Water use efficiency and nitrogen use efficiency of 30 spring wheat genotypes under drought stress and well-watered conditions

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Abstract: Improving water use efficiency (WUE) and nitrogen use efficiency (NUE) of crops is a crucial target of agricultural research. The aim of this study was to evaluate WUE and NUE in different wheat genotypes and investigate the relationships between WUE, NUE and grain yield in two contrasting soil water treatments. Thirty spring wheat genotypes were evaluated for WUE (assessed by flag leaf $\delta^{13}C$) and NUE (assessed by flag leaf C/N ratio) for both drought and well-watered field conditions during the 2009 and 2010 growing seasons. The well-watered treatment decreased WUE ($\delta^{13}C$) and NUE (C/N ratio) compared with the drought treatment, and NUE was more sensitive to supplementary water than WUE. The WUE and NUE decrease under the well-watered conditions for the drought-resistant genotype (DRG) and high-yield genotype (HYG) were much smaller than those for the drought-susceptible genotype (DSG) and low-yield genotype (LYG). The WUE and NUE of DSG were significantly more sensitive to supplementary water than those of DRG. The relationship between WUE and NUE was positive and significant under drought, but non-significant under the well-watered conditions. The WUE and NUE were both negatively correlated with grain yield. A few superior genotypes were identified. Drought-resistant genotype McNeal had high WUE under both drought and well-watered conditions. In HYG group, IDO702 and Alturas had high WUE under well-watered while IDO599 and Alturas had higher NUE under drought and well-watered. Preliminary results suggest that screening WUE and NUE in the HYG and DRG groups may be a feasible way to ameliorate resource use efficiency without yield penalties.

Keywords: wheat, water use efficiency, nitrogen use efficiency, drought stress, well-watered

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1 Introduction

Water and nitrogen (N) are two of the most important resources in plant life and physiological processes. Water use efficiency (WUE) is the ratio of total plant dry mass to cumulative plant water use. Similarly, nitrogen use efficiency (NUE) is the ratio of total plant dry mass to cumulative nitrogen use. In wheat breeding programs, selecting genotypes with high resource use efficiency (WUE and NUE) and grain yield is the aim when breeding for both dryland and irrigated farming (Zhang and Shan, 1998). Since it is difficult to screen large numbers of plants for subtle variation in plant WUE and NUE, the majority of experimental work on WUE and NUE has been restricted to measurements on container grown plants.

Farquhar, O'Leary and Berry (1982) postulated that the discriminate extent of C_3 plants against the carbon isotope ^{13}C during carbon assimilation was associated with WUE. The relationship between $\delta^{13}C$ and WUE has been confirmed in a number of studies with a variety

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of container-grown and field-grown plants including crops (Farquhar and Richards, 1984; Knight, Livingston and Kessel, 1994; Tambussi, Bort and Araus, 2007; Khazaei et al., 2009), trees (Patterson, Guy and Dang, 1997; Livingston, Guy and Ethier, 1999), and grasses (Toft, Anderson and Nowak, 1989; Ebdon, Petrovic and Dawson, 1998; Chen et al., 2005; Tsialtas and Veresoglou, 2007). The correlation between $\delta^{13}C$ and transpiration efficiency was found to be positive in wheat (Ehdaie et al., 1991). Leaf $\delta^{13}C$ was positively related with long-term WUE and has been considered as an effective tool to measure the WUE of C_3 plants (Farquhar, Ehleringer and Hubick, 1989).

In the N-poor soils, species with high NUE were found to dominate communities (Mamolos, Veresoglou and Barbayiannis, 1995). The N concentration of plant leaves was positively correlated with photosynthetic capacity suggesting that most of the N is used for synthesis of components of the photosynthetic apparatus. (Sugiharto et al., 1990). Rubisco (ribulose-1, 5-bisphosphate carboxylase-oxygenase), the primary carboxylase of C_3 photosynthesis, is the most abundant protein in plant leaves. Instantaneous photosynthetic nitrogen use efficiency, the rate of net carbon assimilation per mole of leaf nitrogen, is an indicator of resource capture per unit investment. (Field and Mooney, 1986). The C/N ratio in plant tissues has been widely used to estimate plant long-term NUE. (Patterson, Guy and Dang, 1997; Chen et al., 2005; Tsialtas and Veresoglou, 2007).

To date, limited studies on resource use efficiency of field grown crops have been conducted (Tambussi, Bort and Araus, 2007), and the relationship between WUE and NUE has received even less attention (Patterson, Guy and Dang, 1997; Tsialtas and Veresoglou, 2007). Thus, the objectives of this study were to: (i) evaluate the WUE and NUE for 30 spring wheat genotypes for both drought and well-watered field conditions; (ii) determine the relationships between WUE, NUE and grain yield, again for the drought and well-watered conditions.

2 Materials and methods

2.1 Plant material

Thirty spring wheat genotypes, including 22 cultivars and eight elite breeding lines, were used in this study. The 22 cultivars are well adapted in the Pacific Northwest of the U.S. The 30 genotypes comprised of 12 hard red, nine soft white, eight hard white, and one durum wheat (Table 1). In our previous paper (Li, Chen and Wu, 2011), 13 selected genotypes were classified into four groups: HYG (high-yield genotype), LYG (low-yield genotype), DRG (drought-resistant genotype) and DSG (drought-susceptible genotype), based on their yield performance. The HYG produced greater grain yield under both drought and well-watered conditions; DRG produced greater grain yield under drought and less grain yield under the well-watered conditions; DSG produced less grain yield under drought and greater grain yield under the well-watered conditions; and LYG produced less grain yield than other genotypes under both drought and well-watered conditions.

