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Relationship between carbon isotope discrimination and grain yield of rainfed winter wheat in a semi-arid region

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Abstract Carbon isotope discrimination (CID) has been suggested as an indirect selection criterion for grain yield under drought stress in wheat (*Triticum aestivum* L.). However, the association between CID and grain yield varies greatly among different studies. In this study, conducted in western Kansas, a temperate semi-arid region, the genetic variation of grain CID and its association with grain yield and other agronomic traits were examined using 43 preliminary and 15 advanced breeding lines under dryland conditions. The 43 preliminary breeding lines were tested in three environments while the 15 advanced breeding lines were tested in one environment. Results showed that both preliminary and advanced breeding lines had significant genetic variations and relatively small coefficients of variation in grain CID, indicating grain CID as a promising trait for selection in wheat breeding programs. In the trials for the preliminary breeding lines, the association between grain CID and grain yield was significant ($P < 0.05$) and positive in all three environments with Pearson correlation coefficients ranging from 0.34 to 0.74. This correlation was stronger in a trial with expected post-anthesis drought stress. However, there was only a weak positive correlation between grain CID and grain yield in the trial for the advanced breeding lines. Winter injury may have confounded the grain CID and yield relationship. This study also revealed a significant ($P < 0.05$) and negative correlation between grain CID and grain protein content in all four trials with Pearson correlation coefficients ranging from −0.43 to −0.65, suggesting a possible impact on baking quality while selecting high grain CID. Therefore, our results suggest that grain CID could be useful for grain yield prediction in semi-arid areas with moderate drought stress. However, precaution should be taken for selecting grain CID because of the effect of environment on its association with grain yield and its negative correlation with protein content.

Keywords Wheat · Drought tolerance · Carbon isotope discrimination · Grain yield · Protein content

Introduction

Wheat (*Triticum aestivum* L.) grain productivity is limited by water supply and/or drought in about 50 % of the wheat area in developing countries and 70 % in developed countries (Trethowan and Pfeiffer 2000). As the world leading crop for staple food, new wheat varieties with enhanced drought tolerance will support food security. Direct selection for grain yield in water-

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stressed environments has been used extensively to improve drought tolerance; however, its progress is limited due to the complexity and low heritability of drought tolerance traits (Blum 1988). Indirect selection of drought tolerance associated morpho-physiological traits (physiological approach) has been suggested for wheat breeding programs (Richards et al. 2002; Reynolds et al. 2009). Carbon isotope discrimination (CID) is one physiological trait proposed as an indicator of grain yield under drought conditions (Condon et al. 2002; Rebetzke et al. 2006). Two wheat varieties "Rees" and "Drysdale" were developed in Australia through selection of CID (Condon et al. 2004).

CO_2 assimilation by Rubisco utilizes proportionately less ^{13}C than ^{12}C, resulting in a smaller ^{13}C composition in plant tissues relative to atmospheric isotopic composition (Farquhar and Richards 1984). CID is a measurement of the ^{13}C to ^{12}C ratio difference between plant materials and atmosphere. Genetic variability in CID is substantial (Farquhar and Richards 1984; Monneveux et al. 2005; Rebetzke et al. 2002; Wu et al. 2011). Genetic variation in CID may result from differences in stomatal conductance and/or photosynthetic capacity (Morgan et al. 1993). Moderate to high heritability for CID has been reported (Condon and Richards 1992; Ehdaie and Waines 1994; Rebetzke et al. 2002). Measurement of CID is relatively quick and can use various dry plant tissues, frequently the flag leaf at anthesis and grain at maturity (Monneveux et al. 2005; Shahram et al. 2009; Li et al. 2012; Wang et al. 2013). Other plant components used for analysis include whole plants at stem elongation (Kirda et al. 1992), the fourth seedling leaves (Monneveux et al. 2005), penultimate leaves at heading (Araus et al. 1998), and peduncles at maturity (Condon et al. 1987; Morgan et al. 1993). In wheat, CID can vary among plant components; moderate correlation has been reported between CIDs from different plant components. Condon and Richards (1992) compared CIDs among whole plants at the elongation stage, stems at maturity, flag leaves at anthesis, and grain, and only observed a significant correlation between whole plants at the vegetative stage and flag leaves at anthesis. Merah et al. (2001) found that flag leaf CID was correlated with grain CID, with correlation coefficients ranging from 0.25 to 0.5.

