum spontaneum*, δ¹³C, δ¹⁵N, stress, drought, nitrogen.

Introduction

There is a continuing search for crops tolerant of harsh environments. One strategy is statistical, and involves examining plants from contrasting habitats, correlating their stress responses to habitat characteristics and—in suitable mapping populations—to molecular markers on the genome (Forster et al., 1997; Ellis et al., 1997; Handley et al., 1997). This approach can reveal genotypic and phenotypic facets of stress tolerance, and their interactions. In principle, genes associated with specific physiological processes involved in stress tolerance can be identified and introgressed into breeding lines (Holmberg and Bülow, 1998).

‘Stress tolerance’ comprises many physiological processes which vary quantitatively rather than qualitatively (Yeo, 1998; Zhang et al., 1999). It is often impractical to measure each process individually on many plants. One solution is to measure surrogate variables which integrate many physiological processes. Some of the most useful of these surrogates are the natural abundances (denoted as δ) of biologically important stable isotope pairs, e.g. ¹³C/¹²C and ¹⁵N/¹⁴N.

δ¹³C has been used to screen C₃ genotypes for potential water use efficiency (Ehleringer et al., 1993). A robust theory is available (Farquhar et al., 1982) with which to interpret δ¹³C variations among C₃ plants in terms of measurable physical and physiological processes. Plant δ¹³C reflects mainly the extent to which primary CO₂ assimilation is limited by carboxylation and/or CO₂ diffusion in leaves. Whole-plant δ¹³C is dominated by these processes. Internal partitioning and metabolism of primary assimilate may produce differences in δ¹³C among plant organs (Hubick and Gibson, 1993) and chemical groups (Gleixner et al., 1993; Brugnoli et al., 1998; Schmidt and Kexel, 1998). Environmental stresses (e.g. drought) modify δ¹³C in largely predictable ways, explicable ultimately via effects on the balance between stomatal conductance and carboxylation.

In contrast, δ¹⁵N has been used much less extensively in this way. Plant δ¹⁵N reflects the potentially variable

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δ¹⁵N values of external N sources and ¹⁵N/¹⁴N fractionations which occur during the assimilation, transport and loss of N. The use of δ¹⁵N in plant ecophysiology is currently at the ‘pattern generation’ or ‘hypothesis development’ stage. Taxonomic and environmental variations in δ¹⁵N are being explored and documented in natural and controlled environments (Handley and Scrimgeour, 1997; Handley et al., 1998), despite there being no theory able to explain these variations mechanistically. A theory has been proposed for δ¹⁵N (Robinson et al., 1998) which, despite being restricted to NO₃-grown plants, still demanded information about the δ¹⁵N values of external and internal N pools, information that is difficult to obtain routinely. The ‘decoding’ of plant δ¹⁵N into underlying mechanisms promises to be a non-trivial problem.

An alternative is to find statistical associations between plant δ¹⁵N, growth and specified environmental conditions, and to establish testable hypotheses about the main cause(s) of such associations. Genotypic and environmental variations in plant δ¹⁵N exist. For example, Handley et al. (1997) showed that shoot δ¹⁵N varied by up to 2.4‰ among wild barley (Hordeum spontaneum C. Koch.) genotypes grown on a common N source. Salinity caused shoot δ¹⁵N to become, on average, 2‰ more negative. But to begin interpreting plant δ¹⁵N physiologically, data for whole plants, not just shoots or roots, are required.

The purpose here is to explore further the utility of δ¹⁵N as a physiological integrator. Specifically, the aims are to (1) measure the variations in shoot, root and whole-plant δ¹⁵N in genotypes of one species (H. spontaneum) in relation to experimentally imposed environmental stresses and to site-of-origin conditions; (2) correlate these measurements with stress tolerance; and (3) assess the potential usefulness of δ¹⁵N as an integrator of stress responses.