2.2 Experimental conditions

Experiments were carried out in two seasons of 2009 and 2010 at the research field of University of Idaho Aberdeen Research & Extension Center at Aberdeen, ID, USA (42.96° N, 112.82° W, and elevation 1,342 m). In this area, the annual precipitation is 203 to 279 mm, the mean annual air temperature is 7.2 to 8.3℃, and the frost-free period is 110 to 130 days. The soil at the experimental site was a Declo loam (coarse-loamy, mixed, superactive, mesic, Xeric Haplocalcids) with 0 to 2% slopes and pH of 8.1.

Wheat was planted on 22 April, 2009 and 14 April, 2010, respectively. The planting depth was 3.8 cm and the seeding rate was 300 seeds per m^2. In two seasons, wheat was planted in four-row plots (2009) and seven-row plots (2010), respectively, with the same plot size of 3.0 m long by 1.5 m wide. The row spaces were 0.5 and 0.25 m for 2009 and 2010, respectively. Over the growing season, 15.8 and 10.6 g m^{-2} of N and P were applied based on a soil test before planting. Herbicides including huskie (bromoxynil octanoate, bromoxynil heptanoate, pyrasulfotole) and starane (fluroxypyr 1-methylheptyl ester: ((4-amino-3,5-dichloro-6-fluoro-2-pyridinyl) oxy) acetic acid, 1-methylheptyl ester) were applied at the rates of 0.08 and 0.11 g/m^2, respectively, during jointing stage.

Table 1 Spring wheat cultivars and advanced lines developed by Montana State University (MSU), University of Idaho (U of I), University of California Davis (UCD), Washington State University (WSU), Resource Seeds (RS), and WestBred (WB)

No.	Genotype	Group†	Class‡	Origin	PI No.	Reference
1	Choteau	LYG	HRS	MSU	PI 633974	Lanning et al., 2004
2	Vida		HRS	MSU	PI 642366	Lanning et al., 2006
3	McNeal	DRG	HRS	MSU	PI 574642	Lanning et al., 1994
4	Alzada		Durum	WB	PI 634820	NA§
5	Agawam	DRG	HWS	WB	PI 648027	NA
6	Conan		HRS	WB	PI 607549	NA
7	Hank		HRS	WB	PI 613583	NA
8	WB936		HRS	WB	PI 587200	NA
9	Lassik		HRS	UCD	PI 653535	NA
10	UC1600	LYG	HRS	UCD	Breeding line	NA
11	Louise		SWS	WSU	PI 634865	Kidwell et al., 2006
12	Alpowa	DRG	SWS	WSU	PI 566596	Barrett and Kidwell, 1998
13	WA8039		SWS	WSU	Breeding line	NA
14	UI Winchester		HRS	U of I	PI 642362	NA
15	Jerome		HRS	U of I	PI 632712	Souza et al., 2005
16	IDO702	HYG	HRS	U of I	Breeding line	NA
17	Jefferson		HRS	U of I	PI 603040	Souza et al., 1999
18	Alturas	HYG	SWS	U of I	PI 620631	Souza, Guttieri and O'Brien, 2004
19	Cataldo	LYG	SWS	U of I	PI 642361	Chen et al., 2009
20	Lolo	DSG	HWS	U of I	PI 614840	Souza, Guttieri and McLean, 2003
21	UI Lochsa		HWS	U of I	PI639952	NA
22	IDO694		HWS	U of I	Breeding line	NA
23	IDO686	DSG	SWS	U of I	Breeding line	NA
24	IDO687		SWS	U of I	Breeding line	NA
25	IDO599	HYG	SWS	U of I	Breeding line	NA
26	IDO644		SWS	U of I	Breeding line	NA
27	Klasic	LYG	HWS	RS	PI 486139	Barrett and Kidwell, 1998
28	Snowcrest	LYG	HWS	RS	PI 642376	NA
29	Blanca Grande		HWS	RS	PI 631481	NA
30	Blanca Royale		HWS	RS	PI 655033	NA

Note: † HYG, high-yield genotype; LYG, low-yield genotype; DRG, drought-resistant genotype; DSG, drought-susceptible genotype.

‡ HRS, hard red spring wheat; HWS, hard white spring wheat; SWS, soft white spring wheat.

§ NA, not available.

In each one of the two seasons, the experiment was laid out in a split block design, with three replicates, keeping water treatments in main plots and genotypes in sub-plots. Genotypes were randomized within each main plot. Two contrasting soil water treatments: drought stress (non-irrigated) and well-watered (100%-ET irrigated) (ET, evapotranspiration) were applied by above-ground drip system and determined based on the crop water use information from the Pacific Northwest Cooperative Agricultural Weather Network recommendations (USBOR, 2009-2010). Irrigation was applied once a week and was started several days before heading and during heading for 2009 and 2010 seasons, respectively, and ended at maturity. The amount of water applied per irrigation was determined by the amount of water that plants used and soil surface evaporated (ET) in one week at corresponding growth stages. Irrigated plants received irrigation water and rainfall, while non-irrigated plants only received rainfall water during the growing season (Apr. to Aug.). During the 2009 growing season, all plots (drought and well-watered) received 359 mm of rainfall and irrigated plots (well-watered) received an additional 345 mm of irrigation water. During the 2010 growing season, all plots received 102 mm of rainfall and the irrigated plots received an additional 452 mm of irrigation water.

