Heterogeneity in Carbon Isotope Discrimination in Leaves, Stalks and Spikes of Ten Annual Wild Triticeae Species

J. Zámečník and V. Holubec

Research Institute of Crop Production, 161 06 Praha-Ruzyně, Czech Republic
e-mail: zamecnik@vurv.cz, holubec@vurv.cz

Abstract: The wild Triticeae species are confined mostly to arid regions and thus, they are suitable material for study of their adaptation to dry conditions. Species from semidesert localities of Mediterranean, Balkans and Near East (genera Eremopyrum, Heteranthenium, Taeniatherum, Dasypyrum, and Aegilops) and wild wheats (Triticum) from steppe localities were studied. The studied species can be divided into two groups on the basis of their carbon isotope discrimination (Δ). The first group, which had high Δ, consists of species (Ta. crinitum, A. tauschii, H. piliferum, E. orientale, A. markgrafii, A. comosa) collected in the driest localities such as semidesert sites. The second group from steppe localities, which consists of D. villosum, T. urartu, T. boeoticum, and T. monococcum, had relatively low Δ. We conclude that wild species originally grown in dry regions discriminate more carbon isotope as they are adapted to the dry condition by stomata opening and/or Rubisco activity. We examined from high to low carbon isotope discrimination values of the flag leaves, stalks, and spikes.

Keywords: carbon isotope discrimination; delta; wild Triticeae; heterogeneity; drought adaptation

Wild wheat relatives, members of the Triticeae tribe, could represent a valuable source of genetic variation for improvement of abiotic stress tolerance in cultivated wheat (Triticum aestivum L.). A better knowledge of the adaptive strategies developed by these species is needed. Species of Aegilops (goatgrass) are well adapted to dry environments. Aegilops tauschii is of particular interest, since gene transfer from this drought-adapted species to bread wheat is relatively easy (Vet al. 2004).

Isotopic differences in diverse elements make it possible to demonstrate that fractionation events occur in nature. Fractionation processes are thermodynamic or kinetic reactions that produce different abundances in reactants and products. Two important processes in which $^{13}$CO$_2$ is discriminated are diffusion and carboxylation. Diffusion fractionation is dependent on molecular mass. Diffusion of heavy atoms or molecules is slower than diffusion of lighter ones. Carboxylation by RUBISCO discriminates strongly against $^{13}$CO$_2$ typically in C$_3$ plants. Carbon isotope discrimination (Δ) is a very promising method for evaluation transpiration efficiency (TE). Δ is a measure of the ratio of stable carbon isotopes ($^{13}$C/$^{12}$C) in plant dry matter compared with the same ratio in the atmosphere.

Discrimination against $^{13}$CO$_2$ is closely negatively correlated with the transpiration efficiency integrated over the life of the sampled plant material (Farquhar & Richards 1984; Condon et al. 1990; Condon et al. 1992). Carbon isotope discrimination has been proposed as a useful indicator of TE in wheat (Farquhar & Richards 1984; Ehdai et al. 1991) and in numerous species including other cereals (Condon et al. 1993; Matus et al. 1996). The negative correlation of Δ and water use efficiency (WUE, measured either as net photosynthesis/transpiration or plant biomass produced/water transpired) led to Δ being proposed as selection criteria for WUE (Farquhar & Richards 1984). It is determined by the intercellular partial pressure of CO$_2$, which is largely regulated by variation in stomatal aperture (Farquhar et al. 1989). Under drought stress, Δ can be considered as a good

The main objectives of the present study were (i) to examine the variation in plant carbon isotope discrimination as a tool which represents water use efficiency among accessions of the wild Triticeae collected in their native habitats where they are well acclimated (ii) to analyze relationships in carbon isotope discrimination of different wild Triticeae organs in generative stage important for reproduction at the end of plant ontogenesis and (iii) to evaluate relationship between carbon isotope discrimination and the total carbon content in plant parts.

**MATERIAL AND METHODS**

**Material.** Mature plants of wild Eremopyrum, Heteranthelium, Taeniatherum, Dasypyrum, Triticum and Aegilops species were collected at the end of vegetation period from semidesert and steppe localities of the Mediterranean, Balkans and Near East from steppe localities (Table 1). The localities are characterized by a dry summer season with high temperature and high irradiance.

**Methods.** Collected plants were dried until they reached a constant weight. Twenty flag leaf blades, twenty 10 cm sections from the upper part of the stalk, and twenty spikes per genotype were randomly sampled from fully mature plants. The leaf, stalk and spike samples were separately grounded to a fine powder in a metal ball mill. Carbon isotope ratios were measured to 0.10‰ by isotope ratio mass spectrometer (IRMS). The total carbon content and carbon isotope composition of leaves stalks and spikes were determined by an Elemental Analyzer.

