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Nitrogen remobilization and post-anthesis nitrogen uptake in relation to elevated grain protein concentration in durum wheat

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Suprayogi, Y., Clarke, J. M., Bueckert, R., Clarke, F. R. and Pozniak, C. J. 2011. **Nitrogen remobilization and post-anthesis nitrogen uptake in relation to elevated grain protein concentration in durum wheat**. Can. J. Plant Sci. **91**: 273–282. Grain protein concentration is an important end-use suitability factor in durum wheat [*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.] through its effect on cooking quality. Genetic differences in grain protein concentration are exploited in Canadian durum breeding programs, but the physiological basis of these differences remains unknown. Eighteen durum genotypes varying in grain protein concentration were grown at three pre-selected Saskatchewan locations that differ for soil nitrogen (N). These included check cultivars and six low- and six high-protein doubled haploid (DH) selections from the cross DT695×Strongfield (low- by high-protein). Plants were sampled at the anthesis, milk, dough and physiological maturity developmental stages, and dry matter and N concentration of plant parts were determined. The high-protein selections expressed 0.6 to 1.1 percentage units higher grain protein concentration than the low selections over the three environments ($P<0.05$), but yielded less grain than the low selections in two of the three environments. Remobilization of N from vegetative organs to grain varied with environment and accounted for 73 to 98% of grain N, the remainder made up from post-anthesis N uptake. The high-protein selections showed greater post-anthesis N uptake than the low selections in two of three environments ($P<0.01$), but lower N remobilization from vegetative organs to the grain than the low selections in the same environments ($P<0.05$). Subtle differences in N and dry matter partitioning accounted for the observed differences in grain protein concentration.

Key words: Durum wheat, (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.), nitrogen remobilization, grain protein concentration

Suprayogi, Y., Clarke, J. M., Bueckert, R., Clarke, F. R. et Pozniak, C. J. 2011. **La remobilisation de l'azote et l'absorption d'azote après l'anthèse, et leurs liens avec la concentration élevée de protéines dans le grain du blé dur**. Can. J. Plant Sci. **91**: 273–282. La concentration de protéines dans le grain joue un rôle important dans l'utilité du blé dur (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.), facteur affectant la qualité à la cuisson. Les programmes d'hybridation canadiens exploitent la variation génétique de la concentration de protéines dans le grain, mais on ignore l'origine physiologique des écarts observés. Les auteurs ont cultivé dix-huit génotypes de blé dur à concentration de protéines variable dans le grain. Les essais se sont déroulés à trois endroits de la Saskatchewan, présélectionnés en raison d'une teneur en azote (N) variable dans le sol. Les génotypes incluaient des cultivars témoins et six doubles haploïdes à faible ou à forte teneur en protéines issus du croisement DT695×Strongfield (variété à faible teneur en protéines avec variété à forte teneur en protéines). Les plants ont été échantillonnés à l'anthèse, au stade du grain laiteux, au stade pâteux et à la maturité physiologique, et les auteurs ont déterminé la quantité de matière sèche et la concentration de N dans les organes de la plante. Les sélections à forte teneur en protéines expriment 0,6 à 1,1 point de pourcentage plus de concentration de protéines dans le grain que celles à faible teneur dans les trois environnements ($P<0,05$), mais les premières ont donné moins de grain que les secondes à deux endroits. La remobilisation du N des organes végétatifs vers le grain varie avec l'environnement et explique 73 à 98% du N du grain, le reste venant du N absorbé après l'anthèse. Les sélections à forte teneur en protéines ont absorbé plus de N après l'anthèse que les sélections à faible teneur à deux endroits sur trois ($P<0,01$), mais elles avaient moins mobilisé de N des organes végétatifs que les variétés à faible teneur pour le même environnement ($P<0,05$). De subtiles variations dans la répartition du N et de la matière sèche expliquent les différences notées dans la concentration de protéines du grain.

Mots clés: Blé dur (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.), remobilisation de l'azote, concentration de protéines dans le grain

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Abbreviations: **DH**, doubled haploid; **ZGS**, Zadocks growth scale

Grain protein concentration is an important quality trait in durum wheat for pasta manufacture, through its effect on the firmness of cooked pasta and tolerance to overcooking (Dexter and Matsuo 1977; Grzybowski and Donnelly 1979) under both low- and high-temperature drying regimes (D'Egidio et al. 1990; Dexter and Marchylo 2001). High grain protein concentration is thus one of the primary specifications in durum grain purchased by pasta manufacturers.