2.3 Carbon isotope composition ($\delta^{13}C$) and carbon to nitrogen ratio (C/N)

Carbon isotope composition ($\delta^{13}C$) was analyzed using flag leaf samples collected during the grain filling stage, corresponding to the Feekes growth scale (Miller, 1999) Feekes 11.1 (kernels milky ripe) in the 2009 and 2010 growing seasons. Flag leaves of ten randomly selected plants from each plot were excised, dried at 80℃

for 48 hours, and then ground to pass a 0.5 mm sieve. Ground samples were analyzed for %C, %N, and $^{13}C/^{12}C$ using an isotope rationing mass spectrometer at Augustana College, Sioux Falls, SD. Carbon isotope composition was expressed as $\delta^{13}C$ values (Farquhar, Ehleringer and Hubick, 1989), where $\delta^{13}C$ (‰) = $[(r\ \text{sample}/R\ \text{standard}) - 1] \times 1000$, and R was the $^{13}C/^{12}C$ ratio. Precision of the $\delta^{13}C$ measurements was ±0.1‰. Each sample was analyzed twice. Flag leaf $\delta^{13}C$ was used to assess WUE while flag leaf carbon to nitrogen ratio (C/N ratio) was calculated as an estimate of NUE. Due to the high cost of isotope analysis, $\delta^{13}C$ and C/N ratio was only measured at one growth stage (Feekes 11.1) during growing seasons.

The $\delta^{13}C$ and C/N evaluated at different water treatments (drought and well-watered) were expressed as $\delta^{13}C_d$ and $\delta^{13}C_w$, C/N_d and C/N_w, respectively. The corresponding $\delta^{13}C$ (WUE) and C/N (NUE) percent changes under the well-watered conditions were determined by equations: $(\delta^{13}C_d - \delta^{13}C_w)/\ \delta^{13}C_d$ and $(C/N_d - C/N_w)/\ C/N_d$, respectively.

2.4 Grain yield

In both seasons, plots were harvested using a Wintersteiger Classic small plot combine equipped with a Harvest Master weigh system (Wintersteiger Inc., Salt Lake City, UT). Grain yield was determined from the grain weight of each plot of each genotype. The yield value was expressed as 88% dry matter (DM).

2.5 Statistical analysis

Data were analyzed using SAS Version 9.1 (SAS Institute, Cary, NC) and SPSS 17.0 statistical software. Analyses of variance (ANOVA) for $\delta^{13}C$ and C/N ratio were performed using the Proc GLM procedure. The effect of year between 2009 and 2010 was also tested. Significant differences among genotypes and water treatments were determined using Fisher's protected LSD at prob. = 0.05. Pearsons' correlation was conducted among evaluated traits.

3 Results

The genotype × water treatment interaction effects for $\delta^{13}C$ and C/N ratio were non-significant (Table 2). Differences ($P < 0.001$) over the two contrasting water treatments (drought and well-watered) in $\delta^{13}C$ and C/N ratio were found. Analysis of the variance of the 30 genotypes revealed differences ($P < 0.001$) among genotypes in $\delta^{13}C$. Differences among wheat genotypes for C/N ratio were also significant ($P < 0.01$). There were year effect ($P < 0.01$) and genotype × year interaction effect ($P < 0.05$) for $\delta^{13}C$, but these effects were not significant for C/N ratio (Table 2).

Table 2 Analyses of variance for flag leaf $\delta^{13}C$ (‰) and C/N ratio in 30 spring wheat genotypes

Trait	Source of variation	df	Mean square	*F* value	*P*
$\delta^{13}C$, ‰	Genotype	29	0.28	3.15	<0.001
	Water treatment	1	41.45	461.23	<0.001
	Year	1	14.04	156.2	<0.01
	G × W†	29	0.14	1.54	0.14
	G × Y‡	29	0.27	2.95	<0.05
	W × Y§	1	0.52	5.73	0.07
C/N	Genotype	29	5.9	3.04	<0.01
	Water treatment	1	400.87	206.65	<0.001
	Year	1	10.62	5.47	0.06
	G × W	29	2.53	1.31	0.24
	G × Y	29	2.78	1.43	0.17
	W × Y	1	0.16	0.08	0.78

Note: † G×W, Genotype × Water treatment interaction.
‡ G×Y, Genotype × Year interaction.
§ W×Y, Water × Year interaction.

3.1 Water use efficiency ($\delta^{13}C$)

The flag leaf $\delta^{13}C$ values were different ($P<0.001$) among the 30 genotypes and ranged from -29.0‰ to -26.3‰ in 2009, and from -28.5‰ to -25.6‰ in 2010. Drought stress caused an increase in $\delta^{13}C$ (WUE) for all 30 genotypes. However, the magnitude of this increase varied among genotypes. The increase of $\delta^{13}C$ value was up to 2.1‰ for WA8039 and Jerome in 2010. Combined over two seasons, the WUE ($\delta^{13}C$) of genotypes UI Winchester, IDO686 (DSG), Jefferson, UI Lochsa, Vida, Jerome and Snowcrest (LYG) were greatly increased by drought stress, in other words, the WUE of these genotypes were significantly decreased by sufficient irrigation, indicating these genotypes were more sensitive to supplementary water in WUE. However, the WUE of genotypes UC1600 (LYG), Blanca Royale, Alpowa (DRG), IDO702 (HYG), Lolo (DSG), Blanca Grande, McNeal (DRG), Alzada and WB936 were not significantly affected by irrigation water (Table 3). This indicates these genotypes maintained relatively stable WUE under the well-watered conditions.

Table 3 The mean flag leaf δ^{13}C (‰), flag leaf C/N ratio, and grain yield (GY, g m^{-2}) of 2009 and 2010 for 30 spring wheat genotypes under the drought and well-watered conditions.