**Materials and methods**

Caryopses of *H. spontaneum* plants collected from 30 sites (Table 1) in the Fertile Crescent, the centre of diversity for this species, were bulked under glasshouse conditions. Surface-sterilized caryopses were germinated on moist filter paper in Petri dishes on 13 September 1996. Three days later, when roots were 5–6 mm long, seedlings were transplanted into open-ended Sarsted tubes filled with 0.8% agar containing 200 mg l⁻¹ benzimidazole to suppress fungal pathogens. To minimize root damage and to prevent hypoxia around the embryo, a 3 mm diameter core of agar was removed from each tube, into which a seedling was inserted, and its roots covered immediately with cold agar extruded via a syringe. Seedlings were grown in a controlled environment (4 °C, lit by high-pressure sodium lamps at 300 µmol m⁻² s⁻¹, 8 h daylength) for 7 weeks’ vernalization, during which time the base of each tube was kept in water.

Vernalized seedlings were transferred, on 4 November 1996, into a glasshouse hydroponic system. Air temperature was maintained between 16–24 °C, and the glasshouse was ventilated with outside air to ensure steady [CO₂] and δ¹³C of source CO₂ (c. −8‰). Plants were illuminated by natural daylight, supplemented by sodium lamps. The hydroponic system consisted of three 801 troughs containing aerated half-strength Hewitt’s nutrient solution (Hewitt, 1966) with additional NaSO₄ (Epstein, 1994), changed weekly. N, as Ca(NO₃)₂ and KNO₃, was supplied at 6 mol m⁻³ with an initial mean δ¹⁵N value in solution of +1.4±0.2‰. This value became more positive between solution changes, reaching +2.2 to +4.5‰.

The causes of the gradual ¹⁵N enrichment are unknown, but could reflect the loss from roots of partly assimilated, ¹⁵N-enriched N (Robinson et al., 1998), partial denitrification of NO₃ in the non-sterile solution (Robinson and Conroy, 1999), or both. Mean solution temperature was 16±0.1 °C; mean pH was 6.0±0.1. Solution [O₂] at the end of the experiment (when O₂ depletion would have been greatest) was 92±0.3% saturation. During the experiment, the mean outdoor solar radiation receipt was ~3 MJ m⁻² d⁻¹ (DKL Mackerron, personal communication); about half this amount would have reached the plants growing in the glasshouse.

Three experimental treatments were established: controls, in which plants were maintained in the nutrient solution throughout; drought, in which plants were, 7 d after transfer to the hydroponic system, raised out of the solution to expose their roots to air for 3 h daily (Hendry, 1993); and N starvation, in which plants were deprived of all external N after 7 d growth. Mean solution temperature was 16±2.2 °C, solution [NO₃] ±0.1. Solution [O₂] and [K] were supplied as CaCl₂ 0.3% saturated solution (Robinson and Conroy, 1999), or both. Mean solution temperature was 16±0.1 °C; mean pH was 6.0±0.1. Solution [O₂] at the end of the experiment (when O₂ depletion would have been greatest) was 92±0.3% saturation. During the experiment, the mean outdoor solar radiation receipt was ~3 MJ m⁻² d⁻¹ (DKL Mackerron, personal communication); about half this amount would have reached the plants growing in the glasshouse.

Plants were harvested 16 d after transfer to the nutrient solution, long after seed C and N (and their δ values) had been trivialized in the whole plants (on average, 8 mg C and 0.4 mg N per shoot and 0.3% saturation of internal N pools, information that is difficult to obtain routinely; data for whole plants, not just shoots or roots, are required.

The purpose here is to explore further the utility of δ¹⁵N as a physiological integrator. Specifically, the aims are to (1) measure the variations in shoot, root and whole-plant δ¹⁵N in genotypes of one species (H. spontaneum) in relation to experimentally imposed environmental stresses and to site-of-origin conditions; (2) correlate these measurements with stress tolerance; and (3) assess the potential usefulness of δ¹⁵N as an integrator of stress responses.

In each trough. Each trough contained 170 plants, of which 50 were guard plants of *H. vulgare* cv. Derkado.