Both genetic factors (e.g., Clarke et al. 2009) and environmental conditions such as available N in the soil, N from fertilizer application, water availability, temperature and light intensity influence grain protein concentration in durum wheat (Blanco et al. 2002; Ames et al. 2003) and also in hexaploid wheat (*Triticum aestivum* L.) (Johnson et al. 1972; Kramer 1979; Fowler et al. 1990). Many wheat breeding programs have emphasized selection for grain yield more than selection for grain protein concentration, so that reports on durum (Motzo et al. 2004) and hexaploid wheat (Brancourt-Hulmel et al. 2003) show lower grain protein concentration in modern than in older cultivars. Indeed, there is a widely observed negative correlation of grain yield and protein concentration (e.g., Halloran 1981; Löffler and Busch 1982; Guthrie et al. 1984; O'Brien and Ronalds 1984) attributed to dilution of plant N by higher grain yields. In Canada, however, there has been emphasis on selection for both grain yield and protein concentration due to the end-use quality requirements for the registration and release of commercial cultivars (Clarke et al. 1998a). The registration of high-protein, high-yield cultivars such as AC Avonlea (Clarke et al. 1998b) and Strongfield (Clarke et al. 2005) prompted research to understand the inheritance and improve selection techniques (Clarke et al. 2009) and to develop suitable DNA markers for selection (Suprayogi et al. 2009). The physiological basis of the improvement in protein remains largely unexplored.

At the physiological level, grain protein formation in wheat involves uptake by roots and the accumulation of N in vegetative organs, and subsequent remobilization to the kernel for protein synthesis. These processes are under genetic control in hexaploid wheat (Cox et al. 1985a, b, 1986), but expression is also influenced by growing environment and genotype-environment interactions (Triboï et al. 2000). During the grain-filling period, the amount of N uptake is much smaller than the demand for N accumulation in grain; therefore, a large part of the demand is met by N remobilized from vegetative organs. The proportion of remobilized N in the harvested grain is environment-dependent and can account for 60 to 92% of the total grain N in hexaploid wheat (Austin et al. 1977; Cox et al. 1985a, b, 1986; Papakosta and Garianas 1991). Uptake of N from the soil after flowering also tended to elevate grain protein concentration (Blackman and Payne 1987), especially under conditions of adequate soil water availability (Clarke et al. 1990). In winter wheat, both N remobilization and post-anthesis N uptake contribute to elevated grain protein concentration (Kichey et al. 2007).

Increased grain protein concentration can be achieved by improving uptake or remobilization (Bhatia and Robson 1976). Wang et al. (2003) reported that Canadian hexaploid wheat cultivars with elevated protein concentration were more efficient at N translocation from vegetative tissue to the grains. Elevated protein concentration in the durum wheat introgression line Langdon (DIC-6B) was also due to better N remobilization to the grains (Deckard et al. 1996; Kade et al. 2005). However, there are few studies on the physiology of N accumulation in current durum wheat cultivars showing variable expression of protein concentration. Clarke et al. (1990) found no difference in N uptake or remobilization between a high-protein and a low-protein durum cultivar. Wang et al. (2009) found that the high-protein cultivar AC Avonlea had higher nitrogen harvest index (grain N as a proportion of total plant N) than several other cultivars with lower protein concentration. Grain protein concentration is reported to be generally lower in semi-dwarf than in non-dwarf durum cultivars (McClung et al. 1986; Pinthus and Gale 1990; Clarke et al. 2009), and understanding the physiological basis for this deficiency could allow durum wheat breeders to develop a strategy to elevate grain protein concentration in semi-dwarf types.

The objective of this research was to investigate N remobilization and post-anthesis N uptake in relation to elevated grain protein concentration in a collection of durum wheat genotypes with variable protein concentration.

MATERIALS AND METHODS

Eighteen genotypes varying in grain protein concentration were studied. These comprised Strongfield (high grain protein concentration; Clarke et al. 2005) and DT695 (low grain protein concentration, a breeding line from Agriculture and Agri-Food Canada, Swift Current, Saskatchewan, Canada), the isogenic pair Langdon (DIC-6B) and Langdon, semi-dwarf cultivars Commander and Westbred 881, and a high- and a low-protein group of DH genotypes derived from the cross DT695 × Strongfield. Langdon (DIC-6B) is Langdon with a chromosome 6B substitution from *T. durum* var. *dicoccoides* bearing the *Gpc-B1* gene that confers improved N remobilization in durum wheat (Kade et al. 2005). The six high- and six low-protein DH genotypes were selected based on 2002 and 2003 field trials (Suprayogi et al. 2009); these genotypes did not show significant differences ($P > 0.05$) for grain yield, days to maturity or kernel weight in those studies.

Experiments were carried out at the University of Saskatchewan, Saskatoon, SK, at the Kernen research farm in 2005, and at the Goodale and Nasser research farms in 2006. The farms are located in the black soil climatic zone of Saskatchewan with clay (Kernen), loam

(Goodale), and clay loam (Nasser) textures. A randomized complete block design with three replications was used for each environment. Prior to planting, soil N levels at all experimental locations were determined at 0- to 15-cm, 15- to 30-cm and 30- to 60-cm depths. Soil samples were homogenized and analyzed for NO_3-N content at the Enviro-Test Laboratories Agricultural Services, Saskatoon, SK. Plot size for each experimental unit was 1.2×3.6 m and consisted of five rows with 20-cm row spacing. The seeding rate was 250 seeds m^{-2}. Seeding dates were 2005 May 14 (Kernen), 2006 May 18 (Goodale) and 2006 May 23 (Nasser). Precipitation during the growing seasons was recorded at each site using permanently established weather stations.

