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Communication

Carbon Isotope Ratios in Ear Parts of Triticale[1]

Influence of Grain Filling

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ABSTRACT

Four triticale (×*Triticosecale* Wittmack) genotypes were grown under rainfed conditions with limited irrigation support in Lleida in northeast Spain. For each variety, samples consisting of 10 tillers with half-sterilized spikes were taken three times from anthesis to maturity. Carbon isotope ratios ($\delta^{13}C$) were then determined in water extracts from ear bracts (glumes, paleas, and lemmas), awns and flag leaves, and in powdered kernels. For the half-sterilized spikes, carbon isotope analysis was carried out separately in bracts and awns from fertile and nonfertile spikelets. The $\delta^{13}C$ in the water-soluble fraction of awns, glumes, and glumells from fruitless spikelets was significantly higher than that from fertile spikelets sampled at mid-grain filling. Differences in $\delta^{13}C$ among sterile and fertile spikelets were not significant in samples taken a few days after anthesis or at maturity. These results are in accordance with some degree of refixation by awns and ear bracts of the CO_2 respired by grains during grain filling. There was progressively higher $\delta^{13}C$ from flag leaf blades to awns, glumes, and glumells. This variation in $\delta^{13}C$ along plant parts may be caused by differences in the ratio of assimilation rate to CO_2-diffusive conductance. Values of $\delta^{13}C$ of mature kernels were between the values at anthesis and mid-grain filling for the water-soluble fraction of flag leaves and inner bracts and were fairly similar to those of glumes and awns.

Conservation of respired CO_2 by an efficient recycling mechanism in fruit could provide a significant source of C for yield productivity (2). Cereal crops have received the greatest attention in this regard (4, 9, 16). For example, Kriedemann (9) studied the refixation of respired carbon dioxide in whole ears by measuring CO_2 evolution of ears in a CO_2-free environment in the light and dark, reporting that evolution was lower in the light and suggesting that refixation was taking place. However, evidence of bracts and awns refixing the internal CO_2 released from growing grains remains to be elucidated.

Carbon isotope ratios of plant tissue have been proposed as an indicator not only of water use efficiency or photosynthetic metabolism (5) but also of recycling of respired CO_2 in the forest understory (12). On the other hand, it has been suggested that fractionation of plant carbon may occur during respiration, resulting in a less negative $\delta^{13}C$ for respired CO_2 (7). In the present study, $\delta^{13}C$ is proposed as an indicator of some degree of refixation of internal (respired) CO_2 during grain filling in bracts and awns of triticale (×*Triticosecale* Wittmack) ears. In addition, evidence of differences in $\delta^{13}C$ values among plant parts of this species is discussed.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Four triticale (×*Triticosecale* Wittmack) genotypes were used in this study. Cultivars E1 and E2 had winter habit and European origin, whereas M1 and M2 were spring types released by Centro Internacional de Mejora de Maiz y Trigo in Mexico. All genotypes were grown under rainfed conditions with limited irrigation support in Lleida in northeast Spain. Plants were grown in six-row plots of 3.7 m length and 1.2 m width. Sowing date was early January 1990 at a seeding density of 500 seeds/m^2. Anthesis took place between the end of April for the spring types and mid-May for the winter genotypes. Rain was frequent during grain filling. For each variety, at least 200 main tiller (culms) were tagged at anthesis (yellow anthers). At this stage, half the spikelets of about 100 tiller spikes per variety were subsequently sterilized by piercing the carpel with a sharp needle.

Characterization of Grain Filling

Samples from untreated main tillers (termed controls in this study), consisting of 10 randomly located tillers per genotype, were taken every 4 to 6 d from anthesis to the end of senescence. Grain fresh weight was then measured in 3 to 5 center grains per ear. Grain dry weight was determined after drying to constant weight in a forced-air oven at 80°C. The pattern of grain filling, as determined by the changes in dry weight and water content of individual kernels from untreated spikes, is shown in Figure 1.