Plants were harvested 16 d after transfer to the nutrient solution, long after seed C and N (and their δ values) had been trivialized in the whole plants (on average, 8 and 0.4 mg C and N per seed, versus at least 90 and 7 mg C and N per harvested plant). Shoots were separated from roots. Plant material was oven-dried (60 °C for 48 h), weighed and milled. Concentrations of total C and N, and δ¹³C and δ¹⁵N were determined on subsamples (1 mg dry wt) of shoots and roots using continuous-flow isotope ratio mass spectrometry (Europa Scientific Ltd., Crewe, UK), as described (Handley et al., 1993; Scrimgeour and Robinson, 1999). δ values (%) were calculated as

\[ \delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000 \]  

(1)

where R is the ratio of ¹³C/¹²C or ¹⁵N/¹⁴N.

Data were subjected to a two-way ANOVA with Genotype and Treatment as factors. It was unnecessary to transform data to homogenize variances. Statistical analyses were done with Genstat v. 5 (Genstat 5 Committee, 1993) and Statistica™ v. 5.1 (StatSoft, Norman, Oklahoma) software.

Whole-plant δ¹⁵N (%) was calculated as an average of shoot and root δ¹⁵N weighted by the total N contents (mg) of shoots and roots:

\[ \text{Whole-plant} \delta^{15}N = \frac{\text{Shoot} \delta^{15}N \times \text{Shoot N} + \text{Root} \delta^{15}N \times \text{Root N}}{\text{Shoot N} + \text{Root N}} \]  

(2)

A dimensionless 'stress index' (SI) was calculated for each genotype to account for innate size differences among genotypes.
in their stress responses:

\[ SI = \frac{W_{\text{unstressed}} - W_{\text{stressed}}}{W_{\text{unstressed}}} \]  \hspace{1cm} (3)

where \( W_{\text{unstressed}} \) and \( W_{\text{stressed}} \) are the mean dry weight per plant (mg) of unstressed and stressed plants, respectively. The \( SI \) can range from 0 to 1. \( SI \) values → 0 represent little stress, plants becoming increasingly stressed—in terms of the effect of the environment on growth—as \( SI \) → 1.

**Results**

**Plant growth**

Total dry weight per plant varied significantly \( (P < 0.001) \) with both Treatment and Genotype, but there was no Genotype × Treatment interaction (Table 2). Twelve genotypes showed significant reductions in total dry weight, relative to controls, in response to drought; five genotypes responded significantly to N starvation (Fig. 1). Of those genotypes which did not respond to either stress, almost all had relatively slow growth rates (as indicated by their ranking in Fig. 1, i.e. Genotypes 15 to 11). Exceptions to this were Genotypes 7 and 8 which, notably, were unaffected by N starvation despite being two of the most productive genotypes.

**Whole-plant \( \delta^{15}N \)**

There were highly significant effects of Genotype and Treatment on whole-plant \( \delta^{15}N \) \( (P \leq 0.001; \text{ Table 2}) \). Variations in whole-plant \( \delta^{15}N \) were dominated by the Treatment main effect, but the interaction was significant. Whole-plant \( \delta^{15}N \) in controls (Fig. 2) ranged from −0.9‰ to +0.3‰ (Genotype 12) to +1‰ (Genotype 3). Given that source NO\(_3\) had a \( \delta^{15}N \) value > +1‰, the plants clearly discriminated against \( ^{15}N \) (where discrimination ~ source \( \delta^{15}N \)–whole-plant \( \delta^{15}N \)). However, discrimination could not be quantified because of the temporal variability in source \( \delta^{15}N \) (see Materials and methods).

Whole-plant \( \delta^{15}N \) responded significantly \( (P < 0.05) \) to drought in 16 of the 30 genotypes; 10 genotypes responded significantly to N starvation (Fig. 2). When drought or
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Table 2. Summary analyses of variance of the measured characters of the *H. spontaneum* genotypes (Geno.) in the three treatments (Trt.)