Plants in the first row of each plot were used for tissue sampling and plants in the remaining four rows were used for evaluation of days to heading [59 of the Zadocks growth scale (ZGS); Zadocks et al. 1974], days to maturity (90 ZGS), plant height (cm), grain yield (kg ha^{-1}), 1000-kernel weight and grain protein concentration. Five whole plants were randomly sampled for tissue N-analysis at anthesis (65 ZGS), milk stage (75 ZGS), dough stage (85 ZGS) and physiological maturity (90 ZGS). The plants were pulled and partitioned into bulked samples of lower leaves (all leaf blades except the flag leaf), stem (culm and leaf sheath), flag leaf, and spike (chaff and grain), and dried at 60°C for 72 h. The dried samples were weighed and then ground using a Thomas Willey laboratory grinder (model 4, Arthur H. Thomas Co., Philadelphia, PA) fitted with a 1-mm^2 screen, and stored at room temperature. Nitrogen concentration was determined on 0.2-g subsamples using the combustion method with a FP-528 LECO N Analyzer (LECO Corporation, St Joseph, MI). Moisture concentration (%) was determined by American Association of Cereal Chemists approved method No. 44-15A (AACC 2000b) and sample nitrogen concentration was corrected to a dry weight basis. Nitrogen content (mg) was calculated as dry weight of the respective plant part multiplied by N concentration. Grain protein concentration was calculated as N concentration multiplied by 5.7 (Williams et al. 1998; AACC 2000a) and expressed on a 13.5% moisture basis as is standard in Canada.

Total plant N (mg $plant^{-1}$) was determined by summing the N content (mg) of all individual plant parts. Vegetative N was calculated as the sum of N content (mg) of all individual vegetative organs. The remobilized N (mg $plant^{-1}$) was calculated as total plant N at anthesis not recovered from vegetative tissue at physiological maturity. Post-anthesis N uptake (mg $plant^{-1}$) was calculated as the difference between total plant N at physiological maturity and total plant N at anthesis. Nitrogen harvest index was calculated as the ratio of grain N to total plant N at physiological maturity. All data related to N content are presented on a per-plant basis. Harvest index was calculated as the ratio of grain weight at physiological maturity divided by the sum of the weights of non-grain components, and converted to a percentage.

Diagnostics were performed with Rstudent (Rawlings 1988, p. 250). For each trait, each location was analyzed separately with SAS PROC MIXED (SAS Institute, Inc. 2003) with genotypes considered a fixed effect and replications random. Least square (LS) means, the standard error of differences (SED) among means for genotypes using the "diff" command, and a contrast between the high- and low-protein groups were estimated.

RESULTS AND DISCUSSION

Pre-seeding soil analysis indicated variable soil N levels for the three trial environments (Table 1). Soil N levels were highest at Kernen with 34.6 kg ha^{-1} at the 0- to 15-cm depth. Goodale had lower soil N than Nasser at the 0- to 15-cm depth, but more N at the 15- to 20-cm and 30- to 60-cm depths. Overall, total N at 0- to 60-cm was lowest at the Nasser site and highest at the Kernen site.

May precipitation was above average at Goodale and Nasser, but was lower than the 30-yr average at Kernen (Table 2). Precipitation was above average during vegetative growth in June in all environments. In contrast, precipitation was lower than average during the flowering period in July at Goodale and Nasser, with average precipitation at Kernen. Precipitation during the grain fill period in August was average at Goodale and Nasser, but above average at Kernen (Table 2).

Some genotypes differed significantly in plant height (Table 3). The low-protein selections were consistently taller than the high selections ($P < 0.01$), mainly due to the transgressive segregate A0009&DAQ-02*, which was significantly taller than its tall parent DT695. The isogenic pair Langdon (DIC-6B) and Langdon had similar height. Days to heading were consistently 1 d later for the low- than for the high-protein selections ($P < 0.01$), but maturity did not differ between the groups (data not shown). Days to heading and maturity were also similar for Langdon and Langdon (DIC-6B) except at Goodale, where Langdon matured 4 d later than Langdon (DIC-6B) ($P < 0.05$). The Gpc-B1 high-protein allele carried by Langdon (DIC-6B) is known to cause early senescence in durum wheat (Uauy et al.

Table 1. Soil NO_3-N level based on pre-seeding soil test report (Enviro-Test Laboratories Agricultural Services, Saskatoon, Canada) at the experimental locations Kernen 2005, Goodale 2006 and Nasser 2006

	NO_3-N level (kg ha^{-1})		
Depth (cm)	Kernen 2005	Goodale 2006	Nasser 2006
0–15	34.6	13.6	17.3
15–30	13.6	19.6	13.6
30–60	45.7	32.1	19.8
Total	93.9	65.3	50.7

Table 2. Precipitation at the experimental locations Kernen 2005, Goodale 2006 and Nasser 2006

	Precipitation (mm)				
	May	June	July	August	Total
Kernen 2005	31	193	53	54	331
Goodale 2006	59	113	37	37	246
Nasser 2006	57	102	39	35	233
Saskatoon 30-yr (1975–2005) monthly average[z]	42	61	57	35	195

[z]Adapted from: http://www.worldweather.org/056/c00628.htm.

