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Toward a New Use for Carbon Isotope Discrimination in Wheat Breeding

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Abstract: A major obstacle in the effort to develop drought tolerant varieties of wheat (*Triticum aestivum* L.) is phenotyping. Traits known to contribute to improved drought tolerance, such as water-use behavior, reliance on stem reserve carbohydrates, and the ability to develop deep roots, require time and resource-intensive screening techniques. Plant breeding programs often have many thousands of experimental genotypes, which makes testing for each of these traits impractical. This work proposes that carbon isotope discrimination (Δ) analysis of mature grains may serve as a relatively high-throughput approach to identify genotypes exhibiting traits associated with drought tolerance. Using Δ as a proxy for stomatal conductance and photosynthetic capacity, assumptions can be made regarding fundamental plant physiological responses. When combined with knowledge of the terminal drought severity experienced in a particular environment, genotypes exhibiting conservative and rapid water use, deep roots, and reliance on stem reserve carbohydrates may be identified. Preliminary data in support of this idea are presented. Further verification of this use for grain Δ will better equip wheat breeding programs to develop more drought tolerant varieties.

Keywords: Drought tolerance; wheat breeding; carbon isotope discrimination

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1. Introduction

Drought stress is recognized as a major threat to wheat (*Triticum aestivum* L.) productivity worldwide, reducing yield in more than half of all wheat growing regions [1,2]. The development of tolerant varieties is complicated by the variability associated with drought stress, including timing of onset, duration and severity, as well as environmental contributors, such as high temperature, low humidity, and limited precipitation [3]. A successful breeding approach will define an ideotype possessing a complement of traits that mitigates the drought affecting a particular location [4].

In Mediterranean climates, crops often mature into the hotter, drier and less humid months of summer. An extreme, or a combination, in any of these environmental factors could result in terminal drought. A number of traits are associated with improved yield in a terminal drought environment. These traits include early flowering, reliance on stem reserve carbohydrates, deep roots, and water-use behavior, specifically conservative and high water use [5,6]. Apart from flowering time, all of these traits are challenging to evaluate. Given the many thousands of experimental lines in a breeding program, it is often not practical to evaluate these traits. Rather than screening genotypes for each of these traits individually, it may be more practical to conduct a preliminary screen with a single measurement that can function as an indicator for the more specific traits of interest. Carbon isotope discrimination (Δ) analysis of mature grains may serve as a relatively high-throughput approach to identify genotypes exhibiting these traits.

Plants with C_3 metabolism discriminate in favor of ^{12}C over ^{13}C during the conversion of CO_2 into biomass. For comparison of plant isotopic composition among and between species, carbon isotope discrimination (Δ) was proposed by Farquhar and Richards [7] and is defined as the deviation from unity of atmospheric $^{13}C/^{12}C$ divided by plant $^{13}C/^{12}C$. Early efforts to understand the drivers of discrimination led to the finding that Δ shares a positive and linear relationship with the ratio of internal leaf CO_2 concentration (C_i) and the atmospheric CO_2 concentration (C_a) [8]. This ratio is principally impacted by stomatal conductance and photosynthetic capacity, allowing researchers to make inferences regarding fundamental plant physiology based on Δ analysis [9]. As an integrated measurement of photosynthetic capacity and stomatal conductance over the time that the analyzed tissue developed, high Δ tissue may be assumed to have experienced high stomatal conductance, low photosynthetic capacity, or both, whereas the opposite may be assumed for low Δ plant tissue.

By combining these assumptions with knowledge of the terminal drought environment, it may be possible to classify genotypes based on more specific drought tolerance traits. Instead of directly phenotyping genotypes for water use behavior, reliance on stem reserves, or deep roots, grain Δ may serve as an initial, albeit less accurate, screening tool for genotypes exhibiting these traits. Preliminary data on this novel use of grain Δ are presented.

2. Materials and Methods

This study was performed with collected data from a panel of 480 advanced soft white winter wheat varieties from U.S. Pacific Northwest breeding programs (Oregon State University, University of Idaho, Washington State University, USDA-ARS, and private breeding companies) [10]. The panel was grown in an unreplicated augmented block design near Pullman, WA (46°7′ N; −117°1′ W) during 2015, 2016, and 2017. In 2017, the panel was also grown in an unreplicated augmented block design near Lind, WA (46°8′ N; −118°6′ W) and near Pendleton, OR (45°7′ N; −118°6′ W). In each environment, 20% of the plots were planted to the check cultivar 'Madsen' (PI 511679) [11].