<table>
<thead>
<tr>
<th></th>
<th>Total dry weight</th>
<th>Whole-plant δ¹⁵N</th>
<th>Shoot δ¹⁵N</th>
<th>Root δ¹⁵N</th>
<th>Shoot–root δ¹⁵N</th>
<th>Whole-plant δ¹³C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>F</em></td>
<td><em>P</em></td>
<td><em>F</em></td>
<td><em>P</em></td>
<td><em>F</em></td>
<td><em>P</em></td>
</tr>
<tr>
<td>Geno.</td>
<td>12.7</td>
<td>&lt;0.001</td>
<td>2.73</td>
<td>&lt;0.001</td>
<td>2.38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Trt.</td>
<td>39.4</td>
<td>&lt;0.001</td>
<td>53.2</td>
<td>&lt;0.001</td>
<td>32.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Geno. × Trt.</td>
<td>1.22</td>
<td>0.234</td>
<td>1.80</td>
<td>0.001</td>
<td>1.78</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

N starvation had a significant (*P* < 0.05) effect on whole-plant δ¹⁵N, this almost always became more negative than in controls (Fig. 2); the only exception to this was Genotype 5. The mean difference in δ¹⁵N between control and stressed plants was 0.6 (±0.1 SE) and 0.3 (±0.1 SE) ‰ for the drought and N starvation treatments, respectively. The largest such difference under drought was 1.4‰ in Genotype 15 and 1.1‰ when plants were N-starved (Genotype 26).

### Shoot and root δ¹⁵N

Shoot δ¹⁵N, root δ¹⁵N, and the difference between them, varied significantly with Genotype and Treatment, and there was a significant interaction between these (Table 2). Drought and N starvation caused shoot δ¹⁵N to become, on average, significantly more negative than in controls, N starvation having a larger effect (Table 3). Drought caused root δ¹⁵N to become, on average, 2.1‰
more negative than controls. This trend was reflected in all genotypes except one (Genotype 2) whose root $\delta^{15}N$ was unaffected by drought (data not shown). In contrast, N starvation caused root $\delta^{15}N$ to become significantly less negative than the controls.

All genotypes responded significantly ($P<0.05$) to one or both stresses in terms of the shoot–root $\delta^{15}N$ difference (Fig. 3). In most genotypes, the difference in shoot–root $\delta^{15}N$ was slightly positive in controls (mean $0.7\pm0.1\%$), i.e. shoots were more $^{15}N$-enriched than roots. In response to drought, shoot–root $\delta^{15}N$ usually increased (mean $2.5\pm0.1\%$) compared with the control. By contrast, shoot–root $\delta^{15}N$ usually decreased under N starvation (mean $-0.5\pm0.1\%$). When the absolute shoot–root difference in $\delta^{15}N$ between control and drought treatments was plotted against that between control and N starvation treatments, there was a significant, inverse relation between them (Fig. 4).

There were no significant correlations between shoot $\delta^{15}N$ and shoot N content or concentration, nor between root $\delta^{15}N$ and root N content or concentration (data not shown).

$\delta^{13}C$

Whole-plant $\delta^{13}C$ varied significantly ($P<0.001$) with Genotype and Treatment, and there was a strong interaction (Table 2). As with whole-plant $\delta^{15}N$, Treatment had the dominant effect on $\delta^{13}C$. In controls, whole-plant $\delta^{13}C$ varied by $1.8\%$, ranging from $-32.4\%$ (Genotype 14) to $-30.6\%$ (Genotype 6). Shoot and root $\delta^{13}C$ were both affected significantly by drought, but not N starvation (Table 3). Under drought, $\delta^{13}C$ became less negative than in controls and this effect was more pronounced in shoots than roots.

There were no significant correlations between $\delta^{13}C$ and $\delta^{15}N$ in shoots or roots (data not shown).

**Correlations between plant dry weight and $\delta$ values**

The heaviest plants had the least negative shoot $\delta^{13}C$ values ($P<0.05$; Table 4). Root and whole-plant $\delta^{13}C$ values varied similarly with total dry weight in control and droughted plants, but not when plants were N-starved. Shoot $\delta^{15}N$ was not correlated with total dry weight, although the heaviest plants had the most negative root $\delta^{15}N$ values ($P<0.05$; Table 4). Absolute shoot–root $\delta^{15}N$ differences in droughted or N-starved plants were correlated positively with total dry weight ($P<0.05$; Table 4).

