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

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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, Heteranthelium, 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 (Valkoun *et 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; Ehdaie *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 predictor of stomatal conductance (Condon *et al.* 1990). Carbon isotope discrimination positively correlates with yield and growth cycle duration (Araus *et al.* 1997) and negatively correlates with leaf temperature (Acevedo 1993). The strong correlation between carbon isotope discrimination and yield in durum wheat grown in the Mediterranean conditions has been confirmed (Hafsi *et al.* 2000). It is easier to measure carbon isotope discrimination than transpiration efficiency. Measuring of carbon isotope ratio in dry matter of a plant organ provides a measure of the discrimination of carbon isotope of this organ and carbon isotope ratio of matter coming from other plant organs.

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

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

VH = Vojtech Holubec

(EuroVector 3028/HT) coupled to an Isotope Ratio Mass Spectrometer (IRMS Isoprime). International standards were used so all delta values are intercomparable. We use delta notation (δ) to express carbon isotope ratio conveniently:

$$\delta^{13}C = ([(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}] - 1)\ 1000 \quad (1)$$

delta values are expressed in parts "per mil" (‰) for plant material $\delta\ ^{13}C$ is always a negative number.

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

$$\Delta \approx (\delta_a - \delta_p)/(1 + \delta_p), \quad (2)$$

$$\Delta \approx \delta_{source} - \delta_{product} \quad (3)$$

(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 Δ 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)

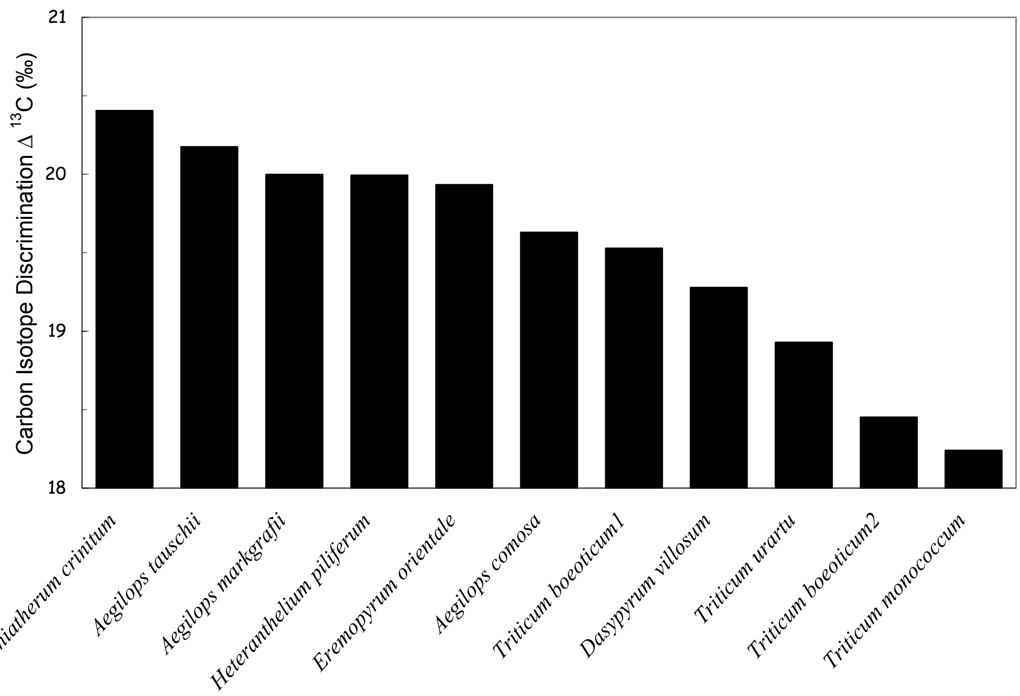

Figure 1 Average carbon isotope discrimination of species collected in their original growing area typical with shortage of precipitation, from semidesert localities of the Mediterranean, Balkans and Near East (genera *Eremopyrum, Heteranthelium, Taeniatherum, Dasypyrum*, and *Aegilops*), wild wheats (*Triticum*) from steppe localities