For each genotype, samples consisting of 10 tillers with half-sterilized spikes were taken three times from anthesis to maturity (see roman numerals in Fig. 1). Samples from fertile ears were also taken at the second sampling date. For each

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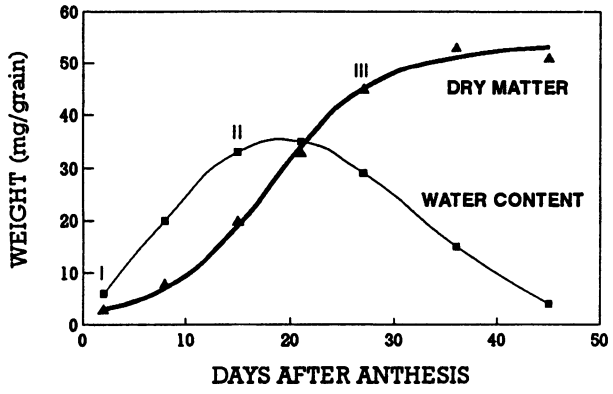

Figure 1. Relationship between water content (■), dry weight (▲), and days after anthesis for a winter triticale cv E1. Continous lines for dry weight represent logistic curve fit to mean dry weight. Roman numerals show sampling dates for carbon isotope discrimination: I, early; II, mid-grain filling; III, late-grain filling.

sample, the $^{13}C/^{12}C$ ratio was determined in water extracts from ear bracts (glumes, paleas, and lemmas), awns, and flag leaves, and in powdered kernels. For the half-sterilized spikes, carbon isotope analysis was carried out separately in bracts and awns from sterile and fertile spikelets.

Carbon Isotope Analysis

The $^{13}C/^{12}C$ ratios were determined mass spectrometrically at Isotope Services, Inc. (Los Alamos, NM). Results were expressed as $\delta^{13}C$ values, where

$$\delta^{13}C(‰) = [(R \text{ sample}/R \text{ standard}) - 1] \times 1000$$

R being the $^{13}C/^{12}C$ ratio. The standard for comparison was a secondary standard calibrated against Peedee belemnite carbonate.

For the ear parts and the flag leaf blade, oven-dried samples were ground, boiled for 15 min in distilled water, and filtered. The filtrate was freeze-dried and powdered for $^{13}C/^{12}C$ analysis. Statistical analyses of grain filling and $\delta^{13}C$ data were completed using PC-SAS/STAT (10).

RESULTS AND DISCUSSION

Differences in $\delta^{13}C$ among Plant Parts

For the set of genotypes assayed, the same pattern of $\delta^{13}C$ values among plant parts was observed. Carbon isotope ratios of mature kernels were between the values at anthesis and mid-grain filling for the water-soluble fraction of flag leaves and inner bracts, and fairly similar to those of glumes and awns. Data appear to indicate that a substantial portion of the photosynthates in the grain come not only from the flag leaf but also from ear parts.

There were progressively higher $\delta^{13}C$ values from flag leaf blades to awns, glumes, and glumells (Table I). In the same way, Hubick and Farquhar (7) reported in barley less carbon isotope discrimination in dry matter of heads than of leaves. This variation in $\delta^{13}C$ along plant parts may be caused by differences in the ratio of assimilation rate to CO_2 diffusive conductance (5), although variations in the photosynthetic metabolism of the plant parts may also be involved (2, 15, 16). With regard to the former possibility, although the CO_2

Table I. *Carbon Isotope Ratios ($\delta^{13}C$) in the Water-Soluble Extracts from Flag Leaves and Different Ear Parts from Fruitless and Fertile Spikelets of Half-Sterilized Ears of Triticale*

Samples were obtained at three grain-filling stages: 2 d after anthesis, mid-grain filling, and physiological maturity. Samples from untreated (check) culms were also obtained at mid-grain filling. Values are means of four genotypes.