Whole-plant $\delta^{15}N$ was not correlated with total dry weight in any treatment (Table 4). Many genotypes, however, showed no dry weight response to either stress (Fig. 1). Excluding the least productive and least responsive genotypes (i.e. Genotypes 15 to 11 in the drought treatment, and 15 to 5 in the N starvation treatment: Fig. 1), revealed significant positive correlations between whole-plant $\delta^{15}N$ and the stress index, $SI$ ($P<0.05$, $n=18$ for drought and $n=8$ for N starvation; Fig. 5). The least stressed of those plants ($SI<0$) had the most negative whole-plant $\delta^{15}N$. In droughted plants, this relation was due to shoot $\delta^{15}N$, root $\delta^{15}N$ showing no correlation with the $SI$. In N-starved plants, however, both shoot and

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**Table 3. Mean ($\pm SE$) $\delta^{15}N$ and $\delta^{13}C$ values (%) and total N concentrations (%) dry weight of roots and shoots in control, droughted and N-starved *H. spontaneum* plants**

Data comprise measurements of all 30 genotypes. Means in the same row followed by different letters are significantly different (LSD, $P<0.001$, $n=30$).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Drought</th>
<th>N starvation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot $\delta^{15}N$</td>
<td>$-0.1\pm0.1$ a</td>
<td>$-0.4\pm0.1$ b</td>
<td>$-0.6\pm0.1$ c</td>
</tr>
<tr>
<td>Root $\delta^{15}N$</td>
<td>$-0.8\pm0.1$ a</td>
<td>$-2.9\pm0.1$ b</td>
<td>$-0.1\pm0.1$ c</td>
</tr>
<tr>
<td>Shoot $\delta^{13}C$</td>
<td>$31.9\pm0.1$ a</td>
<td>$30.2\pm0.1$ b</td>
<td>$32.0\pm0.1$ a</td>
</tr>
<tr>
<td>Root $\delta^{13}C$</td>
<td>$30.8\pm0.1$ a</td>
<td>$30.3\pm0.1$ b</td>
<td>$30.7\pm0.1$ a</td>
</tr>
<tr>
<td>Shoot [N]</td>
<td>$5.5\pm0.04$ a</td>
<td>$3.8\pm0.07$ b</td>
<td>$2.4\pm0.05$ c</td>
</tr>
<tr>
<td>Root [N]</td>
<td>$4.2\pm0.05$ a</td>
<td>$2.6\pm0.03$ b</td>
<td>$1.5\pm0.02$ c</td>
</tr>
</tbody>
</table>

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**Fig. 3.** Mean shoot–root differences in $\delta^{15}N$ of control (■), droughted (○) and N starved (△) *H. spontaneum* genotypes. Genotypes are ranked in order of increasing shoot–root $\delta^{15}N$ of controls. Code numbers (Table 1) of genotypes which responded significantly ($P<0.05$, LSD) to drought are underlined on the right-hand axis, those which responded significantly to N starvation are underlined on the left-hand axis.
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Fig. 4. Absolute differences in shoot–root $\delta^{15}$N between control and N-starved plants in relation to those between control and droughted plants. Genotype numbers (Table 1) are shown against each symbol. The regression $y=2.43-0.679x$ is significant at $P<0.05$ ($r=-0.72$, $n=30$).

**Table 4. Correlations (Pearson product-moment coefficients, $r$) between total dry weights in control, droughted or N-starved plants and $\delta^{13}$C and $\delta^{15}$N values, and total N concentrations measured in those treatments**  