No.	Genotype	Drought			Well-watered		
		δ^{13}C	C/N	GY	δ^{13}C	C/N	GY
1	Choteau	-26.81ab†	13.1def	154.8bcd	-28.22bcdefg	10.4bc	539.6ab
2	Vida	-26.53a	13.66def	183abcd	-28.12abcdefg	10.07c	636.3ab
3	McNeal	-26.3a	13.46def	199.7abc	-27.2a	11.43abc	534.9ab
4	Alzada	-26.62ab	15.28bcdef	181.6abcd	-27.56abcd	11.05abc	560.4ab
5	Agawam	-26.83ab	14.26cdef	245.1ab	-28.13abcdefg	11.79abc	473.2b
6	Conan	-26.8ab	14.28cdef	217.6abc	-27.99abcdef	10.88abc	558.4ab
7	Hank	-27.15ab	14.76cdef	209.9abc	-28.49fg	10.27bc	618.8ab
8	WB936	-27.38b	14.65cdef	183.4abcd	-28.35defg	12.02ab	581.5ab
9	Lassik	-26.61ab	14.12cdef	207.7abc	-27.94abcdef	10.78abc	634.4ab
10	UC1600	-26.76ab	13.48def	149.3bcd	-27.32ab	10.96abc	503.4b
11	Louise	-26.52a	13.67def	193abcd	-27.58abcd	11.26abc	574.9ab
12	Alpowa	-27.3b	11.99f	237.4ab	-27.96abcdef	11.07abc	572.1ab
13	WA8039	-27.07ab	13.04def	257.5a	-28.26bcdefg	12.28ab	636.6ab
14	UI Winchester	-26.79ab	16.86bc	203abc	-28.7g	11.59abc	648.4ab
15	Jerome	-26.69ab	14.17cdef	166.3abcd	-28.26bcdefg	10.67abc	576.1ab
16	IDO702	-26.9ab	12.67ef	216.8abc	-27.62abcd	10.12c	657.9ab
17	Jefferson	-26.62ab	14.8cdef	191.7abcd	-28.43efg	12.17ab	545.5ab
18	Alturas	-27.07ab	15.15bcdef	191.6abcd	-27.88abcdef	11.77abc	659.7ab
19	Cataldo	-26.56a	16.27bcd	118.4d	-27.9abcdef	11.52abc	578.3ab
20	Lolo	-26.56a	15.57bcdef	145.7bcd	-27.29ab	11.11abc	608.4ab
21	UI Lochsa	-26.87ab	14.46cdef	204.5abc	-28.48fg	10.84abc	589.2ab
22	IDO694	-26.72ab	18.73ab	218.8abc	-27.99abcdef	12.42ab	564.3ab
23	IDO686	-26.47a	14.76cdef	132.8cd	-28.28cdefg	11.08abc	644.8ab
24	IDO687	-26.79ab	16.05bcde	191.2abcd	-27.77abcde	12.21ab	577ab
25	IDO599	-27.01ab	15.33bcdef	240.6ab	-28.2bcdefg	11.96ab	742.6a
26	IDO644	-26.83ab	14.42cdef	174.7abcd	-27.94abcdef	11.32abc	645.9ab
27	Klasic	-26.47a	21.13a	134.4cd	-27.6abcd	12.38ab	467.6b
28	Snowcrest	-26.5a	18.72ab	176.9abcd	-27.96abcdef	12.58a	472.2b
29	Blanca Grande	-26.98ab	15.27bcdef	184.4abcd	-27.78abcde	10.73abc	583.5ab
30	Blanca Royale	-26.93ab	16.26bcd	186.3abcd	-27.51abc	11.99ab	535.4ab
	Mean	-26.78	15.01	189.9	-27.96	11.36	584
	SD	0.26	1.92	33.95	0.38	0.72	62.40

Note: † Means followed by different letters are significantly (P < 0.05) different.

Among the 30 genotypes, Hank, WB936 and WA8039 had the lowest WUE while McNeal (DRG), Louise and Klasic (LYG) had the highest WUE under both drought and well-watered conditions (Table 3). In addition, genotypes with high WUE under the well-watered conditions tended to have high WUE under the drought stress, and genotypes with low WUE under drought tended to have low WUE under the well-watered conditions; however, this kind of phenomena was not observed in other cases. For example, IDO686 (DSG) and Jefferson had low WUE under the well-watered conditions but high WUE under drought. IDO686 and Vida had high WUE under the drought conditions but low ones under the well-watered conditions.

3.2 Nitrogen Use Efficiency (C/N ratio)

The flag leaf C/N ratio (NUE) was found to increase under the drought stress compared with the well-watered conditions (Table 3). On average, it increased by about 32% under drought. The C/N ratio was significantly different among genotypes within each water treatment. The C/N ratio of genotypes varied from 11.5 to 25.0 under drought and 9.9 to 14.2 under the well-watered conditions in the 2009 season; it varied from 12.0 to 17.8 under drought and 9.8 to 12.8 under the well-watered conditions in the 2010 season.

Drought stress increased NUE (C/N ratio) for all 30 wheat genotypes. However, the extent of NUE increase was different among genotypes. Having combined two years' data, the NUE of genotypes Klasic (LYG), IDO694, Snowcrest (LYG), UI Winchester, Cataldo (LYG), Blanca Grande, Lolo (DSG), Blanca Royale and Alzada were strongly increased by drought stress, in other words, the NUE of these genotypes were greatly decreased by sufficient irrigation. However, genotypes WA8039, Alpowa (DRG), McNeal (DRG), UC1600 (LYG), IDO702 (HYG), WB936 and Jefferson maintained relatively stable NUE under the well-watered condition (Table 3), indicating the NUE of these genotypes were more tolerant to the supplementary water of well-watered conditions.

In the 30 genotypes, Snowcrest (LYG), IDO694, Klasic (LYG) and IDO687 had the highest NUE while IDO702 (HYG), Choteau (LYG) and Vida had the minimum ones. The results indicated that genotypes with high NUE under drought tended to have high NUE under the well-watered conditions, while genotypes with low NUE under the well-watered conditions tended to have low NUE under the drought conditions; however this kind of trend did not occur in other cases. For example, WA8039 and McNeal (DRG) had low NUE under the drought conditions but high ones when well-watered. WA8039, Jefferson and WB936 had high NUE when well-watered but low ones when the drought conditions were present.