The association between CID and grain yield/biomass, observed in many studies, varied from negative to positive. This discrepancy may result from differences in environmental conditions, including distribution and amount of precipitation. CID was first suggested as an indicator of transpiration efficiency (TE) (Farquhar and Richards 1984); a negative correlation between CID and TE was demonstrated (Condon et al. 1990; Ehdaie et al. 1991; Shahram et al. 2009; Wang et al. 2013). It follows that CID and grain yield should also exhibit a negative correlation, assuming that genotypes use the same amount of water (Morgan et al. 1993) and have similar harvest indices (Passioura 1977). These assumptions may be valid for environments with optimum water or extreme dry conditions. Shahram et al. (2009) noted a negative correlation between CID and grain yield in a pot study under a well-watered treatment. However, most field studies, conducted under moderate water limitations, reported positive correlations between CID and grain yield (Araus et al. 2003; Condon et al. 1987; Li et al. 2012; Kumar et al. 2011; Merah et al. 2001; Misra et al. 2010; Monneveux et al. 2005; Morgan et al. 1993; Shahram et al. 2012; Tsialtas et al. 2001; Wahbi and Shaaban 2011; Wu et al. 2011).

Environmental conditions may greatly affect the correlation between CID and grain yield. Most studies conducted in multiple environments (Li et al. 2012; Kumar et al. 2011; Misra et al. 2006; Monneveux et al. 2005; Tokatlidis et al. 2004; Tsialtas et al. 2001; Xu et al. 2007) found inconsistency in this correlation among environments. Both Monneveux et al. (2005) and Xu et al. (2007) observed consistent correlations only under post-anthesis water stress. Moreover, this correlation is also related to the plant parts analyzed. A negative correlation between CID and grain yield was generally found in plant parts sampled at the early stages (Rebetzke et al. 2002). Merah et al. (2001) found that grain CID is more associated with grain yield than flag leaf CID. Considering both the potential to identify improved drought tolerance and the complexity of the relationship between CID and grain yield, it is appropriate to examine this relationship in target environments where breeding lines are tested and to determine its value to breeding programs.

Kansas is the largest wheat producing state in the U.S.A.; the 3.84 million ha planted in 2013, comprised about 17 % of the total U.S. wheat area (http://www.nass.usda.gov/). Approximately 40 % of the Kansas wheat area is located in the semi-arid western part of the state with annual precipitation of about 430 mm.

Drought can occur at any stage of crop development in this region, depending on the distribution and quantity of precipitation. Similar semi-arid regions spread over most states in the U.S. Central Plains. Therefore, drought tolerance is a major breeding objective for wheat breeding programs in the U.S. Central Plains. However, no previous studies had examined CID in relation to grain yield of breeding lines in this type of semi-arid environment. This study aimed to determine the genetic variation in CID among wheat breeding lines, explore its relationship with grain yield and other agronomic traits, and assess the utility of CID for wheat breeding programs in temperate semi-arid regions.

Materials and methods

Two sets of breeding lines developed by wheat breeding programs at Kansas State University were included in this study. The first set consisted of 43 preliminary breeding lines (F_4 derived) while the second set consisted of 15 advanced breeding lines (F_5 or F_6 derived).

The 43 preliminary breeding lines and seven regionally adapted varieties were tested in 2007 and 2010 at Colby, KS (39.3922°N, 101.0475°W), and in 2007 at Garden City, KS (37.9753°N, 100.8642°W). The advanced breeding lines together with five regionally adapted varieties were tested in 2009 at Hays, KS (38.8794°N, 99.3222°W). These three locations are the main testing sites in western Kansas for the wheat breeding programs at Kansas State University. Soils at Colby and Garden City are Keith silt loams; and at Hays, a Harney silt loam. The mean annual precipitation (from 1981 to 2010) was 486 mm at Colby and Garden City, and 596 mm at Hays.