<table>
<thead>
<tr>
<th>Total dry weight per plant</th>
<th>Control</th>
<th>Droughted</th>
<th>N-starved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot $\delta^{13}$C</td>
<td>0.57</td>
<td>0.52</td>
<td>0.48</td>
</tr>
<tr>
<td>Root $\delta^{13}$C</td>
<td>0.42</td>
<td>0.50</td>
<td>n.s.</td>
</tr>
<tr>
<td>Whole-plant $\delta^{13}$C</td>
<td>0.55</td>
<td>0.53</td>
<td>n.s.</td>
</tr>
<tr>
<td>Shoot $\delta^{15}$N</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Root $\delta^{15}$N</td>
<td>-0.49</td>
<td>-0.38</td>
<td>-0.42</td>
</tr>
<tr>
<td>Whole-plant $\delta^{15}$N</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Shoot–root $\delta^{13}$N</td>
<td>n.s.</td>
<td>0.51</td>
<td>0.42</td>
</tr>
<tr>
<td>Shoot [N]</td>
<td>n.s.</td>
<td>-0.38</td>
<td>-0.59</td>
</tr>
<tr>
<td>Root [N]</td>
<td>0.53</td>
<td>0.48</td>
<td>0.53</td>
</tr>
</tbody>
</table>

Absolute $\delta^{15}$N were significantly ($P<0.05$) correlated with the SI. In contrast, neither shoot nor root $\delta^{13}$C was correlated with the SI (data not shown).

**Correlations between plant dry weight and total N concentrations in shoots and roots**

Total dry weight of control plants was always correlated positively ($P<0.05$) with the concentration of total N in root dry matter, but not in shoots (Table 4). When plants were stressed, however, total dry weight became negatively correlated with total N concentration in shoots: the heaviest plants in the stress treatments had the smallest total N concentrations in their shoots.

**Correlations between isotope natural abundances and long-term meteorological averages**

Of the long-term meteorological averages available for the sites-of-origin (Pakniyat et al., 1997), only mean humidity at 14.00 h local time was correlated consistently with any isotopic data (Table 5). The more humid the site-of-origin, the less negative were the experimentally determined $\delta^{13}$C values of genotypes from those sites.

Absolute shoot–root $\delta^{15}$N values of control and N-starved plants were also correlated positively with humidity at site-of-origin, as was shoot $\delta^{15}$N under N starvation. Only in droughted plants was $\delta^{13}$C correlated with mean annual and mean January temperatures. Mean January temperature was correlated positively with shoot, root and whole-plant $\delta^{15}$N in the N starvation treatment. The only isotopic measurement with which mean annual rainfall was correlated was root $\delta^{15}$N of controls.
Table 5. Correlations (Pearson product-moment coefficients, r) between δ13C and δ15N values and long-term meteorological averages for sites-of-origin (Table 1 in Pakniyat et al., 1997)

Blank cells indicate non-significant correlations (P > 0.05). MAT, MAuT and MJaT: mean annual, August and January temperatures (°C), respectively; MAR: mean annual rainfall (mm). Humid: mean humidity (%) at 14.00 h local time.

<table>
<thead>
<tr>
<th></th>
<th>MAT</th>
<th>MAuT</th>
<th>MJaT</th>
<th>MAR</th>
<th>Humid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control plants</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Shoot δ13C</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Root δ13C</td>
<td>0.39</td>
<td>0.67</td>
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<tr>
<td>Whole-plant δ13C</td>
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<td>0.59</td>
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<tr>
<td>Root δ15N</td>
<td></td>
<td></td>
<td>−0.37</td>
<td></td>
<td>−0.56</td>
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<td>Whole-plant δ15N</td>
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<td>Shoot–root δ15N</td>
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<td>Droughted plants</td>
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<td>Shoot δ13C</td>
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<tr>
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<td>Whole-plant δ13C</td>
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<td>Shoot δ15N</td>
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<tr>
<td>Shoot–root δ15N</td>
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<td>Shoot–root δ15N</td>
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Discussion

Variations in whole-plant δ15N in relation to drought and N starvation

The 1.3‰ range in whole-plant δ15N in controls (Fig. 2) indicates the extent to which genotype may influence 15N/14N fractionations in *H. spontaneum* when plants have access to a common N source. By comparison, whole-plant δ13C varied by 1.8‰ among controls, a larger range than that found for well-watered genotypes of *H. vulgare* (Acevedo, 1993); greater variability is to be expected among individuals from wild populations than among those from genetically narrower breeding lines. Whole-plant δ15N may vary significantly, therefore, for reasons unconnected to changes in the δ15N values of external N source(s).