3.3 Relationships between WUE, NUE and grain yield

Significant relationships were found between WUE ($\delta^{13}C$) and NUE (C/N ratio). In 2009 and 2010, the correlation between WUE and NUE was positive and significant ($P < 0.001$) under drought (Tables 4 and 5). However, no significant correlation was found between them for the well-watered conditions. Both WUE and NUE were negatively correlated with grain yield ($P < 0.05$) under the drought and well-watered conditions. In these correlations with grain yield, the correlations between WUE and grain yield under drought were the most significant ($P < 0.01$) for both seasons (Tables 4 and 5).

Table 4 Pearsons' correlation coefficients between $\delta^{13}C$ (‰), C/N ratio, and grain yield (GY) in 30 spring wheat genotypes within each of the two water treatments: drought (d) and well-watered (w) in 2009 season

	$\Delta^{13}C_d$	C/N_d	$\delta^{13}C_w$	C/N_w
C/N_d	.63***			
$\delta^{13}C_w$	.44*	ns		
C/N_w	ns	.42*	ns	
GY_d	-.72***	-.61***	ns	ns
GY_w	-.45*	-.50**	-.57**	-.63***

Note: * Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† ns, non-significant at the 0.05 probability level.

Table 5 Pearsons' correlation coefficients between $\delta^{13}C$ (‰), C/N ratio, and grain yield (GY) in 30 spring wheat genotypes within each of the two water treatments: drought (d) and well-watered (w) in 2010 season

	$\Delta^{13}C_d$	C/N_d	$\delta^{13}C_w$	C/N_w
C/N_d	.65**			
$\delta^{13}C_w$	.42*	ns		
C/N_w	ns	.53**	ns	
GY_d	-.50**	-.39*	ns	ns
GY_w	ns	ns	-.34*	-.31*

Note: * Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.
† ns, non-significant at the 0.05 probability level.

3.4 Water use efficiency and NUE in different wheat genotypes

In the 30 genotypes, Klasic (LYG) and Snowcrest (LYG) showed the highest WUE and NUE for both drought and well-watered treatments, while Hank showed the lowest WUE and NUE. Besides, Louise and McNeal (DRG) had the highest WUE for both water treatments. IDO686 (DSG) and Jefferson had the highest WUE under drought but the lowest under the well-watered conditions. IDO694 and IDO687 had the highest NUE for both water conditions, but Vida, IDO702 (HYG) and Choteau (LYG) had the lowest NUE for both water conditions. WA8039 had the lowest NUE under the drought conditions versus the highest NUE under the well-watered conditions.

Comparison of the mean WUE ($\delta^{13}C$), NUE (C/N ratio) and grain yield of genotypes in each of the four groups (HYG, LYG, DRG and DSG) under the drought and well-watered conditions based on two years' data is shown in Figure 1. To show the difference among different kinds of genotypes, the mean WUE, NUE and grain yield of the 30 genotypes were used as references respectively.