	Flag Leaf	Awns	Glumes	Lemma + Palea	Grain
			‰		
Anthesis					
Sterilized		−24.65[a]	−23.70	−22.97	
	−25.32				
Fertile		−25.25	−23.71	−23.09	
Organ mean	−25.92 b[b]	−24.95 b	−23.71 a	−23.03 a	
Mid-grain filling					
Sterilized		−23.77[a]	−23.69[a]	−22.17	
	−24.73				
Fertile		−24.69	−24.21	−22.52	−24.08
Check	−24.42	−24.49	−24.69	−22.54	−24.22
Organ mean	−24.58 c	−24.32 b	−24.20 b	−22.41 a	−24.15 b
Physiological maturity					
Sterilized		−25.52	−25.18	−24.21	
	−27.13				
Fertile		−25.70	−25.18	−24.54	−24.14
Organ mean	−27.13 d	−25.61 c	−25.18 bc	−24.38 ab	−24.14 a

[a] Significantly different from the fruitless spikelets for the same organ and sampling date at $P < 0.05$. [b] Within organs and sampling dates, means followed by the same letter are not significantly different at $P < 0.05$.

conductance is basically stomatal in the leaf, stomatal conductance is probably much less important in awns and even more in ear bracts (1). Thus, ear parts from cereals have distinct xeromorphic features, such as thick epidermis and cuticle in the dorsal side (1, 6). Nonetheless, the higher $\delta^{13}C$ values of ear parts compared with flag leaf might be related to a higher water use efficiency (5, 11, 13).

Changes in $\delta^{13}C$ of Ear Parts as Affected by Growing of Grains

The $\delta^{13}C$ in the water-soluble fraction of awns, glumes, and glumells from fruitless spikelets were higher than those from fertile spikelets from the same spike sampled at mid-grain filling (Table I). All the genotypes assayed showed the same pattern of response. Interestingly, differences in $\delta^{13}C$ between sterile and fertile spikelets were not significant in samples taken a few days after anthesis or at maturity (Table I), when respiration from grains is reported to be smallest (4). These results are in accordance with the existence of some degree of refixation by awns and ear bracts of the CO_2 respired by grains during grain filling.

The reported high dark respiration in ears of cereals (3, 6) is well understood to be a consequence of the respiration in the grain and in the nonphotosynthesizing tissues of the ears. Knoppik et al. (8) pointed out that both maintenance respiration of the bracts and respiration in the kernels associated with grain-filling processes contribute to the respiration rates of wheat ears, the latter being quantitatively about twice as much as the former. Diffusive surface conductance of the external epidermis of bracts and awns is small and may cause the accumulation of respired CO_2 in the internal gas space of these ear parts (2, 6). In addition, during grain filling, uptake of external CO_2 may be reduced in bracts as a result of the growing grains, which block a number of (ventral-side) stomata facing the grains. These stomata are excluded from gas exchange with the atmospheric air and take up only the CO_2 respired by the grains.

At first, no further discrimination would occur during refixation of respired CO_2 if the ear parts were gas tight (5). However, some quantities of CO_2 are likely to leak out of these ear parts to the surrounding air. Presumably, inner ear bracts are more gas tight than glumes and awns; thus, discrimination of respired CO_2 would be greater from glumells to glumes and awns. Awns would receive inner CO_2 from respective lemmas through the intercellular air spaces. The increasingly greater differences in $\delta^{13}C$ between glumells (0.35‰), glumes (0.52‰), and awns (0.92‰) from fruitless and fertile spikelets (Table I, mid-grain filling) are in accordance with this theory.

The suggestion raised by Hubick and Farquhar (7) that organs having CO_2 recycling should show $\delta^{13}C$ values that are less negative than those of translocated carbon is not supported by our results. Even when ear tissues show $\delta^{13}C$ values that are less negative than those of flag leaf (Table I), many $\delta^{13}C$ values from ear tissues are similar or more negative than values from growing kernels (i.e. the main source of respired CO_2). In addition, $\delta^{13}C$ values of bracts from fertile florets do not show less negative values than those from sterilized florets.

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