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Carbon isotope discrimination association with yield and test weight in Pacific Northwest–adapted spring and winter wheat

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Abstract

Due to considerable influence of environment on yield, breeding for drought tolerance could benefit from focusing on selection of more heritable physiological traits, such as carbon isotope discrimination (as measured by delta, Δ) for indirectly assessing water use efficiency (WUE) of wheat (*Triticum aestivum* L.). Spring and winter wheat cultivars were assayed for Δ, and these values were used to determine the relationships with performance in over 13 environments in the U.S. Pacific Northwest. The correlation coefficients of Δ values between the wheat cultivars grown in different environments ranged from 0.11 to 0.73 for both spring and winter wheat. There was significant genotypic variation for Δ in soft spring and hard winter wheat but not in hard spring and soft winter wheat. The Δ values were poor indicators of yield for this set of wheat cultivars in most environments, although low values (better WUE) were sometimes correlated with yield. A population of 165 hard spring wheat recombinant inbred lines derived from a cross between two hard spring wheat varieties that differed in Δ was also screened in low-rainfall dryland and irrigated environments. High yields in this recombinant inbred population were weakly correlated with high Δ values or low WUE in most environments.

1 | INTRODUCTION

Dryland cropping in the U.S. Inland Pacific Northwest (PNW) can be divided into low (~1.6 million ha), intermediate (1 million ha), and high (0.8 million ha) annual precipitation regions, generally defined as <300, 300–460, and >460 mm, respectively (Schillinger, Papendick, Guy, Rasmussen, & Van Kessel, 2003). The PNW is also the most important wheat (*Triticum aestivum* L.)–producing area in the far-western United States, but inadequate precipitation is a limiting factor for wheat production in the region, and crop production is dependent on stored soil moisture from winter precipitation (Schillinger et al., 2003). The PNW receives about 70% of its annual precipitation

Abbreviations: Δ, carbon isotope discrimination; DSI, drought susceptibility index; HD, Hollis/Drysdale; LSMEANS, least square means; PNW, Pacific Northwest; RIL, recombinant inbred line; TE, transpiration efficiency; WSU, Washington State University; WUE, water use efficiency; Z, Zadok's growth stage.

during November to April, with dry summers and drought stress occurring during the wheat life cycle in all precipitation zones almost every year (Young & Thorne, 2004). Due to the lack of other suitable cropping alternatives, about 90% of the cropland in the low-precipitation region is based on a winter wheat–fallow rotation system, in which only one crop is grown every 2 yr (Schillinger et al., 2003). This allows the portion of the winter precipitation from the fallow year that is not lost to evaporation to be used for production of the next winter wheat crop planted in late summer or early fall. Spring wheat is grown mainly in the more intensively cropped intermediate- and high-rainfall regions. The winter wheat–fallow system is vulnerable to wind erosion and stores water from the fallow season very inefficiently (Papendick, Lindstrom, & Cochran, 1973). Development of spring, winter, or facultative wheat lines that perform well under drought conditions could help in the development of intensified rotations that more efficiently use available soil moisture (Bewick, Young, Alldredge, & Young, 2008).

Wheat is very sensitive to water stress during tillering and flowering stages (Neibling, Rogers, & Qureshi, 2017). Wheat cultivars exposed to drought at these growth stages produce lower yield. Seasonal total amounts of crop water use range from 483 to 505 mm for most wheat cultivars (Ko, Piccinni, Marek, & Howell, 2009). Wheat requires 60 mm of available water for vegetative growth stages and 163 mm water from flowering to hard dough stages (Bauer, Black, & Frank, 1989; Schillinger, Papendick, & McCool, 2010).

Drought tolerance can be improved by selective breeding of wheat with high water use efficiency (WUE) (Ehdaie, Hall, Farquhar, Nguyen, & Waines, 1991; Farquhar & Richards, 1984). The WUE can be characterized various ways (Condon, Richards, Rebetzke, & Farquhar, 2002; Tambussi, Bort, & Araus, 2007; Vadez, Kholova, Medina, Kakkera, & Anderberg, 2014). Transpiration efficiency (TE) is an important component of WUE and is defined as a ratio of biomass accumulation to water transpired by plants. At the leaf level, the TE is defined as the intrinsic WUE and is determined as the ratio of instantaneous CO_2 assimilation to transpiration (Condon et al., 2002; Vadez et al., 2014). Because direct measurement of TE in the field is very difficult and time consuming, a physiological technique, carbon isotope discrimination (Δ), has been used as a surrogate measure of TE (Farquhar, O'Leary, & Berry, 1982; Hubick, Farquhar, & Shorter, 1986). The net Δ is the function of the ratio of the intercellular (c_i) and the atmospheric (c_a) partial pressure of CO_2 (c_a) in the plant (c_i/c_a)(Farquhar et al., 1982). Of the two stable carbon isotopes in the atmosphere, the abundance of ^{12}C is higher (98.9%) than that of ^{13}C (1.1%). Plant photosynthesis discriminates against the larger ^{13}C isotope both during diffusion into the stomate and during CO_2 fixation. Under optimal water conditions, stomata remain open and incorporate much more ^{12}C than ^{13}C during photosynthesis. Under water-stressed conditions, stomatal closure reduces CO_2 diffusion, and ^{12}C becomes limited, causing relatively more ^{13}C to be incorporated during photosynthesis. This causes lower discrimination against ^{13}C and decreases the value of Δ. Based on this theory, Δ is positively correlated to the ratio of c_i/c_a and is negatively correlated to TE or intrinsic WUE (Condon et al., 2002). The Δ value has been used as a predictor of yield in many cereals, including rice (Gao et al., 2018), barley (Craufurd, Austin, Acevedo, & Hall, 1991), maize (Monneveux, Sheshshayee, Akhter, & Ribaut, 2007), and wheat (del Kumar, Azam-Ali, Snape, Weightman, & Foulkes, 2011; del Pozo et al., 2016). Rebetzke, Condon, Richards, and Farquhar (2002) found that low Δ was associated with high yield of wheat in low-rainfall environments. Conversely, in Mediterranean environments, where post-anthesis water stress is a limiting factor for plant growth, several studies have found a positive relationship between Δ and wheat yield in both water-limited and optimal soil moisture environments (Merah, Monneveux, & Deléens, 2001b; Morgan, LeCain, McCaig, & Quick, 1993). Similarly, Li, Chen, and Wu (2012) found a positive relationship (high Δ or low TE and high yield) between flag leaf Δ and yield in the PNW-adapted spring wheat cultivars tested under rainfed and irrigated conditions in southern Idaho. Under drought conditions, high Δ is indicative of greater water uptake through deep roots, and low Δ is indicative of increased WUE (Sanz-Saez et al., 2019).