Pullman trials were planted on 8 October 2014, 8 October 2015, and 3 November 2016. Lind and Pendleton trials were planted on 7 September 2016 and 4 October 2016, respectively. Pullman trials were harvested on 23 July 2015, 5 August 2016, and 10 August 2017. Lind and Pendleton trials were harvested on 20 July 2017 and 25 July 2017, respectively. The mean annual precipitation (including data from 2010 to 2017) for these locations is 435 mm at Pullman, 235 mm at Pendleton, and 230 mm at Lind. Pullman's mean annual temperature (including data from 2010 to 2017) is 8.8 °C, whereas Lind and Pendleton average 10.2 °C and 11.8 °C, respectively. Location-specific temperature and precipitation data were collected from AgWeatherNet stations (https://weather.wsu.edu/) close to the Pullman and Lind field sites, whereas an AgriMet weather station (https://www.usbr.gov/pn/agrimet/wxdata.html) was used to access environmental data for the Pendleton field site.

Plot heading date was determined as the number of days from 1 January to 50% of fully exposed heads. Plant height was measured from the base of the plant to the top of fully emerged heads, not including the awns. Heading date was collected in all trials except in Pendleton 2017. A ZÜRN 150 plot combine harvester (ZÜRN Harvesting, Schöntal-Westernhausen, Germany) was used to harvest plots at maturity, and grain yield (t ha^{-1}) was computed from the grain weight per plot.

Grain samples (20 g) from each genotype were milled into flour using an Udy cyclone sample mill (Udy Corporation, Fort Collins, CO, USA). Between 3.00 and 5.00 mg of flour for each genotype were packed into sterile tin capsules. An isotopic mass spectrometer at Washington State University's Stable Isotope Core Laboratory was used to determine grain carbon isotope percentages. Carbon isotope composition ($\delta^{13}C$) was calculated by comparing the ratio of ^{13}C to ^{12}C for each sample (R_s) against the same ratio of a Vienna Pee Dee Belemnite (VPDB) standard (R_{VPDB}) by the following formula: $\delta^{13}C$ (per mil) = $[(R_s/R_{VPDB}) - 1] \times 1000$. For conversion to Δ values, the following formula was used: $\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p)$, where $\delta^{13}C_a$ and $\delta^{13}C_p$ are the carbon isotope compositions of the atmosphere and plant samples, respectively [8]. As is convention, the $\delta^{13}C_a$ value was assumed to be −8.0 per mil [12]. Carbon isotope composition values are often expressed in terms of 'per mil' to indicate that the original value was multiplied by 10^3. This conversion is performed as a matter of convenience, allowing Δ values to appear as whole numbers.

All genotypes were ranked for grain yield in each location-year based on standard deviation from the mean. The index ranked genotypes from 1 to 10 based on increments of 0.5 standard deviations from the mean (Table S1), where 1 was equal to genotypes with the lowest yield and 10 was equal to those with the highest yield [13]. Genotypes were considered high for grain yield if their environment-specific yield was at least one standard deviation above the mean. Similarly, genotypes were ranked low for Δ if their environment-specific Δ value was less than negative one standard deviation from the mean, and genotypes were ranked high for Δ if their environment-specific Δ value was greater than one standard deviation from the mean. The same indexing system was used to rate genotypes for heading date and plant height. Heading date has the potential to confound comparisons of Δ between high yielding genotypes. As plants mature into the later months of summer, the threat of drought in Mediterranean climates is often elevated, and, by extension, so too is the likelihood of stomatal closure [5]. Holding all else constant, early heading genotypes may express high Δ as a result of high stomatal conductance, whereas later heading genotypes, having encountered water-deficit conditions, likely close their stomata, reducing C_i/C_a and ultimately lowering the Δ signature of the grain tissue. Controlling for heading date helps to eliminate this confounding effect. Pearson correlations, analysis of variance, and summary statistics (mean, range, standard deviation) were calculated for phenotypic traits using R statistical software (R Core Team, Version 3.4.3, 2017, http://www.r-project.org). Since rankings are presented in terms of standard deviations, calculated least significant differences (LSD) were converted back into rank values based on the standard deviations that were used for each trait in each location-year.