15N/14N discriminations between whole plants and an external N source can have only one general cause: the loss from plants of some isotopically altered N. There is no experimental or theoretical evidence that N uptake itself fractionates 15N/14N. Possible mechanisms of N loss include: the shedding of senescent plant parts; the loss of N volatiles (e.g. NH3, NOx, amines, HCN; Wetselaar and Farquhar, 1980) from leaves into the atmosphere; and the loss of soluble N (e.g. NO3−, amino acids: Jones and Darrah, 1993; Van der Leij *et al.*, 1998) from roots into the rooting medium. Each of these is considered as a possible explanation for the data in Fig. 2.

Senescence can be discounted: the young *H. spontaneum* plants showed little visible leaf senescence. In an annual species such as *H. spontaneum*, there is little root senescence until synchronous mortality occurs towards the end of an individual’s life, as in *Triticum aestivum* L. (Van Vuuren *et al.*, 1997).

NH4-N lost from organic matter can have δ15N values down to −40‰ (Handley *et al.*, 1996, 1999). Such a net loss of 14N will be reflected by an increase of whole plant δ15N in proportion to the fraction of total plant N lost, is most likely to occur via stomata, and to increase with stomatal conductance (which should, in turn, cause shoot δ13C to become more negative). Therefore, if N volatilization is significant, shoot δ15N should increase as shoot δ13C becomes more negative. No such relation was found for *H. spontaneum*, and N volatilization was unlikely to have caused the variations in whole-plant δ15N shown in Fig. 2. A similar conclusion was reached by other authors (Bergersten *et al.*, 1988; Robinson and Conroy, 1999) who were unable to attribute variations in whole-plant δ15N of *Glycine max* (L.) Merrill or *Panicum coloratum* L., respectively, to N volatilization.

The loss of soluble N from roots does occur, as does its partial resorption (Jones and Darrah, 1993). As with
gaseous \( {\delta^{15}N} \) losses, little is known of their magnitude or \( {\delta^{15}N} \) values relative to whole-plant \( {\delta^{15}N} \) (Schmidt and Kexel, 1998). Robinson et al.'s theory suggested that the loss from roots of organic-N which was less \( {^{15}N} \)-enriched than total N was consistent with the \( {\delta^{15}N} \) values measured for other N pools (e.g. root and shoot total N and \( NO_3^- \)) (Robinson et al., 1998). This is not conclusive evidence but, pro tempore, N exudation seems the most likely determinant of variations in whole-plant \( {\delta^{15}N} \) among the \( H. \) spontaneum genotypes under the conditions of the experiment. Direct tests of this possibility are now being done.

In a common, controlled environment in which source N is defined and for which a good estimate of its \( {\delta^{15}N} \) value exists, genotypic differences in whole-plant \( {\delta^{15}N} \) values reflect the extent to which plants retain N in their tissues. Agronomic interest in stress tolerance does not usually concern genotypes whose tolerance involves slow growth rates and, probably, small yields. Rather, the aim is to identify genotypes which have the potential to grow well should conditions allow, and to produce economically and nutritionally acceptable yields when conditions are unfavourable. In Fig. 5, the genotypes which were most productive and stress tolerant (i.e. were heaviest in comparison with their potential growth when unstressed, as indicated by their small \( SI \) values) were those which probably retained most N. Yet, those genotypes expressed the largest discriminations against \( {^{15}N} \), i.e. they had the most negative whole-plant \( {\delta^{15}N} \) values. According to isotope mass balance arguments, if those genotypes lost only small amounts of N, that N must have had 'exotic' \( {\delta^{15}N} \) values significantly different from total plant N (Yoneyama, 1995; Schmidt and Kexel, 1998). It may be that such plants, when stressed, restrict the loss of N from their roots to only one or two amino acids, say, which happen to have 'exotic' \( {\delta^{15}N} \) values (\( NO_3^- \) exudation from the roots of N-starved barley is negligible: Van der Leij et al., 1998).