The trial at Colby in 2010 (2010 Colby) was planted in five-row plots with a plot size of 1.5 m × 3.0 m whereas others were planted in six-row plots with a plot size of 1.8 m × 3.0 m. The trials in 2007 for the preliminary breeding lines were not replicated due to limited amounts of seed. The trial at Hays in 2009 (2009 Hays) and the 2010 Colby trial were arranged in a random complete block design with three replications. In harvest year 2007, the trial at Garden City (2007 Garden City) was seeded (in the previous year) on Oct. 3 and the trial at Colby (2007 Colby) on Sep. 19. The 2009 Hays trial was seeded on Sep. 28 whereas the 2010 Colby trial was seeded on Oct. 7. Seeding rate was 56 kg/ha for each trial. Nitrogen (urea) was applied with a rate of 90 kg/ha before sowing. One application of herbicide Glean® {2-Chloro-*N*-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl] benzenesulfonamide} was made before jointing to control broad leaf weeds. Fungicides were not applied.

Plot heading date was determined as days from January 1 to 50 % of fully emerged heads. Heading date was only recorded in the 2009 Hays trial. After flowering, plant height was measured from the soil surface to the tip of the spike excluding the awns. Weeds and stripe rust infestation were observed in the 2010 Colby trial and were scored with a percentage number for weeds and on a scale of 1–9 for stripe rust (9, as the maximum severity). Plots with weeds rating over 10 % were treated as missing. At maturity, plots were harvested with a combine for grain yield determination. Grain harvested from each plot was measured for test weight. Grain samples (10 g) from each plot were ground and analyzed for carbon isotope percentages and N % using an isotope ratio mass spectrometer in the Stable Isotope and Mass Spectroscopy Laboratory at Kansas State University. Carbon isotope composition ($\delta^{13}C$) was calculated by comparing ^{13}C to ^{12}C composition of each sample (R_s) relative to the composition of a Pee Dee Belemnite (PDB) standard (R_{PDB}) via the formula: $\delta^{13}C$ (‰) = $[(R_s/R_{PDB})-1] \times 1{,}000$. The CID ($\Delta$) of the sample was obtained by the formula: $\Delta = (\delta^{13}C_a - \delta^{13}C_p)/(1 + \delta^{13}C_p)$. The $\delta^{13}C_a$ and $\delta^{13}C_p$ in the formula are the carbon isotope compositions ($\delta^{13}C$) of the atmospheric and plant samples, respectively. $\delta^{13}C_a$ was assumed to be −8 ‰ (Farquhar et al. 1989). Grain protein was calculated from N % by multiplying by a conversion factor of 5.7.

Data were analyzed using SAS 9.3. Analysis of variance was conducted for replicated trials. Broad sense heritabilities based on entry means were estimated using the formula $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/r)$, where σ_g^2 is the genetic variance, σ_e^2 is the error variance, r is the number of replicates. Pearson's phenotypic correlations were calculated to determine relationships between CID, grain yield, and other agronomic traits. Least square means estimated for lines in the replicated trials were used for calculation of phenotypic correlations. In replicated trials, genetic correlations were also estimated using the formula

$r_{g(AB)} = \sigma_{g(AB)}/(\sigma^2_{g(A)*}\sigma^2_{g(B)})^{1/2}$, where A and B represent the two traits.