3. Results and Discussion

All agronomic traits, grain yield, and Δ varied widely within and between environments (Table 1). Precipitation occurring over the growing season totaled (mm) 265 at Pendleton, 294 at Lind, 384 at Pullman 2015, 462 at Pullman 2016, and 377 at Pullman 2017 (Table S2). Average maximum air temperature over the growing season varied across locations. In order from highest average maximum air temperature to lowest—Pendleton 2017 (15.6 °C), Pullman 2015 (14.6 °C), Lind 2017 (14.2 °C), Pullman 2016 (13.4 °C), Pullman 2017 (11.7 °C) (Table S3). There were no significant differences for Δ ($p = 0.7409$), grain yield ($p = 0.6632$), plant height ($p = 0.5257$), or heading date ($p = 0.9984$) for the Madsen check cultivar within each environment, providing evidence for limited spatial field variation. Therefore, no further adjustments or transformation of the data were completed. Heading date confounded comparisons of Δ between high yielding genotypes. In every environment, heading date was found to correlate significantly with Δ (Pullman 2015 $r = -0.25$, $p < 0.001$; Pullman 2016 $r = -0.09$, $p < 0.05$; Pullman 2017 $r = -0.13$, $p < 0.01$; Lind 2017 $r = -0.17$, $p < 0.05$). The negative association between heading date and Δ is reasonable given the expected impact that flowering time can have on grain Δ values collected from a terminal drought environment. Therefore, genotypes with early heading—less than negative one standard deviation from the mean—and late heading—greater than one standard deviation from the mean—were excluded from further evaluation. By removing early and late heading genotypes from further analysis, the influence of heading date on grain Δ was limited. An evaluation of high yielding genotypes from each environment revealed a number of productive low and high Δ genotypes in each location-year (Tables 2 and 3). Across all location-years, a total of 30 high yielding, low Δ and 53 high yielding, high Δ genotypes were observed.

Table 1. Data summary for grain yield (GY, t ha^{-1}), Δ (carbon isotope discrimination, per mil), plant height (HT, cm), and heading date (HD, Julian) collected on a panel of 480 advanced soft white winter wheat lines adapted to the Pacific Northwest.

Pullman 2015	**GY**	**Δ**	**HT**	**HD**
Mean	6.3	17	89.9	151.2
Range	3.7–9.2	15.4–18.5	71.1–127.0	144.0–155.0
SD [a]	0.98	0.53	7.64	2.13
Pullman 2016	**GY**	**Δ**	**HT**	**HD**
Mean	5.4	17.6	89.0	156.5
Range	2.0–7.6	16.4–18.7	68.6–121.9	147.0–160.0
SD	0.83	0.4	7.41	2.12
Pullman 2017	**GY**	**Δ**	**HT**	**HD**
Mean	7.8	18.1	96.5	164.8
Range	6.0–9.4	17.0–19.1	76.2–129.5	154.0–172.0
SD	0.63	0.37	7.59	3.23
Lind 2017	**GY**	**Δ**	**HT**	**HD**
Mean	3.0	16.5	80.2	150.2
Range	1.2–5.0	15.6–17.4	53.3–101.6	142.0–155.0
SD	0.65	0.34	7.41	2.02
Pendleton 2017	**GY**	**Δ**	**HT**	**HD**
Mean	6.6	18.2	109.5	-
Range	3.4–8.9	16.9–19.4	78.7–142.2	-
SD	1.09	0.41	8.41	-

[a] Standard Deviation. -, N/A.

Table 2. Low Δ, high grain yield genotypes from each Pacific Northwest environment, including index ratings for Δ, grain yield (GY), plant height (HT), and heading date (HD).