2006), so incorporation of the allele into breeding programs could reduce grain yields except in terminal drought environments.

Harvest index significantly differed among genotypes within locations (Table 3) The low-protein selections had higher harvest index ($P<0.01$) than the high-protein selections at Kernen and Nasser, but did not differ at Goodale. Strongfield and DT695 did not differ for harvest index, nor did Langdon and Langdon (DIC6B). The harvest index of the semi-dwarf Commander was not statistically different ($P>0.05$) from that observed for the taller checks, Strongfield and DT695.

The high- and low-protein groups did not significantly differ in 1000-kernel weight, nor did Langdon and Langdon (DIC6B), except at Kernen, where Langdon had significantly smaller seeds than Langdon (DIC-6B) (Table 4). Grain yield was higher for the low- than the high-protein selections at Kernen and Nasser, whereas the parent Strongfield yielded more than DT695 at Kernen. Yield did not differ between Langdon and Langdon (DIC-6B). Westbred 881 was consistently the lowest yielding cultivar at all sites.

Significant variation in grain protein concentration was detected among genotypes and growing conditions (Table 4). Langdon (DIC-6B), which carries *Gpc-B1*, had higher grain protein concentration than Langdon except at Goodale (Table 4). Strongfield had significantly higher grain protein concentration than DT695 only at Nasser, whereas Suprayogi et al. (2009) found Strongfield to be significantly greater than DT695 in five of six environments, including in a trial at the Kernen farm in 2005. Grain protein concentration was higher ($P<0.05$) in the high- than the low-protein selections in all environments. The difference between the groups was smallest at Goodale, where some high-protein selections, such as A0009&DAC-04*, had grain protein concentration similar to most of the low-protein selections. Westbred 881 expressed grain protein concentration similar to Strongfield and Langdon (DIC-6B) in all environments, probably due to its low grain yield.

Table 3. Least square means of plant height, and harvest index of durum wheat genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr)

	Plant height (cm)			Harvest index (%)		
Genotypes	Krn	Gdl	Nsr	Krn	Gdl	Nsr
Check cultivars						
Strongfield	108	103	79	41	35	48
DT695	121	113	81	40	31	46
Commander	94	92	70	42	37	45
Westbred 881	82	82	71	41	40	45
Langdon (DIC-6B)	135	143	98	35	39	43
Langdon	134	138	98	37	37	45
Low-protein						
A0009&DAH46*	106	102	76	44	40	52
A0009&DAN08*	118	113	79	45	40	48
A0009&DAE01*	123	113	83	38	34	46
A0009&DAQ02*	133	126	95	40	35	45
A0009&DAD04*	115	110	76	40	39	49
A0009&DAH07*	113	110	78	42	39	50
Mean	118	112	81	42	38	49
High-protein						
A0009&DAD09*	124	116	85	38	35	46
A0009&DAB06*	101	92	71	42	38	50
A0009&DAG02*	123	115	82	41	38	45
A0009&DAD10*	110	103	73	41	37	44
A0009&DAC04*	114	106	77	36	41	46
A0009&DAL08*	111	102	73	36	38	46
Mean	114	105	77	39	38	46
L vs. H contrast[z]	**	**	**	**	NS	**
LSD ($P<0.05$)	6	8	7	4	4	3

[z]Contrast of means of high- and low-protein selections where NS = not significant ($P>0.05$), *significant at $P<0.05$ and **significant at $P<0.01$.

Table 4. Least square means of 1000-kernel weight, grain yield and grain protein concentration of the durum wheat genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr)

Genotypes	1000-kernel weight (g)			Grain yield (kg ha^{-1})			Grain protein concentration (%)		
	Krn	Gdl	Nsr	Krn	Gdl	Nsr	Krn	Gdl	Nsr
Check cultivars									
Strongfield	44.4	45.3	38.2	6117	4130	2484	13.8	13.7	10.9
DT695	47.3	49.0	41.1	5438	4389	2564	13.4	12.7	9.2
Commander	47.0	49.7	41.6	5785	4082	2460	13.0	11.5	10.2
Westbred 881	44.6	45.7	42.9	4505	3529	2048	14.0	12.9	12.2
Langdon (DIC-6B)	48.9	44.5	35.7	4964	3280	1614	15.8	11.6	12.0
Langdon	41.6	43.5	38.5	4713	2838	1895	12.3	10.7	9.9
Low-protein									
A0009&DAH46*	44.9	44.9	37.6	6039	3992	2621	12.4	11.1	9.0
A0009&DAN08*	44.3	45.8	42.1	6254	3717	2503	12.4	11.0	9.8
A0009&DAE01*	40.9	45.1	39.3	5606	3745	2646	14.0	10.6	9.6
A0009&DAQ02*	46.5	45.6	40.9	5172	3583	2563	13.1	12.5	9.1
A0009&DAD04*	38.9	42.1	35.3	5912	4456	2578	12.8	11.0	9.1
A0009&DAH07*	51.2	47.0	40.5	5938	3993	2684	12.6	12.1	9.6
Mean	44.5	45.1	39.3	5820	3914	2599	12.9	11.4	9.4
High-protein									
A0009&DAD09*	41.7	42.8	40.4	5335	4272	2295	14.7	12.4	10.2
A0009&DAB06*	44.8	45.3	38.1	5592	4501	2171	15.0	12.5	10.1
A0009&DAG02*	46.9	48.0	39.1	5646	4165	2430	14.6	11.8	10.2
A0009&DAD10*	47.7	44.7	41.4	6075	3754	2297	13.9	12.7	10.9
A0009&DAC04*	42.4	44.1	40.6	4885	4015	2215	14.9	11.0	11.2
A0009&DAL08*	41.3	44.6	38.9	5345	4136	2318	15.0	11.7	10.9
Mean	44.7	45.3	39.6	5517	3921	2355	13.8	11.9	10.2
L vs. H contrast[z]	NS	NS	NS	**	NS	**	**	*	**
LSD ($P<0.05$)	3.4	3.7	4.6	402	605	281	0.9	1.2	0.8