An important aspect of Δ is that it is thought to be less influenced by the genotype × environment (G × E) interaction than many yield-associated traits. The Δ provides an integrative assessment of leaf TE and exhibits high heritability (Condon & Richards, 1992). It is strongly correlated to aerial biomass and grain yield (Rebetzke et al., 2002). However, due to variability in the relationship (positive to negative) found between Δ and agronomic traits, it

Core Ideas

- Significant genetic variation for carbon isotope discrimination (Δ) was observed on PNW-adapted wheat lines.
- Low Δ or high transpiration efficiency was important contributor to grain yield in drought environment for soft spring and hard winter wheat but not for hard spring wheat lines.
- Grain yield increased by about 6 and 21% in drought-tolerant soft spring and hard winter wheat compared with drought-sensitive wheat.

is important to test these relationships in wheat varieties and breeding materials adapted to specific agroecological regions. Our objectives were (a) to determine Δ and its relationship with grain yield across spring and winter wheat genotypes adapted to the Pacific Northwest, grown in environments spanning the precipitation zones of the region, and (b) to investigate these same relationships in a spring wheat population segregating for Δ.

2 | MATERIALS AND METHODS

2.1 | Field sites and experimental design

To establish the possibility of incorporating Δ as a selection technique in PNW wheat drought breeding programs, two sets of experiments were conducted. Experiment 1 investigated the relationship of Δ with yield and test weight in different market classes (spring and winter; soft and hard) of the PNW-adapted wheat varieties and breeding lines. In this study, Δ was estimated in sampled environments and was associated with agronomic traits in environments with different moisture conditions (low, intermediate, and high) in the field and in a greenhouse. In Experiment 2, a population of hard spring wheat recombinant inbred lines (RILs) segregating for Δ was screened in field environments. The RILs were derived from F_5 progeny of a cross between the cultivars Hollis and Drysdale. Hollis (PI632857) is a hard red spring wheat adapted to the low to intermediate rainfall regions of Washington (Kidwell et al., 2004) that has high grain protein content and high test weight. Drysdale is an Australian hard white spring cultivar that has been widely cultivated in southeastern New South Wales (CSIRO, 2004). It is a semi-dwarf wheat with early to mid-maturity that has high TE (low Δ) and high yield under drought conditions.

2.2 | Experiment 1

For Δ estimations, flag leaves for spring wheat were sampled at Pullman, WA (precipitation, 390 mm; 46°41.711′ N, 117° 08.599′ W; 773 m asl) in 2009 and at Connell, WA (precipitation, 290 mm; 46°37.187′ N, 118°43.558′ W; 393 m asl) in 2010. The cumulative precipitation during the spring wheat growth period was 156.5 mm (April–August) at Pullman and 126 mm (March–July) at Connell (Figure 1). For winter wheat, the sampling sites were located at Lind, WA, in 2010 (average precipitation, 275 mm; 47°00.188′ N, 118°34.294′ W; 507 m asl) and 2011 (average precipitation, 164 mm; 47°08.610′ N, 118°28.401′ W; 506 m asl). The cumulative precipitation during the winter wheat growth period (September–July) was 240.3 mm at Lind in 2009/2010 and

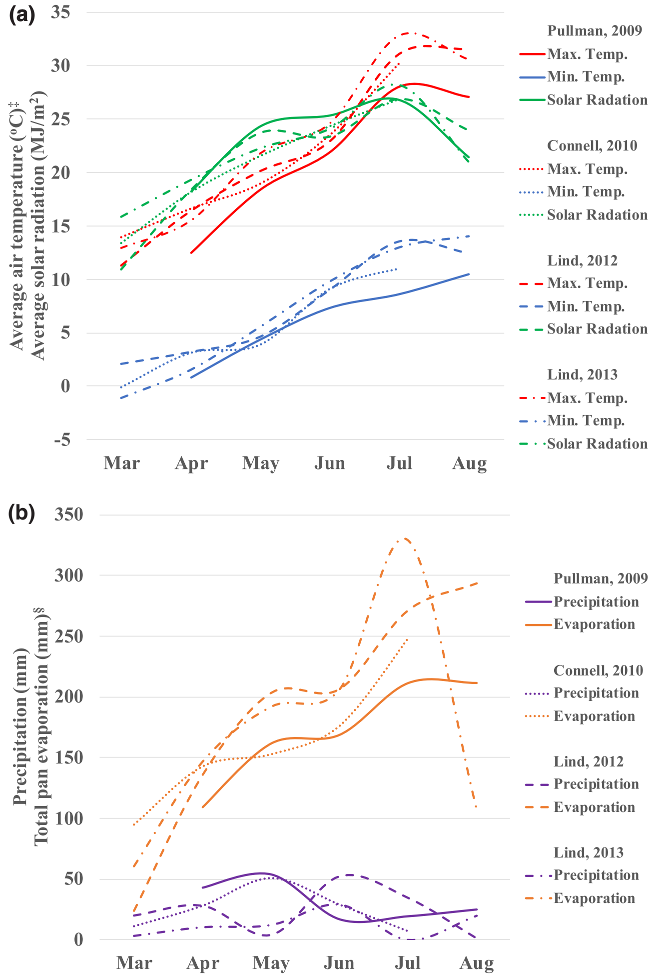

FIGURE 1 Meteorological parameters during spring wheat growing season at Pullman, Connell, and Lind Experiment stations, WA, in March to August 2009, 2010, 2012, and 2013. (a) Average air temperature and average solar radiation. (b) Precipitation and total pan evaporation. Weather data obtained from http://weather.wsu.edu. ‡Maximum and minimum air temperature. §Evapotranspiration in alfalfa

237 mm (September to July) in 2010/2011 (Figure 2). Precipitation during the winter wheat growing cycle was 72.6 mm higher in 2010/2011 than in 2009/2010. All varieties were grown in an alpha lattice design with three replications. All trials were managed through the Washington State University (WSU) Extension Cereal Variety Testing Program (http://smallgrains.wsu.edu/variety/).

Spring wheat was planted on 21 Apr. 2009 in Pullman and on 5 Mar. 2010 in Connell. Spring planting is commonly earlier in Connell than in Pullman due to warmer weather and lower precipitation. The previous crop before the spring wheat in Pullman was peas, whereas in Connell the land was kept fallow. Seeds were sown on silt loam soil at 90 and 67 kg ha^{-1} in Pullman and Connell, respectively, with planting depth of 25–50 mm and a row spacing of 150 mm. The pre-existing soil moisture before sowing

