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Post-anthesis nitrogen accumulation and distribution among grains in spring wheat spikes

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SUMMARY

Knowledge of nitrogen accumulation to the grains is of great importance for identifying breeding and growing strategies to improve cereal yield and quality. The aim of this study was to investigate post-anthesis N translocation in the spike and into different spikelets of two cultivars of spring wheat (*Triticum aestivum* L.) grown to maturity in solution culture. Sport (short grain filling time, low yield and high grain protein concentration) and WL (normal grain filling time, high yield and low protein concentration) were the cultivars used. Plants received stepwise declining rates of N on a daily basis, until 109 days after planting (DAP). Anthesis for cv. Sport was 92 DAP and for cv. WL 78 DAP. On seven occasions (from anthesis in cv. WL until maturity), ^{15}N was given to selected groups of plants for 4 days. Then these plants were sampled and separated into plant parts. Spikelets and grains were separated according to spikelet position. Plant parts were weighed and analysed for N and ^{15}N.

The N concentration in the grains was uniform for the spikelets in cv. WL but differed in cv. Sport. Final N concentration in a spikelet position was obtained at 126 DAP in cv. Sport and 112 DAP in cv. WL and was constant to the end of experiment at 154 DAP in cv. Sport and 147 DAP in cv. WL. A positive correlation between % atom excess ^{15}N to spikelet position numbered from the base of the spike was exhibited in the lowermost part of the spike just after anthesis. Later, during grain filling, a negative correlation between % atom excess ^{15}N to spikelet position was exhibited ($-0{\cdot}991$ $P=0{\cdot}000$). A positive correlation between % atom excess ^{15}N to grain weight in the top of the spike was exhibited. Accumulation of ^{15}N proceeded 14 days longer in the lowermost fertile spikelets than in the uppermost spikelets. It was concluded that the duration of N accumulation in the spikelets was dependent on spikelet position and uppermost spikelets had a shorter N accumulation period and contained lighter grains than lowermost spikelets.

INTRODUCTION

Grain traits are often perceived to be equal in the spike, but the formation of grains and spikelets in the spike is not uniform (Langer & Hanif 1973; Kirby 1974). The amount and concentration of nitrogen for a grain depends on the position along the spike and within the spikelet (Levi & Anderson 1950; Simmons & Moss 1978; Stoddard 1999). The grains in the spikelets in the uppermost part of the spike have the lowest nitrogen concentration and the grains in the lowermost spikelets have the highest nitrogen concentration (Levi & Anderson 1950; Simmons & Moss 1978; Stoddard 1999; Bramble *et al.* 2002). Within the spikelets the uppermost grains have the lowest nitrogen concentration and the two lowermost grains have the highest nitrogen concentration (Levi & Anderson 1950; Bremner 1972; Simmons & Moss 1978; Herzog & Stamp 1983; Stoddard 1999). The levels of other minerals also vary depending on grain position (Calderini & Ortiz-Monasterio 2003).

The reason for the difference in grain N concentration in the spike is not well understood. It has been suggested that the difference in grain N concentration in the spike may be caused by the arrangement of the vascular system in the spikelet (Bremner 1972; Simmons & Moss 1978), or by variation in accumulation rates and accumulation duration of nitrogen and carbohydrates to the grains (Simmons & Moss

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1978; Herzog & Stamp 1983). The N-flow to the grain and amount of N in a grain are more constant than the N concentration, which varies with the carbohydrate accumulation (Triboi & Triboi-Blondel 2002). The present experiment was performed to clarify the N accumulation over time in the spike.

Desired grain protein concentration and quality differ according to the intended end-use of the wheat. In production of bread-wheat, for example, a late nitrogen application is often recommended in order to increase the protein concentration (Lütke Entrup & Oemichen 2000; Fogelfors 2001; Woolfolk *et al.* 2002). A better understanding of uptake, transport and remobilization of nitrogen is important in order to increase the possibilities of producing wheat with the right protein concentration and quality. Good nitrogen efficiency, with low costs for the farmer and low losses to the environment, is also important.

When studying uptake, transport and remobilization of nitrogen in plants, it is convenient to use a ^{15}N-enriched source of N. Nitrogen uptake, transport, and remobilization can be investigated by growing plants in solution culture to full maturity (Ingestad 1982; Mattson *et al.* 1992; Oscarson *et al.* 1995*a*). When the relative addition rate of nitrogen is kept at a growth-limiting level, the nitrogen added daily is taken up within 24 h and is equal to the relative increase in plant nitrogen content (Mattson *et al.* 1992; Oscarson *et al.* 1995*a*, *b*; Oscarson 1996). The combination of solution culture technique and labelled N applied at different times during plant development gives unique possibilities to study the flow of nitrogen in the plant.

The aim of the current study was to investigate the nitrogen flow in the spike over time with the combined technique of solution culture and labelled N. The aim was also to investigate the intrinsic hierarchy during spike maturation of pre- and post-anthesis absorbed nitrogen.