	Pullman 2015			
Name	**Δ Rating [a]**	**GY Rating**	**HT Rating**	**HD Rating**
ARS010780-3C	2	10	6	7
J971186-1	1	9	7	5
J980032-5	1	9	10	7
5J062167-2	3	10	8	5
J950297-003	3	10	7	5
WA8152	2	8	9	5
5J061865-9	3	9	9	4
5J061865-8	3	8	7	4
6J020288-1	3	8	9	6
F/E-39	3	8	7	7
A00122	3	8	6	7
LSD	4	1	2	2
	Pullman 2016			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
X970071-1	3	8	6	5
LSD	-	-	-	-

Table 2. *Cont.*

	Pullman 2017			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
ID00-475-2DH	3	9	5	5
J970057-1	3	8	8	6
ID655	3	9	9	4
LSD	**6**	**1**	**2**	**1**
	Lind 2017			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
5J062167-2	2	9	9	3
X970185-1C	3	10	7	5
X970229	3	10	9	4
X980302-8C	3	10	5	4
ARS98237	1	8	8	5
J950412-01	3	9	9	3
ARS010762-2C	3	8	6	5
SSD02071	3	8	6	4
ARS97230-6C	3	8	4	6
LSD	6	1	2	2
	Pendleton 2017			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
6J020288-1	2	8	7	-
ARS010762-2C	2	8	8	-
X970008-3	2	8	7	-
X010817-4L	3	8	7	-
X970163-3	3	8	5	-
CARA	3	8	4	-
LSD	5	1	2	-

[a] Rating is based on standard deviation from the mean (Table S1), where a score of 1 was assigned to genotypes with the lowest trait value relative to other genotypes in the same location-year. Least significant differences (LSD) ($p < 0.05$) were calculated and then converted back to the rating system based on the standard deviation. -, N/A.

Table 3. High Δ, high grain yield genotypes from each Pacific Northwest environment, including index ratings for Δ, grain yield (GY), plant height (HT), and heading date (HD).

	Pullman 2015			
Name	**Δ Rating** [a]	**GY Rating**	**HT Rating**	**HD Rating**
WA8151	10	8	7	6
J950114-002	8	9	8	6
J970284-2	8	9	7	6
J970536-1	8	9	5	8
98 × 402-1C	8	9	7	6
X010259-2C	8	9	6	6
OR2030554	8	9	3	4
LEWJAIN	8	8	5	9
SSD060252	8	8	7	6
LSD	4	1	2	2

Table 3. *Cont.*

	Pullman 2016			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
WA8117	9	10	3	6
5J062268-1	9	9	5	8
SSD02069	9	8	3	6
SSD02071	9	8	5	7
SSD02072	9	8	5	6
BRUNDAGE96	9	8	5	3
WA8116	9	8	3	8
OR2071071	8	10	3	6
J960793-4	8	9	5	8
J970536-1	8	9	3	8
WA7932	8	9	4	9
XERPHA	8	9	6	6
J981358-2	8	8	5	6
J98408-2	8	8	5	6
A00151	8	8	5	8
WEATHERFORD	8	8	8	6
BRUNDAGECF	8	8	5	4
WA7971	8	8	5	7
LSD	5	1	2	2
	Pullman 2017			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
SSD060530	10	10	4	4
98X371-3C	10	8	3	6
X010729-1L	9	10	3	4
ARS970161-3L(Selbu)	9	9	4	5
X980336-5L	8	10	5	9
X010259-2C	8	9	5	6
X010678-8C	8	9	5	6
V/W-32	8	8	6	4
X970024-4	8	8	3	7
LSD	6	1	2	1
	Lind 2017			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
X970054-2	9	9	4	6
A00122	9	8	6	6
X00277-4L	9	8	5	3
X970170L	9	8	4	5
5J062100-3	8	10	9	3
6J020210-1	8	9	10	6
OR208047P94	8	9	3	5
J960670-1	8	8	7	3
WA7935C	8	8	9	5
LSD	6	1	2	2
	Pendleton 2017			
Name	**Δ Rating**	**GY Rating**	**HT Rating**	**HD Rating**
OR2071073	10	9	2	-
X970054-2	10	8	5	-
WA8153	9	8	6	-
ARS010719-4L	9	8	5	-
OR2070011	8	9	7	-
X010463-9C	8	8	4	-
X010746-4C	8	8	5	-
X970042C	8	8	6	-
LSD	5	1	2	-

[a] Rating is based on standard deviation from the mean (Table S1), where a score of 1 was assigned to genotypes with the lowest trait value relative to other genotypes in the same location-year. Least significant differences (LSD) ($p < 0.05$) were calculated and then converted back to the rating system based on the standard deviation. -, N/A.