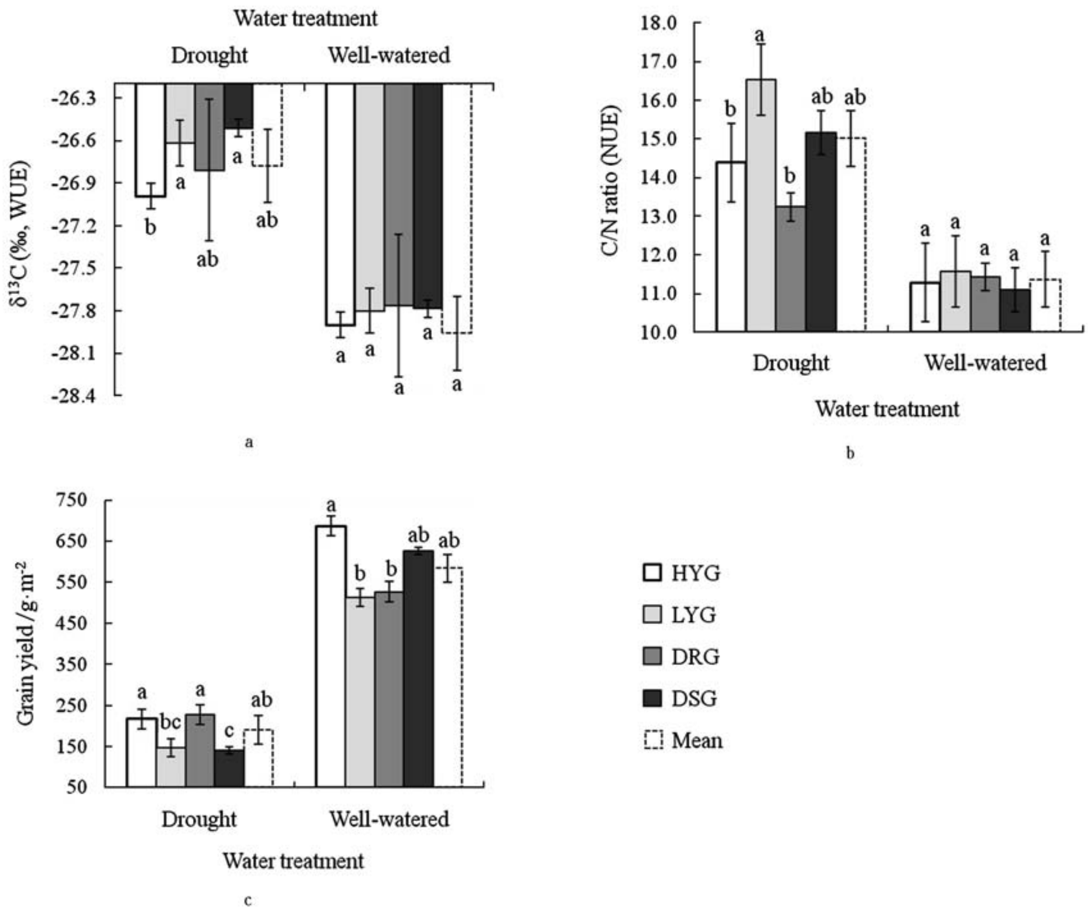

Figure 1 Comparison of the mean (a) flag leaf $\delta^{13}C$ (‰, WUE), (b) flag leaf C/N ratio (NUE), and (c) grain yield (g m^{-2}) of 30 spring wheat genotypes (Mean) and the mean values of genotypes in each of the four groups: high-yield genotype (HYG), low-yield genotype (LYG), drought-resistant genotype (DRG) and drought-susceptible genotype (DSG) under the drought and well-watered conditions based on data from 2009 and 2010. Means with different letters are significantly ($P < 0.05$) different.

For both water treatments, HYG had the lowest WUE while DSG had relatively higher WUE than the other groups. The LYG had the highest NUE while HYG had a relatively low NUE for both drought and well-watered conditions. This confirmed the negative relationships between grain yield and resource use efficiency (WUE and NUE). However, the WUE under well-watered was an exception to this, where the ranking of WUE for the four groups was: DRG (a) > DSG (a) > LYG (a) > HYG (a) > Mean (a). The WUE of all four groups were greater than the mean WUE of 30 genotypes in well-watered environments.

The WUE and NUE decrease under the well-watered conditions for DRG and HYG were much smaller than those for DSG and LYG, which could be used to distinguish DRG and HYG from the other two groups (Figure 2a). The HYG had the smallest WUE decrease followed by DRG, while DRG had the smallest NUE decrease followed by HYG. DSG had the greatest WUE decrease followed by LYG, while LYG had the greatest NUE decrease followed by DSG. The corresponding WUE and NUE percent decreases were compared among the four groups (Figure 2b). The ranking of percent decrease for the four groups was consistent with that of decrease values for both WUE and NUE. DRG was found to be significantly less sensitive to supplementary water in WUE (i.e., maintained relatively stable WUE under the well-watered conditions) than DSG.

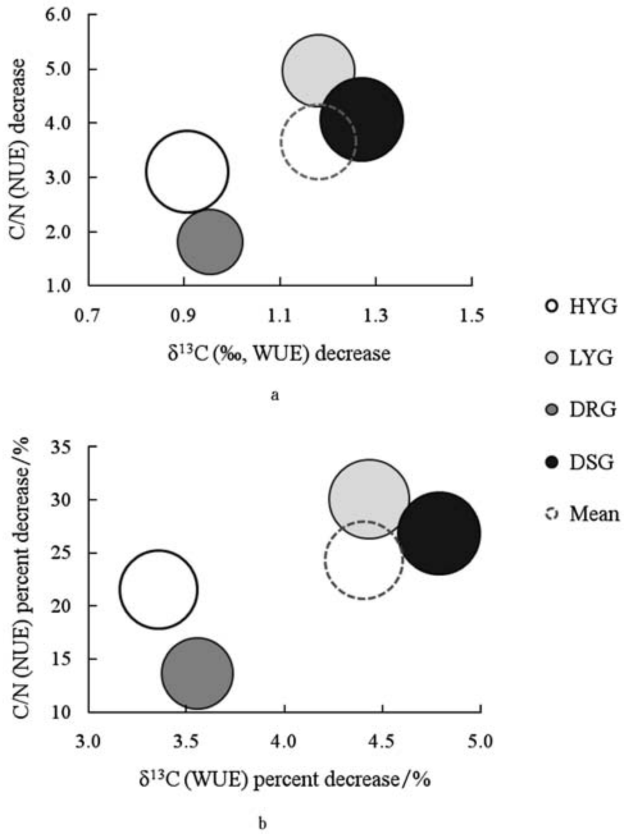

Figure 2 The decreases (a) and percent decreases (b) of flag leaf $\delta^{13}C$ (‰, WUE) and C/N ratio (NUE) under the well-watered conditions compared to drought, and the decrease (a) and percent decrease (b) of grain yield (g m^{-2}) under drought stress compared to the well-watered conditions, averaged from the 30 genotypes and each of the four groups: high-yield genotype (HYG), low-yield genotype (LYG), drought-resistant genotype (DRG) and drought-susceptible genotype (DSG). Grain yield decreases were indicated by the sizes of the bubble markers.

4 Discussion

This study reported that the indirect evaluation of the WUE (assessed by $\delta^{13}C$) and NUE (assessed by C/N ratio) provided powerful information rather conveniently on resource use efficiency under the field conditions. Both the $\delta^{13}C$ (WUE) and C/N ratio (NUE) were significantly affected by the water treatments and genotypes. Drought stress significantly increased the WUE ($\delta^{13}C$) and NUE (C/N ratio) ($P < 0.001$), and plants that suffered drought stress tended to have greater WUE and NUE. The increase caused by drought stress for the C/N ratio was higher than that for $\delta^{13}C$; on average, their values increased by about 32% and 4%, respectively. This indicated that the flag leaf C/N ratio was more sensitive to drought than the flag leaf $\delta^{13}C$.

Under the drought stress, plants are forced to utilize more efficiently soil limited resources (water and nitrogen), and thus they reach the maximum WUE and NUE. As the supplied water increases, WUE and NUE of plants decrease to the minimum levels. Our research confirmed that additional water decreased WUE (Toft et al., 1989). Based on these results, we put forward a testable hypothesis that an optimum water condition should exist between the drought stress and well-watered where both relatively high resource use efficiency and grain yield could be achieved concurrently. Therefore, more water treatments are needed in future studies.