Results

Precipitation amounts during the wheat growing cycle at each test location-year are presented in Table 1. The total precipitation amount during the crop growing cycle for each trial ranged from 362.7 mm at the 2009 Hays trial to 413.5 mm at the 2010 Colby trial. Although there were no large differences in precipitation between trials, the distributions of precipitation were quite different. Both trials at Colby and Garden City in 2007 received much less precipitation than normal in May and June and suffered drought stress during their reproductive stages. However, the 2007 Colby trial received more precipitation at the seedling stage compared to the 2007 Garden City trial. The 2009 Hays trial had good moisture at planting, but received record low precipitation from November through March causing a severe pre-anthesis drought. The 2010 Colby trial was dry after planting and did not establish good stands, leading to weed infestation. However, it had good precipitation during its reproductive stage, which led to a severe stripe rust infestation. The 2007 Garden City trial suffered from several hard freezes in early to mid-April.

In both the 2010 Colby and 2009 Hays trials, significant ($P < 0.05$) genetic variations were found for grain yield, grain CID, kernel protein, test weight, and plant height (Table 2). In the 2010 Colby trial, there was a significant ($P < 0.001$) variation in stripe rust infestation with a range from 4.33 to 9.00. No significant variation in weed infestation was found after eliminating 16 plots with 20–60 % weed infestation. In the 2009 Hays trial, there was a significant ($P < 0.001$) variation in heading date, although only a three-day difference was observed between the earliest and latest entries. In both replicated trials, grain CID had a relatively smaller coefficient of variation (CV) than all other traits except test weight whereas grain yield had the largest CV. However, grain CID had relatively lower broad sense heritability than all other traits.

Among the three trials with the preliminary breeding lines, there were large variations in grain yield, grain CID, grain protein content, and test weight. The mean grain yield varied from 3.73 t/ha in the 2007 Garden City trial to 5.82 t/ha in the 2007 Colby trial. The mean value of grain CID in the 2007 Garden City trial was the smallest (16.07), but it was much larger in the 2010 Colby trial (19.12). However, the range of grain CID was relatively larger in the 2007 trials (3.47 at Garden City and 4.13 at Colby) than the 2010 Colby trial (1.19). In general, the 2007 Garden City trial had a relatively higher grain protein content (16.11 %) and a greater range (from 13.44 to 20.11 %) than the other two trials. The 2007 Colby trial had a relatively larger test weight, but a smaller range than the other two trials. For the advanced breeding lines, the 2009 Hays trial had relatively small grain yields with a mean value of 3.75 t/ha, but a relatively large grain CID with a mean value of 18.51. In general, the 2009 Hays trial had relatively smaller ranges in grain yield, grain CID, and protein content when compared to the trials for the preliminary breeding lines.

Grain yield and grain CID were positively correlated ($P < 0.05$) for the preliminary breeding lines in all three trials, (Table 3; Fig. 1) with Pearson phenotypic correlation coefficients ranging from 0.34 (2010 Colby) to 0.74 (2007 Garden City). The genetic correlation coefficient (0.41) in the 2010 Colby trial was slightly higher than its phenotypic correlation coefficient. However, the association between grain yield and CID in the trial for the advanced breeding lines was not significant. Significant ($P < 0.05$) negative correlations between CID and grain protein content occurred in all four trials (Table 3; Fig. 2) with Pearson phenotypic correlation coefficients

Table 1 Monthly precipitation amount (mm) during the wheat growing season

Year	Location	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	Total
2007	Garden city		56.4	1.5	126.2	14.7	15.7	44.5	73.7	30.2	25.9	388.9
2007	Colby	26.4	79.8	0.5	107.4	16.0	15.5	16.0	87.6	29.2	35.1	413.5
2010	Colby		79.0	10.4	17.8	4.1	9.7	44.2	66.5	67.6	68.6	362.7
2009	Hays		152.9	17.8	6.1	0.8	0.8	0.3	84.8	56.1	43.2	367.8

ranging from −0.43 (2009 Hays) to −0.65 (2007 Garden City). The test weight was not consistently correlated with grain CID across the four trials. Heading date, plant height, and stripe rust reaction were all not significantly correlated with grain CID.