[z]Contrast of means of high- and low-protein selections where NS = not significant ($P>0.05$), *significant at $P<0.05$ and **significant at $P<0.01$.

These results confirm that the high- and low-protein selections were agronomically similar, and the average protein advantage of the high- over the low-selections was 0.5 to 0.9 percentage units for the three environments. There were small differences in dry matter partitioning between the two groups as indicated by the differences in harvest index, with the highest proportion of dry matter in grain in the low-protein group.

Total plant N at anthesis describes the capacity of the plant to accumulate and store N in vegetative organs prior to remobilization to the developing grains, and vegetative (non-grain) N at physiological maturity would indicate the efficiency of remobilization of N to the grain. Total plant N at anthesis was significantly lower for the high- than for low-protein selections at Goodale, but was not different for the other environments (Table 5). The difference in total plant N at Goodale was due to higher N concentration in stems and heads in the low- than in the high-protein genotypes because there were few differences in dry weights of plant parts (not shown). Langdon (DIC-6B) had significantly higher total plant N at anthesis than Langdon at Goodale, but did not differ in the other environments. No significant differences in total plant N at anthesis were detected among genotypes at Nasser, and the differences were relatively minor in the other environments. The high- and low-protein selections did not differ for vegetative N at physiological maturity in any environment. Langdon and Langdon (DIC-6B) did not differ except that Langdon (DIC-6B) had significantly lower vegetative N at physiological maturity at Nasser.

The two selected groups differed in post-anthesis N uptake at Kernen and Goodale, but not at Nasser (Table 6). There was no significant variation among the check cultivars, including between Langdon and Langdon (DIC-6B). The high-protein selections had significantly greater ($P<0.01$) post-anthesis N uptake than the low-protein selections at Kernen and Goodale. A0009&DAE-01* had the highest post-anthesis N uptake of the low group and was statistically similar to that of the high group at Kernen and Goodale. Cox et al. (1985b) found that the heritability of post-anthesis N uptake was low, indicating that improvement of the trait by selection would be difficult. Post-anthesis N uptake was very low at Nasser, with some negative estimates that could be due either to post-anthesis N loss, as observed by Cox et al. (1985b), or sampling error. Post-anthesis N uptake of wheat is known to vary with environmental conditions such available N (Cox et al. 1985b) and available water (Clarke et al. 1990). In the present study, post-anthesis N uptake seems to have been affected by available N, as evidenced by lower uptake in the low N environments Nasser and Goodale than in the high N site Kernen,

Table 5. Least square means of total plant nitrogen (TPN) at anthesis and vegetative nitrogen (VegN) at physiological maturity of durum wheat genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr)

	TPN (mg plant^{-1})			VegN (mg plant^{-1})		
Genotypes	Krn	Gdl	Nsr	Krn	Gdl	Nsr
Check cultivars						
Strongfield	38.8	43.3	35.7	12.8	14.3	8.3
DT695	39.6	40.7	32.5	14.0	15.9	8.3
Commander	38.0	39.8	33.5	13.3	16.3	9.1
Westbred 881	36.5	39.3	32.3	12.6	13.5	8.6
Langdon (DIC-6B)	39.0	39.8	36.9	13.0	12.8	7.9
Langdon	37.1	33.2	37.0	14.7	13.7	9.9
Low-protein						
A0009&DAH46*	40.8	45.7	34.8	12.4	15.1	8.1
A0009&DAN08*	38.6	42.4	35.4	11.5	14.7	8.3
A0009&DAE01*	46.1	44.5	38.4	17.6	19.2	10.5
A0009&DAQ02*	41.1	44.7	35.9	12.2	15.1	9.2
A0009&DAD04*	39.5	46.7	33.1	13.5	16.4	7.6
A0009&DAH07*	41.6	46.6	34.8	13.1	16.3	8.7
Mean	41.3	45.1	35.4	13.4	16.1	8.7
High-protein						
A0009&DAD09*	41.8	43.1	35.9	13.4	15.1	8.7
A0009&DAB06*	42.5	45.0	38.2	15.1	17.9	9.0
A0009&DAG02*	39.4	41.1	33.5	15.3	17.1	9.2
A0009&DAD10*	39.1	43.2	34.2	12.9	17.2	8.8
A0009&DAC04*	39.9	41.3	36.7	14.1	16.0	8.6
A0009&DAL08*	37.9	43.9	37.8	15.7	16.0	9.4
Mean	40.1	42.9	36.0	14.4	16.5	8.9
L vs. H contrast[z]	NS	*	NS	NS	NS	NS
LSD ($P<0.05$)	3.1	5.1	5.5	3.0	3.9	1.4