In this study, the flag leaf $\delta^{13}C$ was only measured once at grain filling stage (Feekes 11.1); however, it may represent the $\delta^{13}C$ level of genotypes over the growing season. For irrigated plants, the $\delta^{13}C$ remained relatively constant throughout the growing season; while for dryland plants, the $\delta^{13}C$ declined in response to the progressive depletion of stored soil water (Knight, Livingston and Kessel, 1994).

Genotypes with high WUE under the well-watered conditions tended to have high WUE under the drought stress, and genotypes with low WUE under drought tended to have low WUE under the well-watered conditions. However, the reverse results were observed for NUE: genotypes with high NUE under the drought conditions tended to have high NUE under the well-watered conditions, and genotypes with low NUE under the well-watered conditions tended to have low NUE under drought. These results suggest a more efficient way for assessing WUE and NUE of genotypes: screening for high WUE and low NUE should be conducted in well-watered environments, while low WUE and high NUE should be selected under drought conditions. In this way, selected genotypes containing either high or low WUE and NUE would perform consistently across different water environments.

The WUE was positively correlated to NUE under the drought conditions, but no correlation was found between them under the well-watered conditions. This infers that the selection of high WUE may benefit high NUE selection only under the drought conditions, and vice versa.

Yield is the most important economic trait, and grain production is the main selection criteria for drought resistance of wheat. However, negative relationships between grain yield and resource use efficiency (WUE and NUE) were found under both drought and well-watered conditions in the current study. This suggests that improving WUE and NUE might lead to yield reductions. Similar results were reported by previous studies (Araus et al., 2002; Condon et al., 2002; Tambussi, Bort and Araus, 2007). Therefore, the yield should also be monitored to avoid possible declines in yield in breeding programs, when the goal is to improve WUE in wheat, and finding ways to ameliorate WUE and NUE without penalties are imperative. For example, screening WUE and NUE of genotypes in HYG and DRG groups might be an easier way to improve resource use efficiency with simultaneous advantage of yield production.

There were some exceptional genotypes: DRG McNeal had higher WUE under both drought and well-watered conditions; HYG IDO702 and Alturas had higher WUE under the well-watered conditions; HYG IDO599 and Alturas had higher NUE under the drought and well-watered conditions. This supported the hypothesis that it is possible to balance high WUE and high grain yield in a wheat breeding process. Due to the significant variations in WUE and NUE among genotypes, the amelioration of resource use efficiency without yield penalties was obtainable. The above-mentioned genotypes can be recommended for planting and appeared to be promising parents and provide great opportunity to obtain elite genotypes for wheat breeding programs.

The WUE and NUE decreases in DRG and HYG were much smaller than those in DSG and LYG, which could be used to distinguish DRG and HYG from the other genotypes. DRG was found to be significantly less sensitive to supplementary water in the WUE (i.e., maintained relatively stable WUE under well-watered conditions) than DSG. This suggests that genotypes with drought resistance tend to be resistant to supplementary water, and vice versa.

5 Conclusions

The results of this study indicate the potential to use the indirect WUE and NUE evaluation of wheat genotypes for assessing procedures of wheat genotype under field conditions, particularly for resource limited regions. This study proposed a more accurate and efficient way to assess the WUE and NUE of genotypes: high WUE and low NUE should be selected in the well-watered environments while low WUE and high NUE should be selected under the drought conditions. The WUE and NUE were positively correlated under drought. WUE and NUE were both negatively correlated with grain yield under the drought and well-watered conditions.

In this study, a few genotypes (McNeal, IDO702, Alturas and IDO599) were identified to possess superior characteristics in both resource use efficiency and yield production. Therefore, improvement of resource use efficiency with simultaneous advantage of yield production might be obtained by screening WUE and NUE of genotypes in HYG and DRG groups. This would be an easier way to achieve both higher resource use efficiency and grain yield.

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[References]

[1] Araus, J. L., G. A. Slafer, M. P. Reynolds, and C. Royo. 2002. Plant breeding and drought in C_3 cereals: what should we breed for? Ann. Bot.-London, 89: 925–940.

[2] Barrett, B. A., and K. K. Kidwell. 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. Crop Sci., 38(5): 1261–1271.

[3] Chen, J., E. J. Souza, R. S. Zemetra, N. A. Bosque-Perez, M. J. Guttieri, D. Schotzko, K. L. O'Brien, J. M. Windes, S. O. Guy, B. D. Brown, and X. M. Chen. 2009. Registration of Cataldo wheat. J. Plant Reg., 3(3): 264–268.

[4] Chen, S., Y. Bai, L. Zhang, and X. Han. 2005. Comparing physiological responses of two dominant grass species to nitrogen addition in Xilin River Basin of China. Environ. Exp. Bot., 53(1): 65–75.

[5] Condon, A. G., R. A. Richards, G. J. Rebetzke, and G. D. Farquhar. 2002. Improving intrinsic water-use efficiency and crop yield. Crop Sci., 42(1): 122–131.

[6] Ebdon, J. S., A. M. Petrovic, and T. E. Dawson. 1998. Relationship between carbon isotope discrimination, water use efficiency, and evapotranspiration in Kentucky Bluegrass. Crop Sci., 38(1): 157–162.

[7] Ehdaie, B., A. E. Hall, G. D. Farquhar, H. T. Nguyen, and J. G. Waines. 1991. Water-use efficiency and carbon isotope discrimination in wheat. Crop Sci., 31(5): 1282–1288.