Table 2 Data summary for grain yield (GY, t/ha), CID (‰), kernel protein (KP,%), test weight (TWT, kg/hl), plant height (HT, cm), heading date (HD, days from Jan 1 to heading), and stripe rust (YR) reaction in trials conducted in 2007 at Garden city, Colby, 2010 at Colby, and 2009 at Hays, KS

	2007 Garden city			2007 Colby			2010 Colby						2009 Hays					
Trait	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	CV[a]	H^{2b}	F^c	Mean	Min	Max	CV	H^2	F
GY	3.76	2.04	5.55	5.87	5.02	6.88	4.94	3.14	6.48	9.96	0.86	7.2***	3.75	3.29	4.29	7.33	0.71	3.5***
CID	16.07	14.75	18.22	17.01	14.99	19.12	19.14	18.46	19.65	1.78	0.40	1.7*	18.51	18.05	18.97	1.55	0.59	2.4**
KP	16.11	13.44	20.11	13.21	10.36	15.22	13.36	11.65	14.90	6.07	0.48	1.9**	13.08	11.59	14.11	4.86	0.70	3.4***
TWT	76.46	69.38	82.69	83.14	79.84	84.88	78.41	71.67	81.33	1.98	0.76	4.4***	82.84	80.37	85.30	0.50	0.97	34.3***
HT	–	–	–	–	–	–	76.35	67.42	88.90	3.78	0.77	4.3***	78.78	72.80	84.70	2.86	0.81	5.3***
HD	–	–	–	–	–	–	–	–	–	–	–	–	133.72	132.33	135.33	0.38	0.89	9.5***
YR	–	–	–	–	–	–	7.58	4.33	9.00	11.45	0.86	7.1***	–	–	–	–	–	–

[a] Coefficient of variation, %

[b] Broad sense heritability

[c] F value for genotypes in analysis of variance

*, **, and ***, significant $P = 0.05$, 0.01 and 0.001, respectively

–, N/A

Discussion

Under dryland or rainfed conditions wheat grain yield is related to available soil water at emergence and precipitation occurring during vegetative and reproductive growth (Aiken et al. 2013). Under typical water-limited conditions, expected grain yield increases with precipitation. In semi-arid regions of the U.S. Central Plains most annual precipitation occurs during the September–June wheat growing cycle. Among the three environments for the preliminary breeding lines, the 2007 Colby trial had the most precipitation, leading to yields of about 2 t/ha more than the 2007 Garden City trial, the site with the least grain yield. However, precipitation distribution could also affect the grain yield since the drought stress during pollination and kernel development stages could cause more grain yield loss than the stress during vegetative stages (Prasad et al. 2008); harvest index can be reduced from 0.40 to 0.04 by severe water deficits (Aiken et al. 2013). In this study the 2007 Garden City trial had about 25 mm more precipitation than the 2010 Colby trial, but produced much less grain. This might be partly explained by the precipitation difference during May and June, the critical reproductive growth period (the precipitation amount in the 2010 Colby trial was almost double that in the 2007 Garden City trial, Table 1). However, several hard freezes in mid-April in the 2007 Garden City trial might also have significantly affected grain yield.

CID may be taken as an indicator of drought stress, with small values of CID indicating more drought stress (Condon and Richards 2002). In C_3 species, CID is positively related with the ratio of leaf internal CO_2 concentration (C_i) to the ambient CO_2 concentration (Farquhar et al. 1989). Under favorable water conditions and full illumination, leaf stomatal conductance can be large, resulting in increased C_i and therefore a large CID; whereas water deficits tend to decrease stomatal conductance, C_i, and CID. In this study grain CID was relatively larger in the 2009 Hays and 2010

Table 3 Pearson phenotypic and genetic (parentheses) correlations between carbon isotope discrimination (CID) and grain yield (GY), kernel protein (KP), test weight (TWT), plant height (HT), heading date (HD), stripe rust (YR) reaction in trials conducted in 2007 at Garden City, Colby, 2010 at Colby, and 2009 at Hays, KS