MATERIALS AND METHODS

Cultivars

The experiments were carried out with two cultivars of spring wheat (*Triticum aestivum* L.) with different agricultural properties in respect of yield and N concentration in the grains. The cultivar Sport has high protein concentration in the grains (170 mg/g) and a normal to low grain yield when grown in Swedish field trials, while cultivar WL-4 (WL in the following) has low protein concentration in the grains (110 mg/g) and a high grain yield (G. Svensson 2003, personal communication). The cultivars were obtained from the Svalöf Weibull AB Plant Breeding Institute, Svalöv, Sweden.

Cultivation

Seeds were germinated on moistened filter paper in complete darkness at a temperature of 8 °C for 3 days and thereafter at 17 °C for 4 days. The seedlings were then placed on black foam plates, which floated on continuously aired N-free nutrient solution in black 2-litre beakers. Each beaker held four plants of one cultivar. The day the seedlings were placed on the plates is referred to as 1 day after planting (DAP). Anthesis for cv. WL was 78 DAP and for cv. Sport 92 DAP.

The plants were grown in a climate chamber in the Biotron at the Swedish University of Agricultural Sciences. A controlled environment with a regime of 16 h light and 18 °C temperature, 8 h darkness and 12 °C, and 70% relative humidity was used. Fluorescent tubes (Sylvania Lumiance, type F 96 T12 VHO cold white 215W) provided a PAR photon flux density of 500 μmol/m^2/s at canopy height. The N-free solution contained (in mM): K_2SO_4, 0·24; K_2HPO_2, 0·12; KH_2PO_4, 0·11; $CaCl_2$, 0·09; $MgCl_2$, 0·18; HCl 25×10^{-3}; $FeCl_3$, $6{\cdot}24 \times 10^{-3}$; H_3BO_4, $9{\cdot}24 \times 10^{-3}$; $MnCl_2$; $3{\cdot}64 \times 10^{-3}$; $ZnCl_2$, $0{\cdot}23 \times 10^{-3}$; $CuCl_2$, $0{\cdot}24 \times 10^{-3}$; Na_2MoO_4; $0{\cdot}04 \times 10^{-3}$; Fe-EDTA, $24{\cdot}1 \times 10^{-3}$. The N-free solution was replaced weekly in order to keep the pH between 6·5 and 5. The N-addition solution contained (in mM): K_2SO_4, 2·8; K_2HPO_4, 1·93; KH_2PO_4, 2·27; KNO_3, 4·87; $Ca(NO_3)_2$, 15·49; $Mg(NO_3)_2$, 17·24; HNO_3, 0·49; $Fe(NO_3)_2$, 0·13; H_3BO_3, 0·185; $Mn(NO_3)_2$, $72{\cdot}8 \times 10^{-3}$; $Zn(NO_3)_2$, $4{\cdot}6 \times 10^{-3}$; $CuCl_2$, $4{\cdot}72 \times 10^{-3}$; Na_2MoO_4, $0{\cdot}72 \times 10^{-3}$.

Nitrogen supply

The plants were grown with a limited N supply and the other nutrients in excess to N. The N amount (NA) in the plants was set to follow the equation:

$$NA_t = NA_{t-1} e^{RA}$$

where NA_t is NA in the plant at day t and NA_{t-1} the NA at the day before day t. RA is the rate at which N is given to the plants (NA added/NA/day). To give an equal daily dose of N to the cultivars, the NA was set to 1·00 mg N/seedling before the first N dose. When RA is kept at a growth-limiting level, the NA added daily is taken up within 24 h and is equal to the relative increase in plant NA (Mattson *et al.* 1992; Oscarson *et al.* 1995*a*, *b*; Oscarson 1996).

In the first week no N was added. From 7 DAP to 109 DAP, the plants were given daily doses of N as nitrate calculated from $NA_t - NA_{t-1}$. RA was reduced stepwise during plant development. From 7 DAP, RA was 0·1/day; on 17 DAP reduced to 0·09; on 27 DAP to 0·08; on 37 DAP to 0·06; on 47 DAP to 0·04; on 57 DAP to 0·02; on 67 DAP to 0·01; on 77

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Table 1. *Schedule of N-supply in mg N/plant at the sampling times, days after planting (DAP)*

	N-supply, mg N/plant							
		as ^{15}N solution						
Sampling time DAP	Total N supplied until 6 days b.s.	at day 5 b.s.	at day 4 b.s.	at day 3 b.s.	at day 2 b.s.	at day 1 b.s.	Total N supplied at sampling time	Atom % excess ^{15}N
84	50·1490	0·2514	0·2526	0·2539	0·2552	0·2565	51·4186	0·8697
98	53·7853	0·2696	0·2710	0·2723	0·2188	0·2197	55·0367	0·7835
112	56·9970	0·2500	0·1875	0·1875	0·1875	0·1875	57·9970	0·5680
126	57·5123	0·2500	0·1875	0·1875	0·1875	0·1875	58·5123	0·5630
140	57·5123	0·2500	0·1875	0·1875	0·1875	0·1875	58·5123	0·5630
147	57·5123	0·2500	0·1875	0·1875	0·1875	0·1875	58·5123	0·5630
154	57·5123	0·2500	0·1875	0·1875	0·1875	0·1875	58·5123	0·5630

b.s. = before sampling day.