**Table 1. Species used for study, their geographic site and ecological characteristic**

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Latin Name</th>
<th>Abbreviation</th>
<th>Geographic Site</th>
<th>Ecology</th>
<th>Collector/donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>C2100705</td>
<td>Aegilops comosa SIBTH. et SM. ssp. heldreichii (BOISS.) EIG</td>
<td>A. com</td>
<td>NW Korfu on Isle of Korfu, Near Potamos River</td>
<td>saline grassland</td>
<td>Gatersleben</td>
</tr>
<tr>
<td>C2100428</td>
<td>Aegilops markgrafii (GREUTER) HAMMER var. markgrafii</td>
<td>A. mar</td>
<td>Hasan Dag, nr. Hamrun, Nigde, Turkey</td>
<td>bazalt, lava pall, 1460 m</td>
<td>VH</td>
</tr>
<tr>
<td>TA2457</td>
<td>Aegilops tauschi COSS.</td>
<td>A. tau</td>
<td>Turkey</td>
<td>dry steppe</td>
<td>Kansas</td>
</tr>
<tr>
<td>C2300014</td>
<td>Dasypyrum villosum (L.) CANDARGY</td>
<td>D. vil</td>
<td>Sopot, 7 km N of Kumanovo, Macedonia</td>
<td>dry steppe, 415 m</td>
<td>VH</td>
</tr>
<tr>
<td>C3400006</td>
<td>Eremopyrum orientale (L.) JAUB. et SPACH</td>
<td>E. ori</td>
<td>Dzrvez, 2 km E of Erevan to Garni, Armenia</td>
<td>semidesert steppe, 1000 m</td>
<td>VH</td>
</tr>
<tr>
<td>C3700001</td>
<td>Heteranthelium piliferum (BANKS et SOLANDER) HOCHST.</td>
<td>H. pil</td>
<td>Iran</td>
<td>semidesert</td>
<td>Logan</td>
</tr>
<tr>
<td>C0104087</td>
<td>Triticum boeoticum BOISS. var. symbolense (Flaksb.) A. Filat.</td>
<td>T. boe1</td>
<td>Izgrev – Peela, Rodopi Mts., Bulgaria</td>
<td>dry steppe, 680 m</td>
<td>VH</td>
</tr>
<tr>
<td>C0106065</td>
<td>Triticum boeoticum BOISS.</td>
<td>T. boe2</td>
<td>Bayindr, Konya, Turkey</td>
<td>dry steppe, 1195 m</td>
<td>VH</td>
</tr>
<tr>
<td>C3300006</td>
<td>Taeniatherum crinitum (SCHRREBER) NEVSKI</td>
<td>Ta. cri</td>
<td>Niftaular nr. Dzebraili, Georgia</td>
<td>semidesert steppe, 1000 m</td>
<td>VH</td>
</tr>
<tr>
<td>C0106421</td>
<td>Triticum monococcum L. var. elusianum L.R. Galara Kiss</td>
<td>T. mon</td>
<td>Transylvania, Romania</td>
<td>old land race</td>
<td>A. Szabo</td>
</tr>
<tr>
<td>C0106076</td>
<td>Triticum urartu THUM. ex GANDIL.</td>
<td>T. ura</td>
<td>Sanli Urfa, Turkey</td>
<td>dry steppe to semidesert, 620 m</td>
<td>ICARDA</td>
</tr>
</tbody>
</table>

VH = Vojtech Holubec
(EuroVector 3028/HT) coupled to an Isotope Ratio Mass Spectrometer (IRMS Isoprime). International standards were used so all delta values are inter-comparable. We use delta notation (\(\delta\)) to express carbon isotope ratio conveniently:

\[
\delta^{13}C = \left(\frac{\text{[}{^{13}}C/^{12}C\text{]}_{\text{sample}}}{\text{[}{^{13}}C/^{12}C\text{]}_{\text{standard}}} - 1\right) 1000
\]

(1)

Delta values are expressed in parts “per mil” (‰) for plant material \(\partial^{13}C\) is always a negative number.