[z]Contrast of means of high- and low-protein selections where NS = not significant ($P>0.05$), *significant at $P<0.05$ and **significant at $P<0.01$.

although the lower July and August precipitation at Nasser and Goodale than at Kernen could also have contributed to the lower N uptake. In addition to N fertilizer application, the quantity of N available for uptake was also likely influenced by mineralization, which is affected by moisture and temperature (Ellert and Bettany 1992; Sierra 1997).

Similar amounts of N were remobilized in the three environments (Table 6). Genotypic differences were greatest at Goodale, relatively minor at Kernen and non-significant at Nasser. Langdon (DIC-6B) had greater N remobilization than Langdon at Kernen and Goodale. The high-protein selections remobilized less N than the low selections at Kernen and Goodale. This agrees with the findings of Cox et al. (1986) that cultivars with greater post-anthesis N uptake translocated less from vegetative tissue to grain. There was no significant variation between the parents Strongfield and DT695 or among the other Canadian check cultivars.

Remobilized N accounted for an average of 98% of grain N in the Nasser environment, 85% at Goodale and 73% at Kernen (data not shown). Similarly, reports from hexaploid wheat (Peoples and Dalling 1988; Feller and Fischer 1994; Kichey et al. 2007) show N remobilization as the primary contributor to grain N. The proportion of remobilized N in the grain was inversely proportional to available soil N in the present study, as observed by others (Papakosta and Garianas 1991; Barbottin et al. 2005). The environmental differences in grain N at physiological maturity were due to post-anthesis N uptake. Low available N at Nasser inhibited post-anthesis plant growth and N uptake. Comparison of non-grain plant dry weights at anthesis and maturity suggests that remobilization of dry matter from vegetative organs to grain accounted for 65% of grain weight at Nasser, compared with 27% at Goodale and 0% at Kernen (data not shown).

Langdon (DIC-6B) had significantly higher ($P<0.05$) N harvest index than Langdon at Kernen and Nasser (Table 6). The isogenic pair did not differ significantly at Goodale due to the high variability at that site. The low- and high-protein selections had similar N harvest index in all environments. Strongfield had higher N harvest index than DT695 and the other check cultivars at Nasser.

The results do not point to a clear reason for the higher grain protein concentration of Strongfield and the high-protein selections. On average, the high-protein selections had similar or slightly lower total plant N at anthesis, similar vegetative N at physiological maturity and lower post-anthesis remobilization of N compared with the low selections. The higher post-anthesis N uptake of the high- than the low-protein selections offset this imbalance, but was not sufficient to explain

Table 6. Least square means of post-anthesis nitrogen uptake, remobilized nitrogen and nitrogen harvest index of durum wheat genotypes at Kernen 2005 (Krn), Goodale 2006 (Gdl) and Nasser 2006 (Nsr)

	Post-anthesis N uptake (mg plant^{-1})			N remobilization (mg plant^{-1})			NHI[z] (%)		
Genotypes	Krn	Gdl	Nsr	Krn	Gdl	Nsr	Krn	Gdl	Nsr
Check cultivars									
Strongfield	10.2	4.5	2.0	26.0	29.0	27.4	74.0	70.1	78.1
DT695	8.6	4.0	0.9	25.6	24.8	24.3	71.1	64.3	75.2
Commander	7.5	4.6	0.2	24.8	23.6	24.3	71.0	63.4	73.0
Westbred 881	6.4	1.1	0.8	23.9	25.8	23.6	70.7	66.6	74.0
Langdon (DIC-6B)	9.4	4.4	−2.0	28.9	27.0	29.0	73.1	70.9	77.2
Langdon	4.9	5.3	−1.0	22.4	19.5	27.0	65.1	64.5	72.4
Low-protein									
A0009&DAH46*	4.8	1.5	1.5	28.4	30.6	26.7	72.9	67.8	77.6
A0009&DAN08*	11.7	2.5	−0.2	27.2	27.7	27.1	77.0	67.4	76.4
A0009&DAE01*	13.4	5.4	3.1	28.5	25.4	27.9	70.3	61.7	74.6
A0009&DAQ02*	7.1	5.4	0.3	28.9	29.6	26.7	74.8	69.8	74.6
A0009&DAD04*	8.9	0.1	−2.2	26.0	30.3	25.5	72.0	65.0	75.5
A0009&DAH07*	8.4	4.2	3.7	28.5	30.3	26.1	73.7	67.8	77.3
Mean	9.0	3.2	1.0	27.9	29.0	26.7	73.5	66.6	76.0
High-protein									
A0009&DAD09*	9.8	5.1	1.4	28.4	28.1	27.2	74.0	68.6	76.4
A0009&DAB06*	15.1	9.2	3.0	27.4	27.1	29.2	73.8	67.1	78.1
A0009&DAG02*	13.7	7.9	2.2	24.0	24.1	24.3	70.8	65.6	74.3
A0009&DAD10*	13.2	9.2	1.2	26.2	25.9	25.4	75.3	67.3	75.1
A0009&DAC04*	8.1	4.3	0.2	25.8	25.3	28.1	70.8	65.1	76.7
A0009&DAL08*	14.3	4.7	0.8	22.2	28.0	28.4	69.9	67.2	75.7
Mean	12.4	6.7	1.5	25.6	26.4	27.1	72.4	66.6	76.0
L vs. H contrast[y]	**	**	NS	*	**	NS	NS	NS	NS
LSD ($P<0.05$)	5.2	4.3	6.0	4.2	4.4	5.0	4.8	7.2	2.7