There are a number of theories to explain how these genotypes may have attained their high yield. The Δ signature of plant tissue shares a positive relationship with the internal carbon dioxide concentration of the leaf (C_i/C_a) [12]. This C_i/C_a ratio is impacted primarily by stomatal conductance and photosynthetic capacity [14]. High Δ tissue can be assumed to have experienced high stomatal conductance, low photosynthetic capacity, or a combination of the two over the course of tissue development, and the reverse can be expected of tissue registering as low Δ [14]. In terms of yield, high photosynthetic capacity is likely to be a favorable trait, perhaps explaining certain productive low Δ genotypes [15]. Less straightforward is the yield advantage associated with stomatal conductance. Here, it is important to consider the field environment and, specifically, the severity of terminal drought experienced at each location (Figure 1).

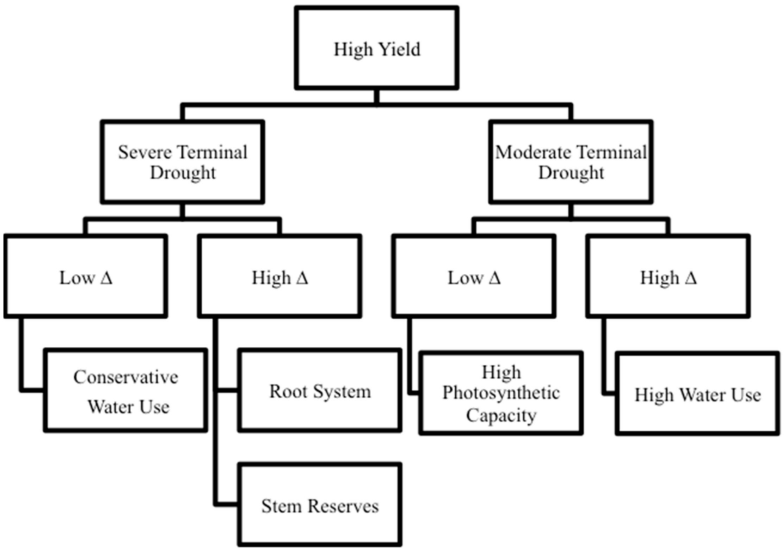

Figure 1. Flow chart of beneficial traits associated with low and high Δ genotypes in distinct terminal drought environments. A severe terminal drought may favor low Δ genotypes expressing conservative water use or high Δ genotypes with the ability to develop deep roots or store carbohydrates pre-anthesis for later translocation to the grain. A moderate terminal drought may favor low Δ genotypes with high photosynthetic capacity or high Δ genotypes able to use all of the available soil moisture.

Yield potential in a severe terminal drought environment is influenced by a genotype's ability to safeguard sufficient water for grain fill. Severe drought stress during grain fill can reduce the harvest index, underscoring the importance of water supply during this time [16]. Low Δ, driven by low stomatal conductance, may allow conservative water use throughout the growth cycle. These genotypes are able to meter out soil water reserves pre-anthesis in order to ensure sufficient water during the post-anthesis period [6]. Additionally, by maintaining access to water despite water-deficit conditions in the upper soil horizons, deep rooting genotypes are able to sustain relatively high stomatal conductance, ultimately leading to the development of high Δ tissue. Yield potential in a severe terminal drought environment may be influenced by the genotype's ability to avoid the drought altogether. In these environments, genotypes with the ability to develop a surplus of stem reserve carbohydrates prior to drought onset could be advantageous. During grain fill, these reserves are translocated to the grain, providing an effective means to adequately fill grain without abundant water. If these reserves are assimilated at a time of high stomatal conductance, the Δ signature of these carbohydrates may also be high [17]. Later translocation to the grain will elevate the overall Δ signature of grain tissue. Stem reserves can contribute anywhere from 10% to as much as 80% of final grain carbon, emphasizing the influence that reserves can have on the mature grain Δ signature [18].