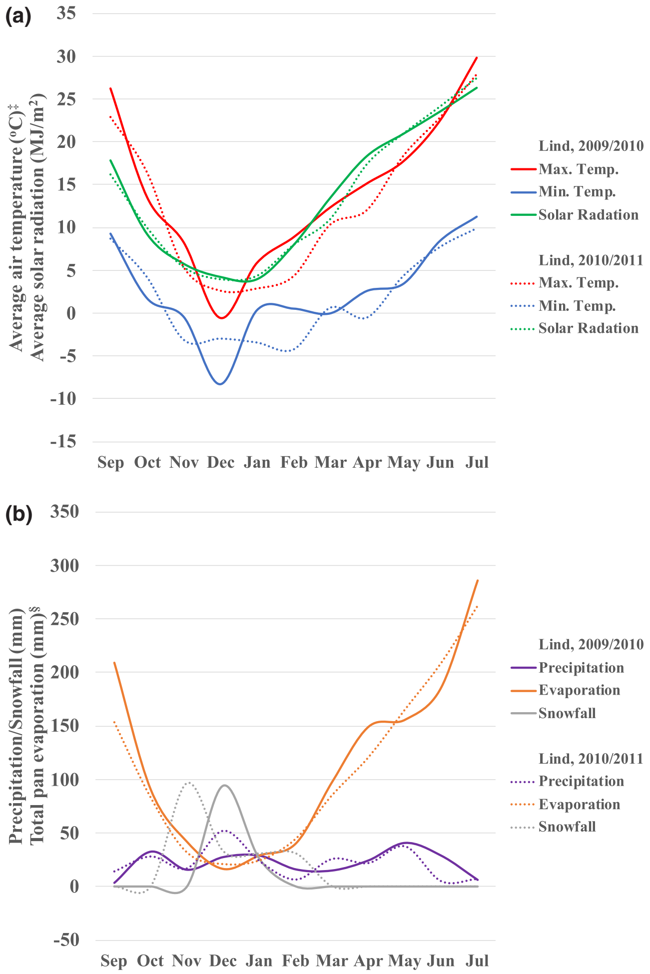

FIGURE 2 Meteorological parameters during winter wheat growing season at Lind Experiment station, WA, in September 2009 to July 2010 and September 2010 to July 2011. (a) Average air temperature and average solar radiation. (b) Precipitation/snowfall and total pan evaporation. Weather data obtained from http://www.wrcc.dri.edu. ‡Maximum and minimum air temperature. §Evapotranspiration in alfalfa

was 105 mm in Pullman and 103 mm in Connell. Base fertilizer was applied before planting at 50 kg ha^{-1} N, 11 kg ha^{-1} P, and 11 kg ha^{-1} S in Pullman and 67 kg ha^{-1} N in Connell. Based on soil tests, an additional 72 kg ha^{-1} N and 11 kg ha^{-1} S were applied in the spring using a broadcast surface application in the hard spring wheat nursery in Pullman. Winter wheat was planted at Lind on 1 Sept. 2009 and 3 Sept. 2010 at approximately 150 mm of soil depth. Fields were fallow before planting winter wheat in both years. The seed sowing rate was 50 kg ha^{-1}, with a row spacing of 380 mm. Base fertilizer application was 56 kg ha^{-1} N and 11 kg ha^{-1} S in both years.

Based on the total precipitation during the studied period (2009 and 2010 for spring wheat and 2010 and 2011 for winter wheat), the WSU varietal testing sites were categorized into low- (<150 mm), intermediate- (150–310 mm), and high-precipitation (>310 mm) regions (Table 1). In 2010, due to greater annual precipitation (>150 mm), the low-precipitation region was not categorized in both soft spring and hard winter wheats. Due to a higher precipitation rate in 2011 (192 mm) compared with 2010 (119 mm), the total moisture was greater in 2011 than in 2010. The WSU's uniform wheat variety trials are conducted over a total of 19 environments for winter wheat and 15 environments for spring wheat. For the purposes of estimating yield and test weight in each of the precipitation zones, yield and test weight values of the genotypes were averaged over the environments within each of the low-, intermediate-, and high-precipitation regions. Thus, yield and test weight values for soft spring wheat (2009) were averaged over two, four, and three environments for low, intermediate, and high rainfall zones, and for soft spring wheat (2010), yield and test weight values were averaged over four and two environments for intermediate and high rainfall zones, respectively. Similarly, yield and test weight values for hard winter wheat (2010) were averaged over three and three environments for intermediate and high rainfall zones, and for hard winter wheat (2011), the yield and test weight were averaged over one, three, and three environments for low, intermediate, and high rainfall zones, respectively. The Pearson correlation was used to determine the relation of Δ with yield and test weight in each precipitation region for spring and for winter wheat.

2.3 | Experiment 2

A Hollis/Drysdale (HD) RIL population (165 inbred lines) was assayed for Δ at Lind, WA, in 2012 and 2013. The trials were planted in a randomized complete block design with three replications for each of two water regimes, dryland (rainfed) and drip irrigated, in both 2012 and 2013. Plants were sampled for Δ analysis from two replications in the rainfed plots in 2012 and the irrigated plots in 2013 and from one replication in the irrigated plot in 2012.

The cumulative precipitation at Lind during the spring wheat growth period was 138.9 mm (March–August) in 2012 and 74.7 mm (March–August) in 2013 (Figure 1). Approximately 71 and 97 mm soil moisture (at <1.2 m depth) was available during planting in the rainfed environments in 2012 and 2013, respectively. Thirty grams of seed were sown in each plot (1.52 m by 1.52 m) using a seven-row cone planter with double disc openers spaced at 180 mm. Before sowing, seeds were treated with a mixture of tebuconazole and metalaxyl (Allegiance and Raxil MD) and imidacloprid (Gaucho Bayer CropScience) and water (1 ml per 100 g seed) to reduce infestations with soil-borne pathogens and pests. Two field water regimes were established: one as rainfed, where stress occurred due to low

TABLE 1 Precipitation at the varietal testing locations categorized into low-, intermediate-, and high-precipitation regions during 2009–2011

Locations[a]	Precipitation[b] 2009	2010	2011	5-yr avg.[c]
	mm			
Almira	187.7i	376.4h	225.3i	290.8
Bickleton (Roosevelt)	43.4l	279.1i	–	207.3
Connell	194.3i	288.0i	134.4l	213.1
Dayton (Huntsville)	240.5i	–	474.0h	442.5
Horse Heaven (WSU Hamilton)	119.1l	253.5i	–	152.4
Lind	215.4i	275.6i	163.8i	213.9
Pullman	390.4h	351.5h	423.7h	410.0
St John	353.6h	299.0i	–	208.3
St. Andrews	–	304.5i	216.2i	256.3
Walla Walla	418.6h	–	419.6h	432.3
Avg. precip. (low)	81.3	–	134.4	
Avg. precip. (intermediate)	216.7	283.3	201.8	
Avg. precip. (high)	387.5	351.5	439.1	

[a]The locations in parentheses represent the closest weather stations to the respective varietal testing sites. [b]Precipitation regions: l, low; i, intermediate; h, high.
[c]Long-term averages calculated using data from 2009 to 2004 (source: http://weather.wsu.edu).

TABLE 2 Agronomic and physiological traits measured on spring and winter wheat in the carbon isotope discrimination (Δ) sampled environment[a]

Wheat	Type	Location	Year	No. of genotypes	Growth (Zadok's)	Yield[b]	Test wt.	Δ[b]
						kg ha^{-1}	kg m^{-3}	‰
Spring	soft	Pullman	2009	16	Z53	5,090 ± 91 ns	799***	20.2 ± 0.08***
Spring	hard	Pullman	2009	19	Z53	4,309 ± 87 ns	790***	19.8 ± 0.05*
Spring	soft	Connell	2010	22	Z53	3,058 ± 40***	776***	18.7 ± 0.06***
Spring	hard	Connell	2010	24	Z53	2,464 ± 39***	773***	19.0 ± 0.04***
Winter	soft	Lind	2010	57	Z53	2,928 ± 38***	800 ns	17.3 ± 0.03***
Winter	hard	Lind	2010	28	Z53	2,651 ± 65***	801***	17.5 ± 0.06***
Winter	soft	Lind	2011	58	Z45	2,860 ± 43***	790***	18.7 ± 0.04 ns
Winter	hard	Lind	2011	29	Z45	2,797 ± 78***	789***	18.4 ± 0.08***

[a]Washington State University wheat variety trials data (http://variety.wsu.edu). [b]Means of all genotypes ± SE.
*Significant at the .05 probability level. **Significant at the .01 probability level. ***Significant at the .001 probability level. ns, not significant.

summer rainfall, and the other as irrigated, where a limited supply of irrigation water was provided through dripline irrigation. Under drip irrigation, plants were watered at a uniform rate for 3 h every other day beginning 1 mo after planting.