[z]Nitrogen harvest index.
[y]Contrast of means of high- and low-protein selections where NS = not significant ($P>0.05$), *significant at $P<0.05$ and **significant at $P<0.01$.

the difference in grain protein concentration of the two groups. Kernel N concentration was higher in the high- than the low-protein selections throughout the sampling period at Nasser and Kernen, but not at Goodale (Fig. 1). This suggests that differences in N partitioning contribute to part of the protein concentration difference between the high- and low-protein selections. Differences in dry matter partitioning also contribute to the protein difference, with the slightly higher grain dry matter of the low-protein selections reducing protein concentration at physiological maturity (Fig 2); Clarke et al. (1990) reached a similar conclusion. The genotypic variation in post-anthesis N uptake and remobilization within the high- and low-protein groups suggests that either different physiological mechanisms or variation in partitioning produce the same final protein concentration.

Langdon (DIC-6B) was previously shown to have higher protein than Langdon due to greater post-anthesis remobilization of N (Deckard et al. 1996; Kade et al. 2005). This was confirmed in the present study, with Langdon (DIC-6B) expressing greater N remobilization except at the low N site Nasser. The gene responsible for the higher protein concentration of Langdon (DIC-6B), *Gpc-B1* (Olmos et al. 2003), appears to have a different physiological mode of action than the gene(s) responsible for high grain protein concentration in Strongfield and its progeny.

This study did not provide any clear insights into the lower protein concentration of semidwarf genotypes. Westbred 881 in fact expressed high protein concentration due to its low grain yield, the latter reflecting its poor adaptation to the local environment manifested largely by extreme susceptibility to leaf diseases. The semidwarf Commander is adapted to local conditions and expressed similar grain yield and relatively low grain protein concentration compared with Strongfield. Commander tended to remobilize less N than Strongfield, significant ($P<0.05$) at Goodale (Table 6), due to a tendency to slightly lower total plant N at anthesis and higher vegetative N at physiological maturity than Strongfield (Table 5), although none of these differences reached the 5% significance threshold. Further research with appropriate populations would be required to determine the effect of the dwarfing allele on protein concentration.

We previously found that marker *barc108* was associated with the high-protein concentration QTL *QGpc.usw-A3* on chromosome 7A of the DT695 × Strongfield population, and accounted for approximately one-third of the protein difference between the two parents (Suprayogi et al. 2009). The selections used in