Conversely, genotypes expressing the smallest discriminations against \( {^{15}N} \) were smaller, contained less N and, perhaps, lost relatively more N from their tissues. They may have less capacity to restrict N loss from their roots when stressed than did more stress-tolerant genotypes. Lost N would then comprise a diverse mixture of N compounds with a correspondingly wide range of \( {\delta^{15}N} \) values. The average \( {\delta^{15}N} \) of lost N would then be closer to that of the total N, resulting in smaller whole-plant discriminations against \( {^{15}N} \). These intriguing possibilities also require explicit testing.

**Variations in \( \delta \) values in relation to conditions at sites-of-origin**

No consistent or strong associations were found between plant \( {\delta^{15}N} \) measured under the experimental conditions and habitat characteristics (Table 1) or long-term meteorological averages (Table 5). \( {\delta^{13}C} \) values, by contrast, varied consistently with site-of-origin humidity. The association between less negative \( {\delta^{13}C} \) (measured at a common ambient vapour pressure deficit; vpd) and greater site-of-origin humidity is opposite to that found between ambient vpd and \( {\delta^{13}C} \) discrimination in several C3 species (Madhavan et al., 1991; Masle et al., 1993). As commonly reported for other plant species, Handley et al. found that shoot \( {\delta^{13}C} \) was most negative in \( H. \) spontaneum genotypes from sites receiving the least rainfall annually (Handley et al., 1994). That correlation was not found in this hydroponic experiment.

A strong inverse relation \( (r = -0.59, P < 0.001) \) has been found between mean annual rainfall and site-averaged foliar \( {\delta^{15}N} \) for a wide range of ecosystems (Handley et al., 1999). Such was the variability in \( {\delta^{15}N} \), however, that many samples were required to reveal significant correlations with environmental factors. Discrepancies between samples collected from the field and those produced in common, controlled environments have been well-documented for \( {\delta^{13}C} \) (Condon and Richards, 1993); similar constraints will, no doubt, apply to research with \( {\delta^{15}N} \).

Some genotypes which originated from one locality expressed similar \( {\delta^{15}N} \) responses to stress, while others from another site showed very different responses. For example, the Tabigha genotypes (2 and 27) showed opposite shoot–root \( {\delta^{15}N} \) responses to drought and N starvation (Fig. 4), despite there being no significant difference in their growth or whole-plant \( {\delta^{15}N} \) whether stressed or unstressed (Figs 1, 2). By contrast, the three Neve Ya’ar genotypes (1, 25 and 28) had very similar shoot–root \( {\delta^{15}N} \) responses in the two stress treatments (Fig. 4). While Genotypes 1 and 28 grew similarly (Fig. 1) and had similar whole-plant \( {\delta^{15}N} \) values (Fig. 2), Genotype 25 differed from these in shoot–root \( {\delta^{15}N} \) when droughted, but not when N-starved.

**The utility of plant \( {\delta^{15}N} \) as a physiological integrator**

Although the statistically significant correlations between \( {\delta^{15}N} \) and total plant dry weight were not particularly strong (Table 4), they were of similar magnitude to some which have been measured between total dry weight and \( {\delta^{13}C} \) (Condon and Richards, 1993). The correlations between whole-plant \( {\delta^{15}N} \) and \( SI \), especially for N starvation, but less convincingly for drought (Fig. 5), suggest that causal links exist between \( {\delta^{15}N} \) and stress tolerance.

The possibility that, in controlled experiments, whole-plant \( {\delta^{15}N} \) may reflect N retention suggests that whole-plant \( {\delta^{15}N} \) could be used to screen plants for this agriculturally and ecologically important trait. It will not be easy to apply such an idea in field settings because of the uncertainties which continue to surround the identity
and δ15N value(s) of plant-available N species in soil (Handley and Scrimgeour, 1997; Handley et al., 1999). Whole-plant δ15N in droughted or N-starved *H. spontaneum* was always more negative than in controls, confirming the report for shoot δ15N of hydroponically grown plants in response to salinity (Handley et al., 1994). The whole-plant δ15N of many genotypes did not respond to either drought or N starvation, whereas shoot–root δ15N did. Shoot–root δ15N may, therefore, be a more sensitive indicator of incipient stress than shoot, root or whole-plant δ15N.

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**References**