2.4 | Flag leaf collection and sample preparation for carbon isotope discrimination

For Δ analysis of the variety trials, 5–10 flag leaves of each spring wheat genotype were sampled at Zadok's growth stage (Z)53 (1/4 of inflorescence emerged) (Zadoks, Chang, & Konzak, 1974) from Pullman in 2009 and Connell in 2010, and winter wheat genotypes were sampled during Z45 (booting stage) and Z53 in 2010 and 2011 from Lind, WA, in Experiment 1 (Table 2). A total of 56 spring (Supplemental Tables S1 and S2) and 115 winter (Supplemental Tables S3 and S4) wheat genotypes were screened, of which 26 spring genotypes (including 12 soft and 14 hard) and 59 winter genotypes (including 39 soft and 20 hard) were common in both environments. In Experiment 2, leaves from the RILs were sampled at jointing stage (Z31) in three environments, including Lind rainfed (two replications) and Lind irrigated (one replication) in 2012 and Lind irrigated in 2013 (two replications). The sampled leaves were dried at 60 °C for 72 h and ground using a Foss Tecator Cyclotec Sample Mill (Fischer Scientific Inc.). Samples (0.20–0.50 mg) of the ground leaves were combusted to release pure C to analyze carbon isotope composition (^{12}C and ^{13}C) using a continuous flow isotope ratio mass spectrometer (Delta PlusXP, Thermofinnigan). We calculated $\delta^{13}C$ (‰) as [(R sample/R reference − 1) × 1,000], where R is the $^{13}C/^{12}C$ of plant sample and the reference material (Pee Dee Belemnite) (Farquhar, Ehleringer, & Hubick, 1989). The following formula was used to determine Δ (Farquhar et al., 1989): Δ (‰) = $[(\delta_a - \delta_p)/(1 + \delta_p)] \times 1{,}000$, where δ_a is the $\delta^{13}C$ of the atmospheric CO_2 (−8%), and δ_p is the $\delta^{13}C$ of the plant sample.

2.5 | Greenhouse experiments

A greenhouse study was conducted to examine the effect of water restriction alone on plant growth and grain production. Eighteen spring wheat genotypes were selected based on a range of Δ values, and yield in the field and their grain yield performance was evaluated. Genotypes included the soft spring wheat varieties Nick, Louise, WA8089, Diva, Alpowa, and Alturas; the hard spring wheat varieties WA8078, Lassik, Hollis, Jefferson, WA8074, Scarlet, Clear White, Buck Pronto, WA8100, and Patwin; and spring club wheat varieties Eden and JD. Seeds were sown in 3-L pots containing a mix of No. 1 Sunshine Peat Moss Mix (Sun Gro Horticulture) and sand (3:1 ratio). The experiment was arranged in a randomized complete block design with three replications. Plants were thinned to two per pot after emergence. Plants were watered uniformly until growth stage Z31 (jointing stage), after which water treatments, defined as low (50 ml d^{-1}), intermediate (150 ml d^{-1}), and high (275 ml d^{-1}), were applied every other day. The low-moisture treatment was supplemented with 50 ml of water starting at Z59 (complete inflorescence emergence stage), which was continued until all leaves and stems senesced. To avoid pot water loss through soil evaporation, the top of the soil of each pot was covered with Turface MVP (Profile Products, LLC), a calcined nonswelling illite with bulk density 540 kg m^{-3}, before water treatments were applied. Plants were grown in a greenhouse under a 16 h photoperiod and were fertilized with approximately 250 mg N per pot per month.

The drought susceptibility index (DSI) was calculated to determine the stability of grain weight potential of genotypes in the greenhouse. Drought susceptibility index was calculated as DSI = $(1 - Y_{s1}/Y_{p1})$/SI (Fischer & Maurer, 1978); SI (stress intensity) = $(1 - Y_{s2}/Y_{p2})$, where Y_{s1} is the mean grain weight under low moisture regime (T3), Y_{p1} is the mean grain weight under high moisture regime (T1), Y_{s2} is the mean grain weight over all genotypes under T3, and Y_{p2} is the mean grain weight over all genotypes under T1. Genotypes with DSI values ≤1 were considered as drought tolerant, and DSI values >1 were considered as drought susceptible (Li et al., 2012).

2.6 | Statistical analysis

The yield, test weight, and Δ values from the field experiments were analyzed using mixed models within each environment considering genotype as fixed and replication as a random effect to obtain least square means (LSMEANS). In addition, a combined mixed models analysis of yield, test weight, and Δ over environments was performed to test the effect of genotype (G), environment (E), and G × E interactions for the soft and hard spring and winter wheat. The genotypes were considered as fixed effects and the environments and G × E interactions as random effects for the G × E test in these analyses. The correlations of Δ from sampled environments with yield, test weight, and other traits were assessed within each environment using Pearson's correlation coefficient. The Δ measured in each sampled environment was also used to determine the correlation with yield, test weight, and other traits averaged over environments in each of the low-, intermediate-, and high-precipitation regions. In the greenhouse, the LSMEANS of grain weight of spring wheat genotypes were derived through mixed models analysis, and the genotypes were ranked in each low, intermediate, and high moisture regimes. Fisher's LSD ($P \leq .05$) was used to compare the differences between LSMEANS.

For Experiment 2, the best linear unbiased predictors of genotypes in each environment were obtained using restricted maximum likelihood (REML) tests. Genotypes and replications were considered as random effects. In the recombinant inbred population, broad-sense heritability (h^2) closely estimates the narrow-sense heritability and was calculated for the studied traits using the method described in Cullis, Smith, and Coombes (2006) and Piepho, Möhring, Melchinger, and Büchse (2008).

$$h^2 = 1 - \frac{(\text{avesed})^2}{2V_g}$$

where avesed is the average standard error of the difference between best linear unbiased predictors, and V_g is the REML variance component estimate for the genotypes. Pearson correlations of Δ with yield and test weight were also performed. All statistical analysis was performed with JMP 10 software (Sall, Lehman, Stephens, & Creighton, 2012).