In moderate terminal drought environments, grain yield potential is influenced by the ability of genotypes to capitalize on the additional soil moisture. Genotypes that express high water use, perhaps as a result of stomatal insensitivity to water-deficit, may convert this excess water into photosynthates and ultimately into grain weight. The high transpiration of these genotypes may be a valuable, yield-enhancing trait and could explain the observed high yielding, high Δ genotypes in moderate terminal drought environments [19–21]. Regarding the high yield, low Δ genotypes observed in this environment, it may be that these genotypes express high photosynthetic capacity. As low Δ may result from either low stomatal conductance or high photosynthetic capacity, this hypothesis is born out of the expectation that low stomatal conductance is likely not yield-enhancing in a moderate terminal drought environment, leaving high photosynthetic capacity as a viable explanation.

Along with helping to explain how low and high Δ genotypes attain high yield, taken together, these theories form a broader hypothesis. Combining knowledge of the terminal drought environment with grain Δ analysis allows identification of genotypes that exhibit specific drought tolerance traits, including water-use behavior (conservative or high water use), reliance on stem reserve carbohydrates, and the ability to develop deep roots. In order to test the validity of this hypothesis, it is necessary to first characterize the field environments by terminal drought severity. The severity of terminal drought in a particular environment is a function of multiple factors involving environmental (precipitation, temperature, humidity), agronomic (weed pressure, straw cover), and soil health parameters, particularly water holding capacity. An evaluation of terminal drought severity by any one of these factors alone would be incomplete. For example, of the three Pullman locations, Pullman 2016 received the highest amount of precipitation at 463 mm, yet averaged the lowest mean yield of 5.4 t ha^{-1}. This may be a result of more precipitation occurring over the winter months in Pullman 2016 versus the month of May, when genotypes would be flowering and using water more rapidly. Furthermore, in 2017, Lind received more precipitation and had cooler temperatures, on average, than Pendleton. Despite this, the mean grain yield of Pendleton was double that of Lind. This could potentially point to soils with better water holding capacity in Pendleton, allowing more water to be available during the full growing season.

Instead of evaluating terminal drought severity by the limiting variables of temperature and precipitation, average Δ of all genotypes in a particular environment can serve as a more holistic measure of the terminal drought severity experienced in that environment. An elevated C_i due to high stomatal conductance can be expected in a well-watered environment, ultimately resulting in high overall Δ values. Conversely, soil moisture deficits tend to reduce stomatal conductance, lowering C_i and eventually decreasing average Δ values [14]. Measurements of Δ from unstressed tissue typically register between 19.0 and 22.0 per mil, whereas very stressed tissue can be as low as 12.0 per mil [14]. Average Δ values in this study ranged from 16.5 to 18.2 per mil, indicating that every environment experienced a certain degree of terminal drought stress. In order from most severe to least severe, the environments ranked as follows: Lind 2017 ($\Delta = 16.5$), Pullman 2015 ($\Delta = 17.0$), Pullman 2016 ($\Delta = 17.6$), Pullman 2017 ($\Delta = 18.1$), and Pendleton 2017 ($\Delta = 18.2$). In the more severe terminal drought environment of Lind 2017, high yielding, high Δ genotypes may be expected to rely heavily on stem reserves, or the ability to access water by deep roots, whereas the productive, low Δ genotypes may express conservative water use. Similar hypotheses can be made of the more moderate terminal drought environments of Pullman 2017 and Pendleton 2017. High water use by high Δ genotypes may be favorable, and improved photosynthetic capacity could explain the observed high yielding, low Δ genotypes in moderate terminal drought environments (Figure 1).

The data collected in this study provide some evidence for the hypotheses given above. While conservative water use is expected to be beneficial in a severe terminal drought environment, this trait is likely detrimental in a moderate terminal drought environment. Genotypes suspected of conservative water use may leave soil moisture behind at harvest in a moderate terminal drought environment, representing an opportunity cost in terms of yield. It is then reasonable to predict that the yield advantage of conservative water use will diminish as terminal drought stress becomes less severe, and, indeed, this seems to be the case ($r = -0.87$, $p = 0.0545$) (Figure 2). A similar analysis can be applied to high water use genotypes. These genotypes may exhaust the soil moisture supply prior to grain fill in a severe terminal drought environment. The yield advantage of this trait may then be expected to diminish as the terminal drought stress becomes more severe. Genotypes suspected of high water use identified in this study show a reduction in yield as the terminal drought environment becomes more severe ($r = 0.95$, $p = 0.0130$) (Figure 2). These analyses suggest that by combining our understanding of the terminal drought environment with grain Δ analysis we may have identified a group of genotypes exhibiting conservative water use and another group of genotypes exhibiting high water use.