	GY	KP	TWT	HT	HD	YR
2007 Garden city	0.74***	−0.65***	0.64***	–	–	–
2007 Colby	0.46**	−0.46**	0.45**	–	–	–
2010 Colby	0.34* (0.41**)	−0.49*** (−0.33*)	0.22 (−0.14)	−0.01 (−0.28)	–	−0.11 (−0.26)
2009 Hays	0.25 (0.34)	−0.43* (−0.46*)	0.40 (0.51**)	−0.15 (−0.21)	0.15 (−0.09)	–

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

–, N/A

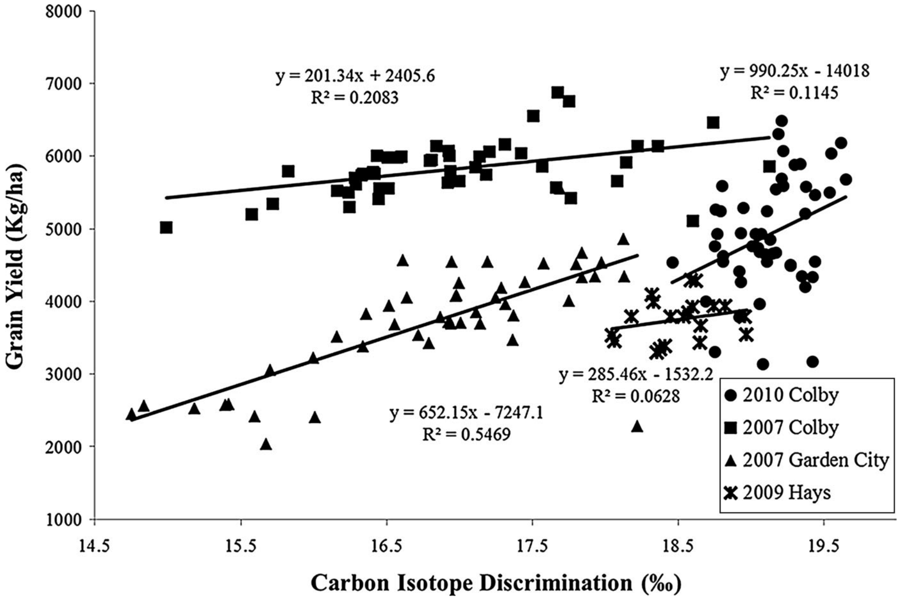

Fig. 1 Correlation between grain yield and carbon isotope discrimination in trials conducted in 2007 at Garden City and Colby, 2010 at Colby, and 2009 at Hays, KS

Colby trials, which could be related to favorable water conditions during their reproductive development. Also, the range in grain CID among genotypes was much smaller in these two trials. This is not surprising since all genotypes might have had similar large stomatal conductances under favorable water conditions, and therefore the variation in CID among genotypes was limited. Most variation in grain CID in the 2010 Colby trial might have been due to drought stress in the early growth stage and/or intermittent drought stress during the reproductive development. Other investigators reported that trials with large CID values also produced high grain yields (Araus et al. 2003; Kumar et al. 2011; Monneveux et al. 2005). In this study, the 2010 Colby trial, with the largest grain CID, produced only an intermediate grain yield. This might have been a consequence of stripe rust infection. Stripe rust in this trial had a negative impact on grain yield as indicated by a significant and negative correlation (−0.65, $P < 0.001$, data not shown) between disease severity and grain yield.

Significant genetic variation in grain CID was observed in both sets of experimental lines, indicating a possibility for selection among breeding lines. The broad sense heritability for CID in this study was moderate, and close to those reported by Ehdaie and Waines (1994). A high heritability (>0.83) in grain CID was noted by Condon and Richards (1992)