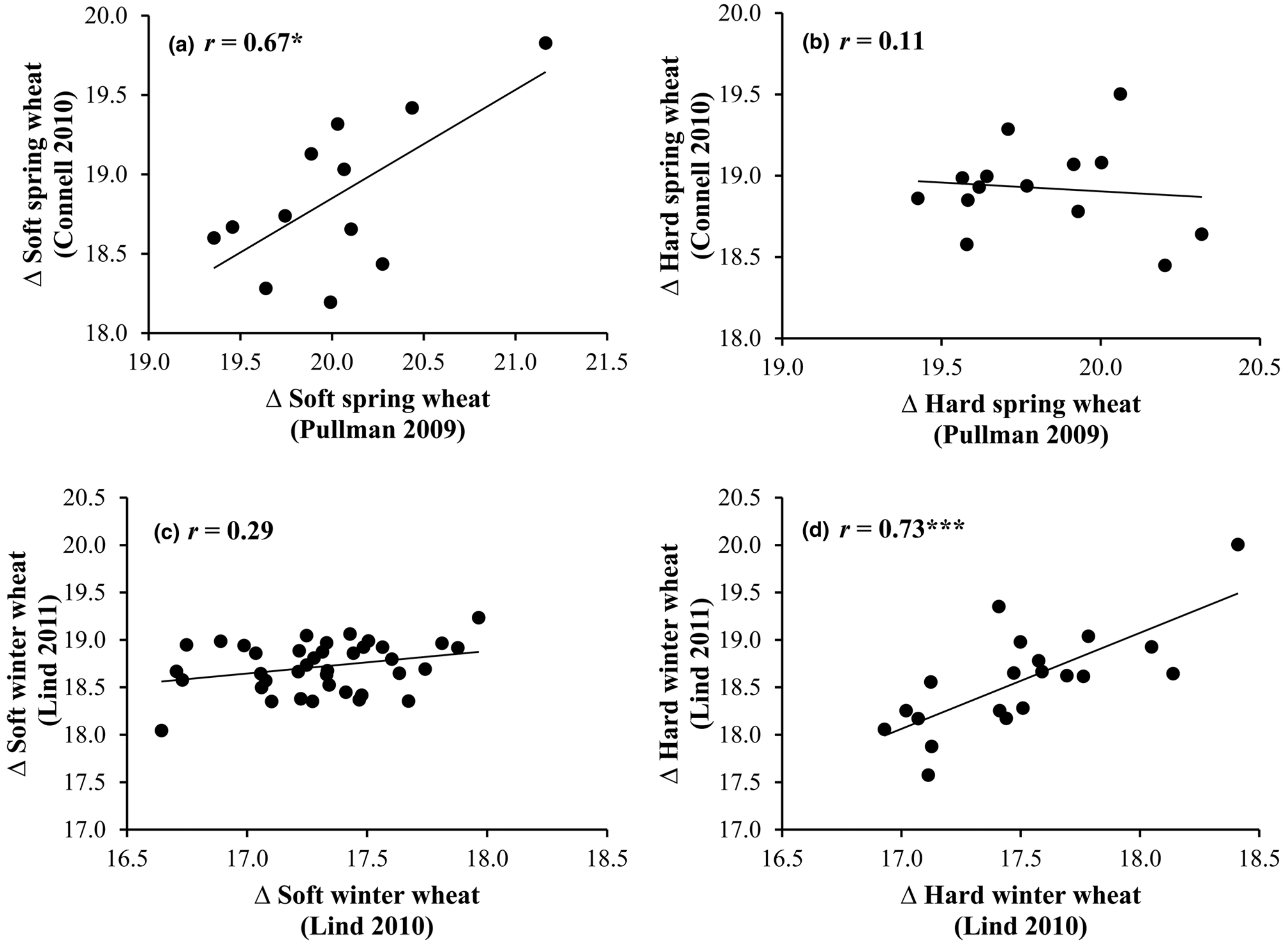

FIGURE 3 Correlation of carbon isotope discrimination (Δ) values of the same wheat lines grown in different environments of (a) soft spring ($n = 12$), (b) hard spring ($n = 14$), (c) soft winter ($n = 39$), and (d) hard winter ($n = 20$) wheat lines. *Significant at $P \leq .05$; ***significant at $P \leq .001$

3 | RESULTS

Significant variation within the different wheat classes was detected for yield, test weight, and Δ in most of the environments in which flag leaves were sampled for Δ analysis (Δ sampled environments; Table 2). Spring wheat yield, test weight, and Δ values were generally higher in the high-precipitation environment (Pullman) than in the low-precipitation environment (Connell). Winter wheat was sampled only in the low-precipitation environment (Lind) in 2010 and 2011. The Δ values of the same wheat lines were significantly positively correlated across environments for both soft spring and hard winter wheat (Figures 3a,d) and had significant variation due to genotype (Table 3). The correlations between the Δ values across environments were not significant for hard spring wheat lines (Figure 3b; Table 3). The relative Δ values among different wheat lines were inconsistent between the two environments. For example, the variety Clear White had the highest Δ value in 2009 (20.20) and the lowest in 2010 (18.45). Genetic variation was, therefore, not significant when tested across environments. Soft winter wheat lines had small variation in Δ values (significant at $P > .10$) due to genotype across environments (Table 3); the Δ values were poorly correlated between 2010 and 2011 ($r = .29$) (Figure 3c). The reason for this may be due to the lack of significant differences between Δ values among the soft winter wheat lines in 2011 (Table 2). In all cases, except in the hard spring wheat, the G × E interaction was low relative to genotype or environment. Although a significant G × E interaction existed in spring wheat, the main effects were more important (Table 3).

Because significant genetic variation for Δ was found in the soft spring and hard winter wheat lines, the relationship between Δ and yield was examined (Figure 4). The soft spring wheat lines Louise and WA 8089 (Pullman in 2009 and Connell in 2010) had low Δ and high yield (Figures 4a,b). The other soft spring wheat

TABLE 3 Genotype, environment, genotype × environment interactions and error for yield, test weight (TW), and carbon isotope discrimination (Δ) mean sum of squares for different wheat classes in Δ sampled environments

Source of variation	Soft spring				Hard spring			
	DF	Yield ('000')	TW	Δ	DF	Yield ('000')	TW	Δ
		kg ha^{-1}	kg m^{-3}	‰		kg ha^{-1}	kg m^{-3}	‰
Genotype (G)	11	364.34*	528.58*	1.19*	13	224.21	509.61	0.20
Environment (E)	1	82,900*	10,829*	23.99**	1	75,700*	6,009.98*	16.40*
G × E	11	118.56	196.4*	0.24*	13	158.22	295.50*	0.24*
Error	46	187.84	24.38	0.10	54	108.76	42.25	0.07
	Soft winter				**Hard winter**			
G	38	651.32*	266.97*	0.32	19	1,467.16*	617.02*	1.12*
E	1	570.12	8,021.19*	113.78*	1	1,522.63*	3,207.65*	33.89*
G × E	38	233.39	118.22*	0.20	19	372.32*	186*	0.20
Error	154 (*152*)	*164.71*	*34.81*	0.18	78 (*77*)	129.49	22.52	*0.18*

Note. Italicized numbers indicate some data points are missing.
*Significant at the .05 probability level.

varieties, including Diva, Eden, Nick, and Cataldo, had low Δ, and Babe and Alpowa had high Δ in both environments. The grain yield of Diva, however, was relatively high in the low-precipitation environment and low in the high-precipitation environment, whereas the grain yield of Nick, Eden, Babe, and Alpowa was high in the high-precipitation environment and low in the low-precipitation region. The yield of Cataldo was poor in both environments. Grain yield increased by 5.88% in drought-tolerant Louise and WA8089 compared with drought-sensitive Alpowa and Babe. Among the hard winter wheat lines, Bauermeister, Boundary, MDM, and WA8096 had low Δ and high yield in both 2010 and 2011 (Figures 4c,d). The other hard winter wheat lines, including Northwest 553, OR2080156H, and UICF-Grace, had low Δ, and WA8119, WA8120, WA8070, Finley, AgriPro Paladin, Hatton, and WA8118 had high Δ in both years. The grain yield of Northwest 553, OR2080156H, UICF-Grace, and AgriPro Paladin was low, and that of WA8119, WA8120, WA8070, and Finley was high in these environments. The yield of Hatton and WA8118 varied across the two different years. In hard winter wheat, grain yield increased by 21.27% in drought-tolerant Bauermeister, Boundary, MDM, and WA8096 compared with drought-sensitive AgriPro Paladin, Hatton, and WA8118.