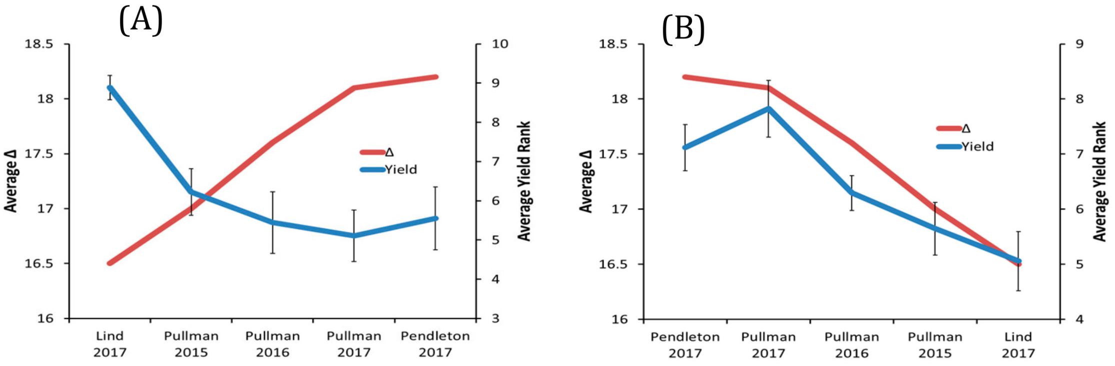

Figure 2. Change in average yield rank of suspected conservative water use genotypes (high yield and low Δ genotypes from Lind 2017; $n = 9$) as terminal drought stress (estimated by average Δ per environment) becomes less severe (**A**). Change in average yield rank of suspected high water use genotypes (high yield and high Δ genotypes from Pullman 2017 and Pendleton 2017; $n = 17$) as terminal drought stress (estimated by average Δ per environment) becomes more severe (**B**).

Grain Δ may also be able to help distinguish deep rooting genotypes from genotypes that rely on stem reserve carbohydrates. By allowing access to water, it is reasonable to suspect that the stomatal conductance of deep rooting genotypes will remain high throughout the plant lifecycle, despite exposure to mild vegetative drought. Genotypes relying heavily on stem reserves, however, may close their stomata in the event of a vegetative drought, resulting in a lower Δ signature of stem carbohydrates fixed during this time. Translocation of these low Δ carbohydrates to the grain will reduce the overall grain Δ signature. Distinguishing between deep rooting and stem reserve genotypes may then be possible by observing the change in Δ signature for a particular genotype across environments. Genotypes suspected of deep roots should demonstrate a relatively high Δ signature across environments, whereas the grain Δ signature of those genotypes relying more on stem reserves may fluctuate depending on the prevailing environmental conditions over the period that stem reserves are assimilated.

4. Conclusions

This study proposes that grain Δ may be useful as a screening tool for the identification of genotypes expressing more specific drought tolerance traits. Preliminary evidence in support of this idea was presented. Moving forward, it is necessary to test the identified high yielding genotypes for their suspected traits. This validation step will provide important insight into the robustness of the hypotheses presented in this work. If future studies can confirm the water use behavior, reliance on stem reserves, and deep roots of the suspected genotypes, grain Δ may then be leveraged as an initial step in the identification of beneficial drought tolerance traits. Equipped with a reasonably high-throughput way of evaluating genotypes for these traits, plant breeding programs will be more capable of advancing the development of drought tolerant cultivars.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/7/385/s1, Table S1: Index for relative comparison of Δ, grain yield (GY), plant height (HT), and heading date (HD) between environments; Table S2: Monthly and total precipitation (mm) occurring over the growing season for each location-year; Table S3: Average maximum temperature (°C) per month during the growing season for each location-year and average maximum temperature (°C) over the entire growing season for each location-year.

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