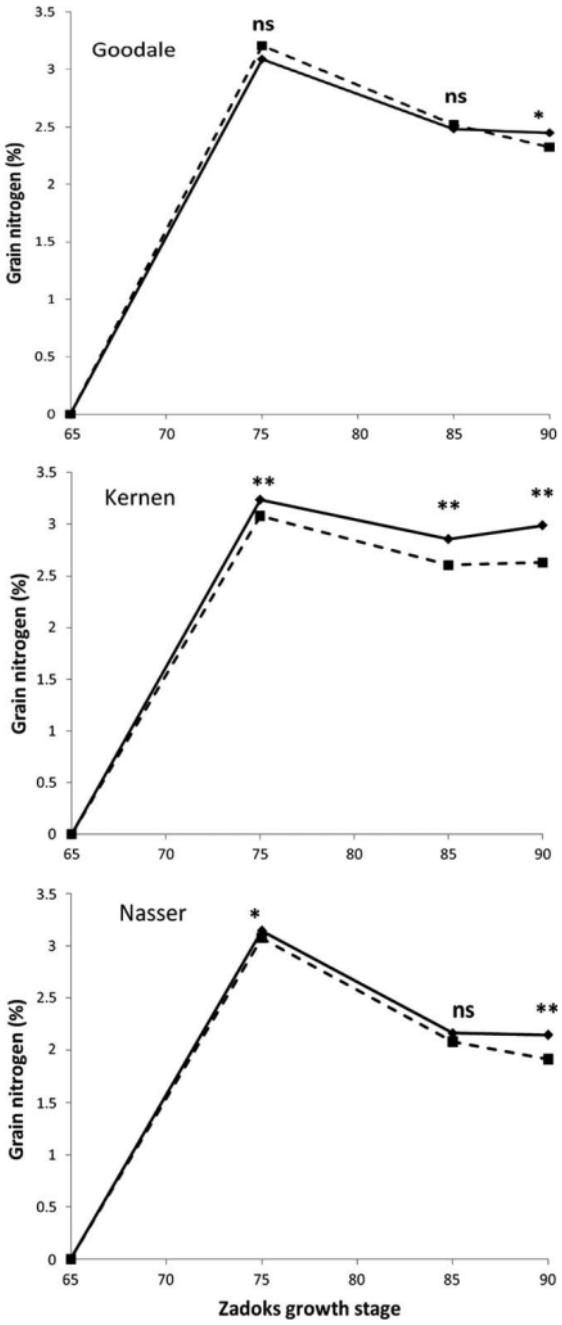

Fig. 1. Mean kernel nitrogen concentration of high-protein (—) and low-protein (---) selections at Kernen 2005, Goodale 2006 and Nasser 2006 sampled at Zadoks growths stages 65 (anthesis), 75 (milk), 85 (dough) and 90 (physiological maturity); ns: non-significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$.

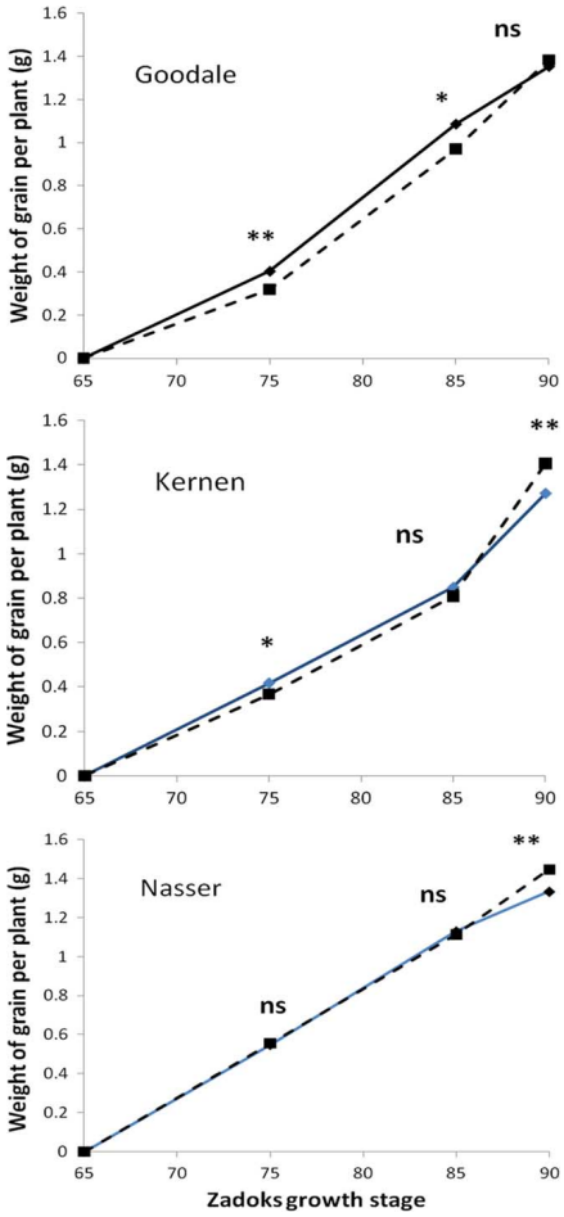

Fig. 2. Mean kernel weight of high-protein (—) and low-protein (---) selections at Kernen 2005, Goodale 2006 and Nasser 2006 sampled at Zadoks growths stages 65 (anthesis), 75 (milk), 85 (dough) and 90 (physiological maturity); ns: non-significant ($P > 0.05$), *$P < 0.05$, **$P < 0.01$.

this study were analyzed for *barc108* and all the high-protein selections carried the Strongfield allele at *barc108*, while the majority of low-protein selections (except A0009&DAH-46* and A0009&DAQ-02*) had the *barc108* allele similar to DT695. This result coincides well with the previous finding that Strongfield contributed the positive allele for grain protein concentration QTL *QGpc.usw-A3* on 7A (Suprayogi et al. 2009). However, more genotypes would have to be assessed in a detailed QTL study to confirm this.

Further study would be required to fully elucidate the mechanisms underlying the grain protein concentration difference in the DT695×Strongfield population. Pairs of near-isogenic genotypes would perhaps be a better way to study the phenomena, given the apparent variation in how high protein was achieved in the genotypes studied here. In the meantime, breeding progress will continue through concomitant selection for yield and protein, as used to develop the high-grain-protein-concentration, high-yield cultivars AC Avonlea and Strongfield, which optimizes the partitioning of N and dry matter and post-anthesis N uptake.

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