To assess the predictive capability of Δ, values obtained in sampled environments were used to determine the relationships with yield and test weight in the low-, intermediate-, and high-precipitation regions (Table 4). Only the two wheat classes that had significant genetic variation for Δ and significant correlations for Δ values between sampled environments (soft spring and hard winter) were used (Table 4). In regions with low and intermediate precipitation, Δ values were negatively correlated with yield of soft spring wheat in 2009 only and were not correlated with test weight. Only a weak association was observed between Δ and yield in the intermediate precipitation region in 2010 ($P < .10$). The Δ values for hard winter wheat in the zones with low and intermediate rainfall were not correlated with yield but were positively correlated with test weight in both 2010 and 2011. In the high-precipitation region, Δ values were negatively correlated with yield of hard winter wheat in 2011 only and were not associated with soft spring wheat in either year. Over both classes of wheat, the relationships observed between Δ and yield was negative or nonsignificant, and relationships between Δ and test weight were positive or nonsignificant.

A subset of spring wheat lines was evaluated in the greenhouse under three levels of moisture to determine the relationship between Δ values and grain yield in a carefully controlled moisture regime. The effects of moisture treatment and the genotype response in each treatment were significant ($P \leq .05$) (Figure 5). The mean grain weights combined over moisture treatments of Nick, Louise, WA8089, Diva, Eden, WA8078, Lassik, Hollis, Jefferson, WA8074, and JD were significantly higher than Alturas, Alpowa, and Patwin (Table 5). These low-yielding lines had later heading than other lines tested in the greenhouse. These lines may all have a requirement for vernalization, which would make them late in the greenhouse but would not show up in the field because, even in spring, the nighttime temperatures are low enough to meet the requirement. The ranking of all spring wheat genotypes for grain weight averages among moisture treatments found that the top five were soft spring wheat varieties. Nick ranked the highest among the 18 wheat lines and was followed by Louise, WA8089, Diva, and Eden (Table 5). In

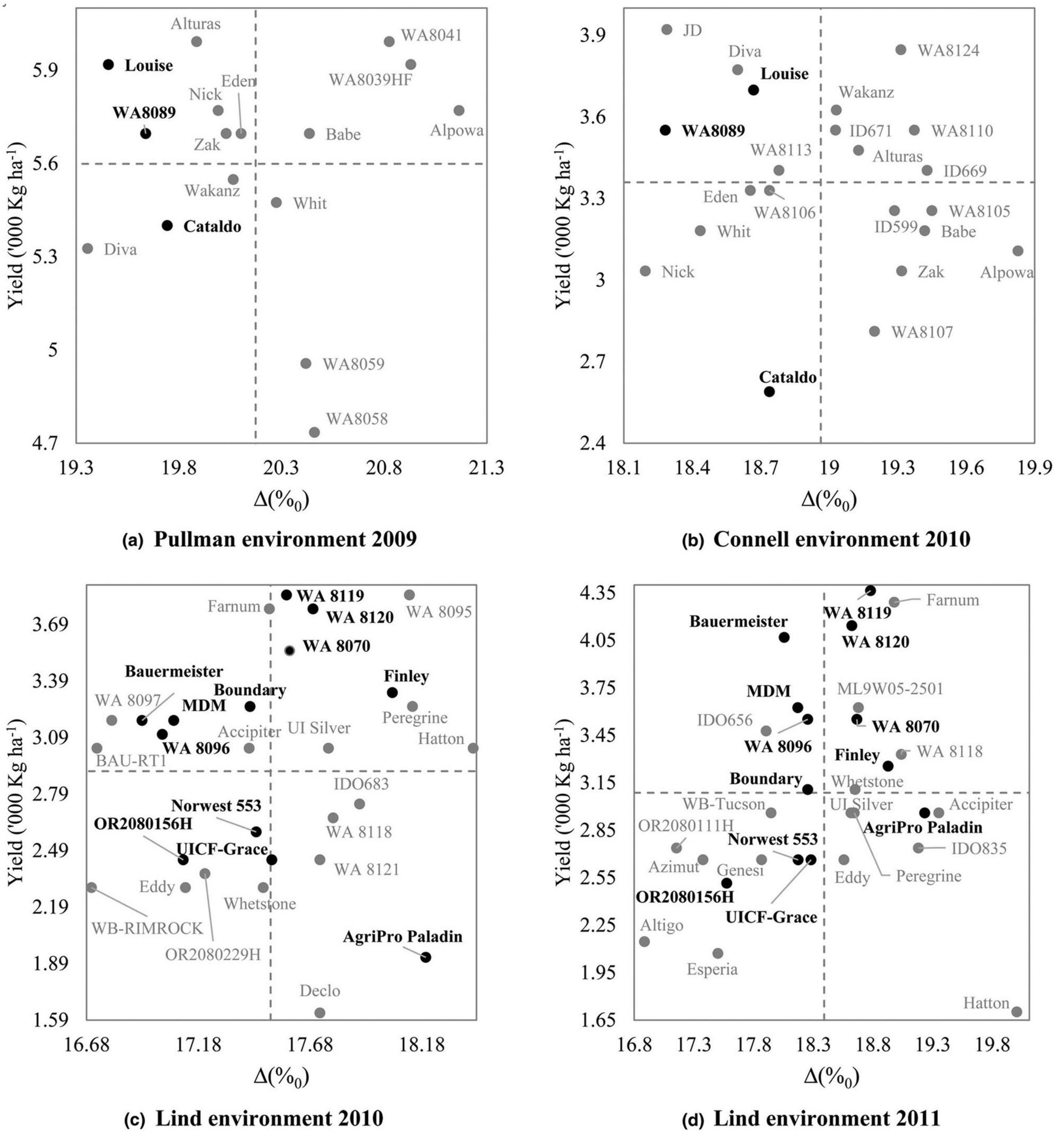

FIGURE 4 Distribution of (a and b) soft spring wheat and (c and d) hard winter wheat in relation to carbon isotope discrimination (Δ) and grain yield in each sampled environment. Mean values of Δ and yield are indicated with dashed lines that separate four distinct quadrants (high yield and low Δ, high yield and high Δ, low yield and low Δ, and low yield and high Δ). The wheat lines in each quadrant common in both years are highlighted in bold letters

the low-moisture treatment, Louise, WA8089, Diva, and Eden performed better than Nick. Through a separate grain weight analysis of soft spring and hard spring wheat in an average moisture regime, Alturas and Alpowa had lower yield than other soft spring wheat lines, but no significant difference was found in hard spring wheat lines.