[8] Farquhar, G. D., M. H. O'Leary, and J. A. Berry. 1982. On the relationship between carbon isotope discrimination and inter cellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol., 9: 121–137.

[9] Farquhar, G. D., and R. A. Richards. 1984. Isotopic composition of plant carbon correlates with water-use efficiency of wheat genotypes. Aust. J. Plant Physiol., 11: 539–552.

[10] Farquhar, G. D., J. R. Ehleringer, and K. T. Hubick. 1989. Carbon isotope discrimination and photosynthesis. Ann. Rev. Plant Phys. Plant Mol. Biol., 40(1): 503–537.

[11] Field, C., and H. A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. In On the Economy of Plant Form and Function, ed. T.J. Givnish, 25–55. Cambridge: Cambridge University Press.

[12] Khazaei, H., S. D. Mohammady, M. Zaharieva, and P. Monneveux. 2009. Carbon isotope discrimination and water use efficiency in Iranian diploid, tetraploid and hexaploid wheats grown under well-watered conditions. Genet. Resour. Crop Evol., 56(1): 105–114.

[13] Kidwell, K. K., G. B. Shelton, V. L. Demacon, J. W. Burns, B. P. Carter, X. M. Chen, C. F. Morris, and N. A. Bosque Perez. 2006. Registration of Louise wheat. Crop Sci., 46: 1384–1385.

[14] Knight, J. D., N. J. Livingston, and C. Van Kessel. 1994. Carbon isotope discrimination and water-use efficiency of six crops grown under wet and dry land conditions. Plant Cell Environ., 17(2): 173–179.

[15] Lanning, S. P., L. E. Talbert, C. F. McGuire, H. R. Bowman, G. R. Carlson, G. D. Jackson, J. L. Eckhoff, G. D. Kushnak, R. N. Stougaard, and G. F. Stallknecht. 1994. Registration of McNeal wheat. Crop Sci.. 34(4): 1126–1127.

[16] Lanning, S. P., G. R. Carlson, D. Nash, D. M. Wichman, K. D. Kephart, R. N. Stougaard, G. D. Kushnak, J. L. Eckhoff, W. E. Grey, and L. E. Talbert. 2004. Registration of Choteau wheat. Crop Sci., 44: 2264–2265.

[17] Lanning, S. P., G. R. Carlson, D. Nash, D. M. Wichman, K. D. Kephart, R. N. Stougaard, G. D. Kushnak, J. L. Eckhoff, W. E. Grey, A. Dyer, and L. E. Talbert. 2006. Registration of Vida wheat. Crop Sci., 46(5): 2315–2316.

[18] Li, P., J. Chen, and P. Wu. 2011. Agronomic characteristics and grain yield of 30 spring wheat genotypes under drought stress and non-stress conditions. Agron. J., 103: 1619–1628.

[19] Livingston, N. J., R. D. Guy, and G. J. Ethier. 1999. The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca* (Moench) Voss) seedlings. Plant Cell Environ., 22(3): 281–289.

[20] Mamolos, A. P., D. S. Veresoglou, and N. Barbayiannis. 1995. Plant species abundance and tissue nitrogen concentrations of limiting nutrients in low-nutrient grassland: A test of competition theory. J. Ecol., 83(3): 485–495.

[21] Miller, T.D. 1999. Growth stages of wheat: identification and understanding improve crop management. Texas agricultural extension service, the Texas A&M university system, SCS-1999-16.

[22] Patterson, T. B., R. D. Guy, and Q. L. Dang. 1997. Whole-plant nitrogen- and water-relations traits, and their associated trade-offs, in adjacent muskeg and upland boreal Spruce species. Oecologia, 110: 160–168.

[23] Souza, E., J. Windes, D. Sunderman, and K. O'Brien. 1999. Registration of Jefferson wheat. Crop Sci., 39(2): 296–297.

[24] Souza, E., M. Guttieri, and R. McLean. 2003. Registration of Lolo wheat. Crop Sci., 43(2): 734–735.

[25] Souza, E. J., M. J. Guttieri, and K. O'Brien. 2004. Registration of Alturas wheat. Crop Sci., 44(4): 1477–1478.

[26] Souza, E. J., N. A. Bosque-Perez, M. J. Guttieri, D. J. Schotzko, S. O. Guy, B. Brown, and R. Zemetra. 2005. Registration of Jerome wheat. Crop Sci., 45(3): 1161–1162.

[27] Sugiharto, B., K. Miyata, H. Nakamoto, H. Sasakawa, and T. Sugiyama. 1990. Regulation of expression of carbon-assimilating enzymes by nitrogen in maize leaf. Plant Physiol., 92(4): 963–969.

[28] Tambussi, E. A., J. Bort, and J. L. Araus. 2007. Water use efficiency in C_3 cereals under Mediterranean conditions: a review of physiological aspects. Ann. Appl. Biol., 150(3): 307–321.

[29] Toft, N. L., J. E. Anderson, and R. S. Nowak. 1989. Water use efficiency and carbon isotope composition of plants in a cold desert environment. Oecologia, 80(1):11–18.

[30] Tsialtas, I. T., and D. S. Veresoglou. 2007. Nitrogen use efficiency in a semi-arid community and its relationship with water use efficiency and soil water and nitrogen. World J. Agric. Sci., 3(4): 485–488.

[31] U.S. Bureau of Reclamation. 2009-2010. AgriMet: The Pacific Northwest Cooperative Agricultural Weather Network, Idaho Crop Water Use Charts. http://www.usbr.gov/pn/agrimet/id_charts.html (accessed weekly from April 2009 to September in 2009 and 2010).

[32] Zhang, Z., and L. Shan. 1998. Comparison study on water use efficiency of wheat flag leaf. Chinese Sci. Bull., 43(14): 1205–1211.