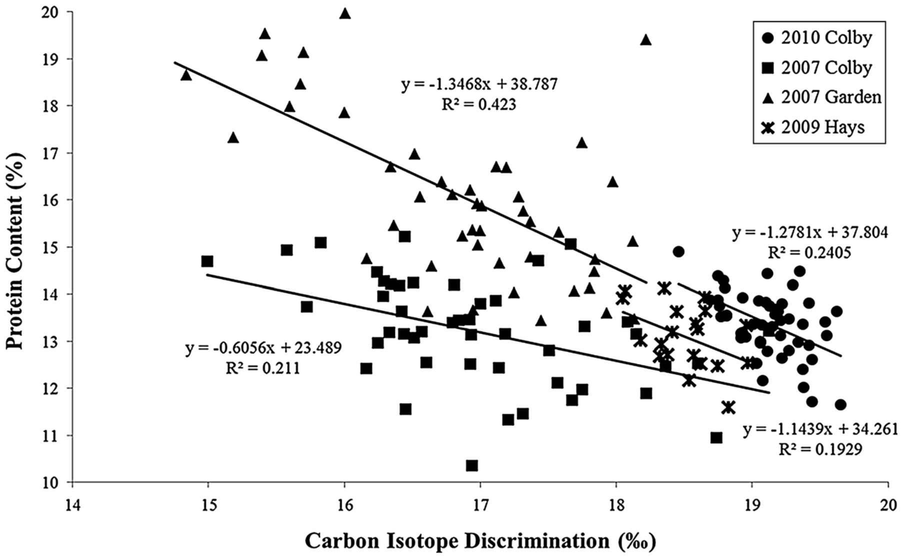

Fig. 2 Correlation between kernel protein content and carbon isotope discrimination in four trials conducted in 2007 at Garden City and Colby, 2010 at Colby, and 2009 at Hays, KS

whereas that reported by Wu et al. (2011) was much lower (0.23). This variation in reports of broad-sense heritability likely resulted from different genetic materials. According to its definition, broad sense heritability is defined by the relative size of genetic variation in relation to the experimental error variance. The small CV for CID reported in our trials, indicating a small error variance, is in agreement with other studies (Morgan et al. 1993; Rebetzke et al. 2002). However, our breeding lines had low genetic variability as indicated by the small ranges in grain CID in both replicated trials. This small genetic variability might have contributed to the relatively low heritability in our study. However, the small ranges of CID in our replicated trials could be due to the favorable water conditions during the reproductive stage as discussed above. Therefore, it is possible that heritability estimates for CID under post-anthesis drought stress might be higher than the values we actually obtained. Our results suggest that it might be more efficient to make selection for CID in environments with post-anthesis drought conditions.

Most previous studies reported a positive correlation between CID and grain yield under moderate drought conditions (see "Introduction"). Under moderate drought stress, genotypes differing in water capture could differ in cumulative transpiration; these differences in water use may impact grain yield more than TE (Morgan et al. 1993). Genotypes capable of greater water capture could keep their stomata more open, resulting in greater discrimination against ^{13}C and larger CID; the larger assimilation rates and associated yield increase could result in a positive association between CID and grain yield. Previous genetic studies also provided evidence for this positive correlation. Wu et al. (2011) identified a QTL for CID, which was close to a QTL for spike number per plant. Through analysis of substitution lines, chromosome 2B was found to be associated with both CID and grain yield (Shahram et al. 2012). In our study, each trial had about 400 mm of precipitation during its growing season, which could be characterized as moderate drought stress. The correlations between grain CID and yield were positive in all four trials, supporting the interpretation of CID under moderate water stress conditions. In our study, for the preliminary breeding lines, the strongest correlation between grain CID and grain yield was found in the 2007 Garden City trial whereas a weak correlation occurred in the 2010 Colby trial. The 2007 Garden City trial had a relatively more severe post-anthesis drought stress whereas the 2010 Colby trial mostly suffered pre-anthesis drought stress. Monneveux et al. (2005) also reported that correlation between grain CID and grain yield was relatively stronger and more consistent in environments with post-anthesis drought stress than the environments with pre-anthesis drought stress. Like the 2010 Colby trial, the 2009 Hays trial had drought stress mostly in its early growing stage. However, the correlation between gain CID and grain yield in 2009