The field Δ values (averaged over the two field environments Pullman and Connell) of soft spring wheat were used to predict grain weight in different moisture regimes in the greenhouse. The cultivar JD was not included in the test because Δ values were available in just one environment. The correlation between Δ and grain weight of soft

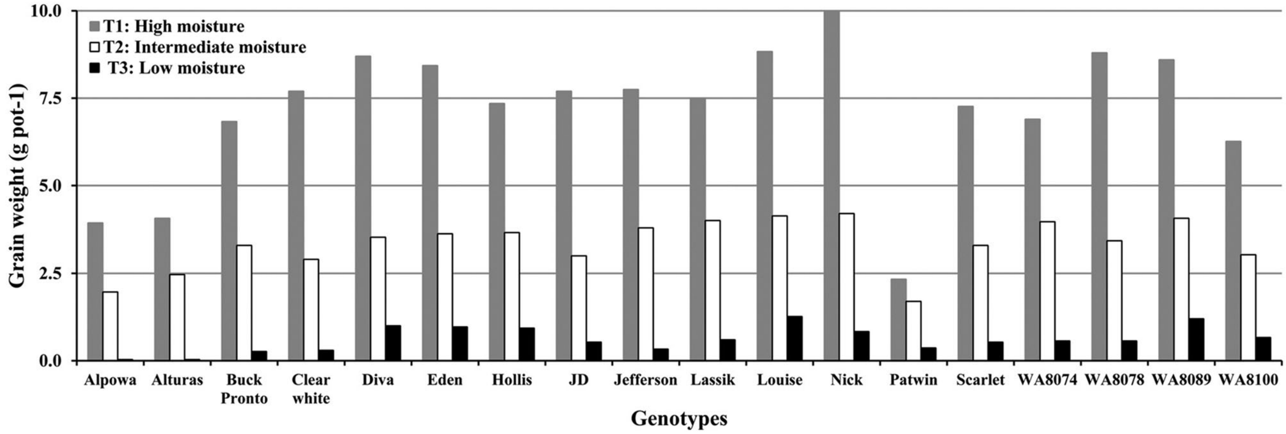

FIGURE 5 Grain weight of spring wheat lines tested in three different moisture regimes (T1, high; T2, intermediate; T3, low) in the greenhouse. Both treatment and genotype effects were significant at $P \leq .05$

spring wheat was significant ($P < .05$) under low ($r = -.81$), intermediate ($r = -.88$), and high ($r = -.81$) moisture regimes. The Δ values were positively associated with DSI calculated from greenhouse data ($P = .05$; $r = .46$). Louise, WA8089, Diva, Nick, Eden, WA8078, and Jefferson had DSI values ≤1, indicating drought tolerance in these varieties.

The parents of the HD-RIL population varied for yield (Hollis, 247.11 g m^{-2}; Drysdale, 297.83 g m^{-2}) and Δ (Hollis, 18.87; Drysdale, 18.39) when the values were averaged over all environments. Higher yield and higher TE were observed in Drysdale than in Hollis in both rainfed (yield: Hollis, 154.51 g m^{-2} and Drysdale, 172.23 g m^{-2}; Δ: Hollis, 17.43 and Drysdale, 16.54) and irrigated (yield: Hollis, 293.41 g m^{-2} and Drysdale, 360.64 g m^{-2}; Δ: Hollis, 19.59 and Drysdale, 19.32) environments. Significant genetic variation for yield was found among the HD-RILs in both the irrigated and dryland conditions when genotype was considered as a fixed factor in ANOVA. Genetic variation for Δ values was also observed in two of the three environments in which they were sampled in rainfed environments (2012; $P = .11$) and irrigated environments (2013; $P \leq .05$). The leaves for Δ analysis were sampled at the same growth stage (Z31) in all environments. Carbon isotope discrimination values were about 18% higher in irrigated environments than in the rainfed environment. When the Δ values for the different lines were correlated with the yield performance from the same environment, significant associations were observed in two of three environments. High yields were significantly, but weakly, correlated with high Δ values (low WUE) in the 2012 rainfed environment ($r = .42$) and in the 2013 irrigated environment ($r = .41$) (Table 6).

4 | DISCUSSION

Genetic variation of Δ was estimated to be low in some wheat classes. For example, the significant and larger G × E interaction found in hard spring varieties was particularly apparent because some cultivars had relatively high Δ values in one environment and relatively low Δ values in the other. Alternatively, the soft winter wheat cultivars showed significant differences in Δ values in the Lind 2010 environment but not in Lind the following year, indicating either that a low level of genetic variation exists for the trait in this germplasm or that the trait is not expressed in all environments. The poor consistency of Δ values of cultivars between environments can lead to difficulty in breeding for TE. This is also supported by Dixon, Godoy, and Carter (2019), who found that Δ was not beneficial for increasing yield of PNW-adapted soft winter wheat varieties. In addition to this, the G × E interaction for Δ was significant in both classes of spring wheat but not in winter wheat, likely because flag leaf samples were collected from different (high and low) precipitation environments for spring wheat and from the same (low) precipitation region for the winter wheat. Li et al. (2012) also found a significant G × E interaction of Δ when flag leaf samples of spring wheat were collected from three irrigation regimes.

High Δ values observed for spring wheat in a high-precipitation environment (Pullman in 2009) and low Δ values in a low-precipitation environment (Lind in 2010) reflect the different response of Δ to available moisture. The Δ has been found to be affected by the amount and distribution of precipitation and pre-existing soil moisture (Condon et al., 2002; Raimanova, Svoboda,

TABLE 4 Correlation coefficients of carbon isotope discrimination (Δ) in sampled environments with mean yield and test weight in regions with low, intermediate, and high precipitation for soft spring and hard winter wheat

	Yield			Test wt.		
Wheat type/environment	Low (<150 mm)[a]	Intermediate (150–310 mm)[b]	High (>310 mm)[c]	Low (<150 mm)[a]	Intermediate (150–310 mm)[b]	High (>310 mm)[c]
Soft spring 2009	−0.64**	−0.59*	−0.17	0.25	−0.12	−0.08
Soft spring 2010	–[d]	−0.39	−0.07	–	−0.37	0.02
Hard winter 2010	–	0.06	−0.04	–	0.56*	0.49*
Hard winter 2011	−0.25	0.06	−0.68**	0.53*	0.57**	0.30

[a]Low: Bickleton and Horseheaven for soft spring wheat in 2009 and Connell for hard winter wheat in 2011. [b]Intermediate: Almira, Connell, Dayton, and Lind for soft spring wheat in 2009; Bickleton, Connell, Lind, and St. John for soft spring wheat in 2010; Connell, Horse Heaven, and St. Andrews for hard winter wheat in 2010 and Lind, St. Andrews, and Almira for hard winter wheat in 2011. [c]High: Pullman, St. John, and Walla Walla for soft spring wheat in 2009; Pullman and Almira for soft spring wheat in 2010; Dayton, Pullman, and Almira for hard winter wheat in 2010 and Dayton, Pullman, and Walla Walla for hard winter wheat in 2011. [d]Data not presented for soft spring wheat and hard winter wheat in low-precipitation region in 2010 because of the higher-than-normal (>150 mm) precipitation in these locations.