The standard error of determination was 0.10‰ on average. Fractionation is also known as carbon isotope discrimination (\(\Delta\)). The \(\Delta\) value of the samples was obtained according to the formula:

\[
\Delta \approx (\delta_a - \delta_p)/(1 + \delta_p),
\]

(2)

\[
\Delta \approx \delta_{\text{source}} - \delta_{\text{product}}
\]

(FARQUHAR et al. 1989), where \(a\) and \(p\) refer to air and plant carbon isotope composition, respectively. The carbon isotope composition of air was \(-8.3\)‰, \(\delta^{13}C\) of \(C_3\) plants is reported approximately \(-25\)‰. Each sample was analyzed at least 2 times. Direct transpiration efficiency was not measured in this study. Statistica 6.1 (StatSoft) software was used for statistical evaluation of the data.

**RESULTS AND DISCUSSION**

*Taeniatherum crinitum* exhibited the highest overall carbon isotope discrimination and *Triticum monococcum* the lowest (Figure 1). In general, *Dasypyrum* and all studied *Triticum* had the lowest carbon isotope discrimination. Interestingly, *Ta. crinitum* had values of more than \(\Delta 20\)‰, which is characteristic for species with low water use efficiency. The *Triticum* spp. on the other hand, had low carbon discrimination, corresponding to the high WUE.

For most of the species studied, the carbon composition of the leaves was low in comparison to rest of the plant (Figure 2). Most species had a carbon composition of 48–50% in their stalks and spikes. *Aegilops comosa* and *Heteranthelium piliferum* were exceptional in this respect, having values of only 37% and 30%, respectively. The low carbon composition of the leaves of *H. piliferum* contrasts with the values obtained for its stalks and spikes (Figure 3)
Carbon isotope discrimination proved to be a valuable tool for evaluating contribution of different organs to grain filling and yield. We examined the ratio of carbon isotope discrimination of individual organs (Figure 3) by plotting the individual organ Δ against the average Δ of these organs. There is a significant linear trend for all organs. There is low Δ of spike and higher for stalk and leaf at low values of Δ average. The difference in Δ of stalk and spike is smaller with higher Δ average. The importance of spike Δ increased and Δ of stalk decreased for the higher average values of Δ. The Δ of leaves increased and was higher for all range of Δ average. Similarly Teulat et al. (2002) found higher Δ in leaves than in kernels in field grown barley. The relative contributions of the Δ values of flag leaf, peduncle and spike to Δ grain of durum wheat genotypes were 51, 29, and 21%, respectively (Harri et al. 2000). With the exception of Aegilops comosa, we obtained the highest carbon
isotope discrimination in the leaves of all species (characterised by the highest WUE). The two accessions of one species *T. boeitcum* seem to behave like the majority of other species regardless *T. boe1* originating from Bulgaria had higher Δ on average than *T. boe2* originating from Turkey.

The wild wheat relative *Aegilops geniculata* Roth. had highly significant correlations among carbon isotope discrimination (Δ) and plant temperature depression, biomass and grain weight per plant (Zaharieva et al. 2001). Strong negative correlations between transpiration efficiency and carbon isotope discrimination in wheat (*Triticum aestivum* L.) suggest that selection of progeny with low Δ may increase transpiration efficiency and aerial biomass under water-limited conditions (Rebetzke et al. 2002). The results of QTL study of carbon isotope discrimination measured in mature grains from plants grown in the Mediterranean field conditions indicate that several chromosomal regions are involved in Δ variation (Teulat et al. 2002).

The studied species can be divided in to two groups on the basis of their carbon isotope discrimination (Δ). The first group, which had high Δ, consists of *Ta. crinitum*, *H. piliferum*, *E. orientale*, *A. tauschii*, *A. markgrafii*, and *A. comosa* collected in the driest localities such as semidesert sites. The second group, which came from steppe localities, had relatively low Δ. It included *D. villosum*, *T. rartu*, *T. boeoticum* and *T. monococcum*. The wild *Triticaceae* species from dry areas had similar carbon isotope discrimination as the advanced wheat cultivars grown under full water supply. The first group had Δ on average close to the Δ average value (21.4%) of 14 winter wheat cultivars grown under full water supply. The second group were closer to the average value of 19.8‰ of the same cultivars grown under water stress (Zámečník unpublished). The indigenous wild species growing in dry regions had higher discrimination values. These comparisons suggest a better adaptation of wild *Triticaceae* species to dry conditions. The wild *Triticaceae* species had stomata open and/or high activity of RUBISCO even at very dry conditions. The carbon isotope discrimination was higher in leaves than in stalks and lowest in spikes in most species.

**Acknowledgements.** This work was supported by Project No. QD1352 and by the National Programme for Plant Genetic Resources Conservation of the Ministry of Agriculture of the Czech Republic.

**References**


of rainfed bread wheat. Crop Science, 42: 739–745.