Hays was not significant. This might be explained by the winter injury caused by a cold winter and very little snow cover. The winter injury might have affected grain yield and/or grain CID, and thus affected their correlation. In the 2009 Hays trial, the second and third replication might have had more winter injury than the first replication as indicated by their mean grain yields (3.55 and 3.56 t/ha for the second and third replication vs. 4.14 t/ha for the first replication). Without much winter injury, the correlation between grain CID and grain yield in the first replication was actually significant (0.43, $P < 0.05$, data not shown). Therefore, our results suggest a consistent correlation between grain CID and grain yield in environments with either post-anthesis drought stress or early drought stress when possible effects of winter injury are eliminated. This finding is in agreement with reports of positive correlation of CID and grain yield across two or three environments under rainfed conditions (Condon et al. 1987; Kumar et al. 2011; Li et al. 2012; Merah et al. 2001). The relatively stronger correlation between grain yield and CID in the 2007 trials further suggests that it might be more effective to use grain CID as an indicator for yield when post-anthesis drought stress occurs. There might be a concern for the cost of sample analysis when using CID as a selection criterion. However, this sample analysis cost ($10–15) is still relatively cheaper when compared to a yield trial ($33 per line for a three-replicated trial). To reduce costs in breeding programs, grain CID might be used to screen head rows or preliminary breeding lines, which have been selected for other desirable traits, to further eliminate lines before entering replicated yield trials or advanced yield trials at multiple locations.

Phenology traits might affect CID. Li et al. (2012) reported significant and positive correlations for both plant height and heading date with CID in the flag leaf collected at the milk grain stage (Feekes 11.1). Monneveux et al. (2005) reported a moderate correlation between heading date and grain CID in certain environments. Kumar et al. (2011) found that semi-dwarf breeding lines had higher grain CID than the tall ones. However, in our study, grain CID was not correlated with either plant height or heading date. The non-significant association between grain CID and heading date might result from the small variation (3 days) in maturity among the advanced breeding lines evaluated. As increased wheat yields are favored by earlier flowering, further studies on the effect of heading date on grain CID are warranted.

Our study is the first report on the relationship between grain CID and test weight. A positive and significant ($P < 0.01$) phenotypic or genetic correlation between grain CID and test weight was observed in the 2007 Colby, 2007 Garden City, and 2009 Hays trials, but not in the 2010 Colby trial. Under moderate drought stress genotypes with more capability to capture the soil water would have a higher CID and have more assimilates from photosynthesis to fill the grain, and therefore higher test weight. Moreover, drought-tolerant genotypes might have more capability to translocate more carbohydrates from stem reserves for grain fill under drought stress. Those carbohydrates stored in stems are usually synthesized during pre-anthesis or shortly after flowering and would be expected to have a higher CID than those fixed later in grain filling when plants are more stressed, and therefore a positive correlation between test weight and CID. The lack of a significant correlation between grain CID and test weight in the 2010 Colby trial might have resulted from stripe rust infection, which negatively affected both yield and test weight.

A consistent and negative correlation was found between grain CID and protein content in this study. This correlation is in agreement with the finding of Tokatlidis et al. (2004). An early study by McNeal et al. (1972) demonstrated that plants with either high or low protein contents absorbed and translocated similar amounts of nitrogen; grain protein percentages entirely depended upon the amount of carbohydrate transported into the grain. In our study, genotypes with large grain CID might have synthesized more carbohydrates during grain filling or translocated more pre-anthesis reserves into the grain than genotypes with lower grain CID, resulting in a relatively lower percentage of protein in the grain. This negative correlation might be a challenge for breeding wheat varieties with high protein content. However, this moderate and negative correlation might be overcome by selecting lines with enhanced N uptake and/or utilization capacity to obtain genotypes with both high CID and high protein content.

In conclusion, this study found significant genetic variations for grain CID among our breeding lines and a significant and positive correlation between grain CID and yield across three of four field trials conducted under various environmental conditions. These results indicate that grain CID could be a useful selection criterion for grain yield improvement under moderate drought stress, especially post-anthesis drought, in western Kansas and similar temperate semi-arid regions. The positive relationship of CID and test weight suggests that further investigation of CID as an indicator of enhanced translocation capacity should be undertaken.

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