*Significant at the .05 probability level. **Significant at the .01 probability level.

Kuresova, & Haberle, 2016; Zhu, Liang, Xu, Hua, & Monneveux, 2009).

Leaf samples for Δ from Pullman in 2009 showed good correlation with yield in regions with low and intermediate rainfall, whereas leaf samples from Lind did not have good association with yield in regions with low and intermediate rainfall. Therefore, the Δ values used should be from a region with high rainfall or from an irrigated environment where soil moisture is uniformly distributed. In Pullman, yield was not significant, but Δ was; this could be because Δ is a more highly heritable trait than yield.

The association between Δ and yield in regions with low and intermediate precipitation was negative. Such negative associations between Δ and yield in moisture-limited environments have been reported by other studies (Ferrio et al., 2007; Rebetzke et al., 2002). A low Δ value indicates high photosynthetic capacity or high TE, which is a desirable trait for plants growing in environments with low soil moisture (Condon, Richards, & Farquhar, 1992; Farquhar et al., 1989). The negative correlation was greater in 2009, which was a much drier year than 2010. The poorer correlation between Δ and yield in spring wheat in 2010 might be due to reduced drought stress because of the higher precipitation during the study period. Merah, Deléens, Souyris, Nachit, and Monneveux (2001a) found that the correlation between Δ of flag leaves and grain yield decreased with increasing precipitation. However, contrary to this expectation, the only other significant correlation between high yield and good WUE was with hard winter wheat in the high-precipitation region in 2011. A general trend toward low grain test weight with good WUE was also observed for this germplasm in most environments. Lanning et al. (2010) found that high summer temperatures lowered wheat test weight by decreasing the duration and efficiency of grain filling. In our study, post-flowering drought and heat stresses in summer likely affected the grain filling duration and lowered test weight.

The poor association between Δ and yield in wheat classes like the hard spring class was likely due to a lack of significant genetic variation in the current varieties. Significant genetic variation was found in the HD-RIL lines for these traits in rainfed and irrigated environments. When Δ values were associated with grain yields from the same low-rainfall environment the leaves were sampled from, the correlations were either nonsignificant or weakly positive in spite of having the high TE line Drysdale from Australia as a parent. Christy et al. (2018) found that the drought-tolerant parent Drysdale transpired less water compared with drought-susceptible parent Hartog. Despite the higher transpiration efficiency of genotypes in dry areas, higher yield was found mostly in wet areas,

TABLE 5 Mean grain weight of spring wheat lines in the greenhouse and their rank in low, intermediate, and high moisture regimes

			Rank		
Genotypes	Wheat type[a]	Grain weight least square mean[b]	Low moisture	Intermediate moisture	High moisture
Nick	SWS	5.03a	6	1	1
Louise	SWS	4.74ab	1	2	2
WA8089	SWS	4.62ab	2	3	5
Diva	SWS	4.41ab	3	9	4
Eden	SC	4.34ab	4	8	6
WA8078	HWS	4.27ab	10	10	3
Lassik	HRS	4.03ab	8	4	10
Hollis	HRS	3.98ab	5	7	11
Jefferson	HRS	3.96ab	14	6	7
WA8074	HRS	3.81ab	9	5	13
JD	SC	3.74ab	11	14	9
Scarlet	HRS	3.7abc	12	12	12
Clear white	HWS	3.63abc	15	15	8
Buck Pronto	HRS	3.47bcd	16	11	14
WA8100	HWS	3.32bcd	7	13	15
Alturas	SWS	2.19cde	18	16	16
Alpowa	SWS	1.98de	17	17	17
Patwin	HWS	1.47e	13	18	18

[a]HRS, hard red spring; HWS, hard white spring; SC, spring club; SWS, soft white spring. [b]Different letters indicate significant difference at $P \leq .05$.

TABLE 6 Mean, maximum, minimum, broad sense heritability (h^2), and correlation of carbon isotope discrimination (Δ) with yield and test weight in the Hollis/Drysdale population evaluated in rainfed (2012) and irrigated (2012 and 2013) environments of Lind, WA

	Δ[a]				Δ correlation with	
Location	Mean	Max.	Min.	h^2	Yield	Test wt.
					g m^{-2}	kg m^{-3}
Lind rainfed 2012	16.58	17.13	16.30	0.59	0.42***	0.07
Lind irrigated 2012	19.27	20.41	18.20	na[b]	0.08	0.01
Lind irrigated 2013	19.67	20.73	18.56	0.92	0.41***	−0.21*

[a]Carbon isotope discrimination at jointing stage (Z31) in Lind rainfed and irrigated in 2012 and Lind irrigated in 2013 environments. [b]Not available because Δ was measured only in one replication in this environment.
*Significant at the .05 probability level. ***Significant at the .001 probability level.

where both transpiration efficiency and higher transpiration can occur. Araus et al. (2003) found a weak but positive association between Δ and yield in wet years or under supplemental irrigation. In our study, the Lind 2012 environment had high precipitation during grain filling, which could have led to a positive association between Δ and yield in this rainfed environment. However, excessive water during a wet season may have affected the relationship between Δ and yield and could have resulted in no significant association in the irrigated environment in the same year. Seasonal weather conditions affect the Δ relation with yield (Wang et al., 2016). Similarly, the Lind 2013 environment was dry, with less precipitation during the growing season. Additional water provided through irrigation could have benefited plant performance, resulting in a positive association between Δ and yield in the irrigated environment. Furthermore, the lower TE line Hollis was the adapted parent. Thus, in this population, TE, at least as measured by leaf Δ analysis, may not have been the most important contributor to grain yield in low-rainfall PNW environments.

The benefits of high TE were apparent in some genetic backgrounds. A positive association was found between Δ values (from field samples) and DSI of the genotypes tested in the managed greenhouse environment. Drought susceptibility index values indicated that soft spring and club wheat, including Louise, WA8089, Diva, Nick, and Eden, were relatively tolerant to drought, whereas

Alpowa and Alturas were susceptible. A greenhouse evaluation for grain weight found a consistently high performance of Nick, Louise, WA8089, Diva, and Eden averaged over moisture treatments. The lines that exhibited significant differences that were repeatable may be exploited in breeding for drought tolerance. Examples included the spring wheat varieties Louise, WA8089, Diva, Nick, and Eden and the winter varieties Bauermeister and MDM.

5 | CONCLUSIONS

Genotypic variation of Δ exists in PNW-adapted soft spring and hard winter wheat lines. Low Δ is important in soft spring wheat lines in regions with low and intermediate precipitation, where crop growth is supported through efficient use of water to sustain yield. In hard winter wheat, no significant association was observed between Δ and yield in the region with low precipitation; however, some WUE genotypes had high yield in moisture-limited environments. This is consistent with the hypothesis that genotypes with low Δ have better adaptation in drought environments than genotypes with high Δ. Future research on other physiological traits, including leaf stomata conductance and leaf photosynthesis, may help to understand physiological mechanisms of WUE in the PNW wheat germplasm.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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