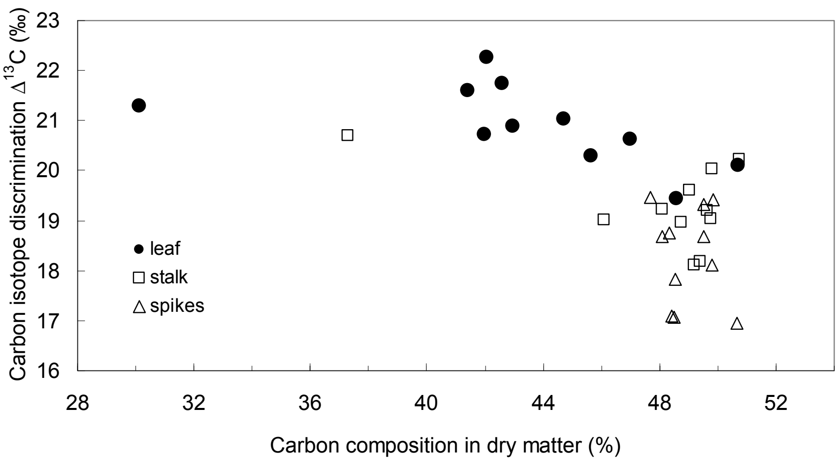

Figure 2. Relation of carbon isotope discrimination of leaf, stalk and spike of wild *Triticeae* species to their total carbon composition in dry matter

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 (HAFSI *et al.* 2000). With the exception of *Aegilops comosa*, we obtained the highest carbon

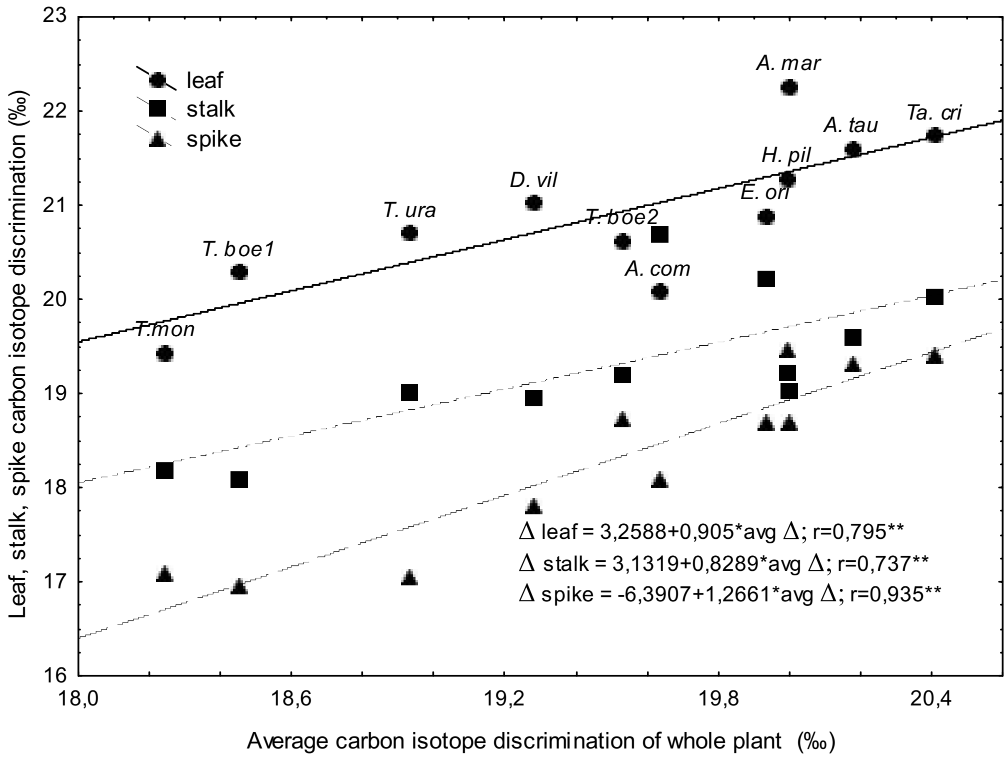

Figure 3. Relation of carbon isotope discrimination of separate plant parts (leaf, stalk and spike) on the average carbon isotope discrimination of tested plant parts. Coefficients of linear regression are significant on $P < 0.99$ levels

isotope discrimination in the leaves of all species (characterised by the highest WUE). The two accessions of one species *T. boeticum* 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 *Triticeae* 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 (Záměčník unpublished). The indigenous wild species growing in dry regions had higher discrimination values. These comparisons suggest a better adaptation of wild *Triticeae* species to dry conditions. The wild *Triticeae* 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.

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