DAP to 0·005; on 97 DAP to 0·004; and on 107 DAP to 0·003/day.

To supply 0·15 of the total N after anthesis of the earliest cultivar WL, no IV was added to any cultivar from 110 DAP, except on the five days prior to each sampling (Table 1).

Together with the daily N dose, the plants were also given other nutrients so there was little or no change in concentrations of nutrients in the nutrient solution between the weekly changes.

Samples, ^{15}N supply and analyses

The first sampling day was 84 DAP, which was before anthesis for cv. Sport, and 6 days after anthesis for cv. WL. Three beakers (12 plants) were selected randomly from each cultivar for ^{15}N supply before each sampling day. Each day for the 4 days immediately preceding each sampling day, the daily N dose to the selected plants contained ^{15}N nitrate (43·92 atom % ^{15}N) (Table 1).

At each sampling time, the plants from each beaker were divided into roots, main shoot (MS) spikelet grains, the rest of MS (including chaff and rachis), tillers (T) vegetative parts (including aborted T), and T grains. In the following, the average of all grains/spikelet is denoted as grains/spikelet. Spikelet positions were numbered from base to top in the spike. At each sampling time, number of spikelets in MS spike, number of grains in spikelets, number of T/plant, and number of T spikes/plant were counted.

Plant parts were dried at 70 °C for 48 h, weighed, ground and analysed for N and ^{15}N. N and ^{15}N were determined according to the Dumas method using a Carlo Erba NA 1500 elemental analyser (Carlo Erba Strumentazione, Milan, Italy) in combination with a mass detector (Europa Scientific, Crewe, UK).

Calculations and statistical analysis

Total NA found in the plant was calculated as:

$$\sum(\text{dry weight} \times \text{N concentration}) \text{ for all plant parts.}$$

The experiments were carried out with three replicates and repeated twice. At 84 DAP, cv. Sport was only sampled in the first run of the experiment; samples at 147 DAP were taken only in the repeat experiment; and samples at 154 DAP were only taken for cv. Sport in the first run. To ensure enough material for analysis, grains from each spikelet position in each repeat were pooled. Only small differences appeared between the repeat experiments and therefore root data were statistically analysed as six replicates and aboveground plant parts as two replicates.

Statistical analyses were carried out using ANOVA, Pearson correlation coefficients and standard errors (S.E.) using Minitab Statistical Software (release 13:31). One-way ANOVA was used to test differences in N concentration between sampling times in the spikelet position regions, and N concentration between spikelet positions for each sampling time (Table 2a); and in ^{15}N amount between spikelet positions for each sampling time (Table 3). Standard errors in tables are based on pooled standard deviations from one-way ANOVA; standard errors in figures are based on pooled standard deviations from GLM ANOVA. The degrees of freedom (D.F.) from the pooled standard deviations are given.

RESULTS

Dry weights and vegetative pattern

The grain growth in spikelets was smaller in the uppermost half spikelet positions compared with the lowermost half spikelet positions. The dry weight for

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Table 2a. *Average grain nitrogen concentration* (*mg/g dry weight*) *for spikelet positions 5–9 and 15–19, and standard error* (*S.E.*) *with corresponding degrees of freedom in parentheses in two cultivars at different sampling times, days after planting* (*DAP*)

Cultivar and spikelet position region	84 DAP	98 DAP	112 DAP	126 DAP	140 DAP	147 DAP	154 DAP
Sport 5–9		31·67	24·36	17·51	17·11	17·01	17·05
Sport 15–19		30·75	23·71	16·75	16·08	15·17	15·85
S.E.		1·208 (18)	0·225 (18)	0·186 (18)	0·179 (18)	0·196 (8)	0·178 (8)
WL 5–9	31·60	20·54	14·38	13·93	13·79	14·11	
WL 15–19	30·95	19·85	14·34	13·87	13·54	13·32	
S.E.	0·856 (18)	0·299 (14)	0·108 (17)	0·122 (18)	0·162 (18)	0·173 (8)	

Table 2b. *Pearson correlation coefficients* (*corr*) *between N concentration and spikelet position in the spikelet region 8–19 and* P-*value in two cultivars at different sampling times, days after planting* (*DAP*)

Cultivar and spikelet position region	84 DAP	98 DAP	112 DAP	126 DAP	140 DAP	147 DAP	154 DAP
Sport 8–19 corr		−0·295	−0·673	−0·882	−0·942	−0·831	−0·739
P		0·351	0·016	0·000	0·000	0·001	0·006
WL 8–19 corr	−0·265	−0·681	−0·198	−0·233	−0·395	−0·690	
P	0·405	0·015	0·538	0·467	0·203	0·013	

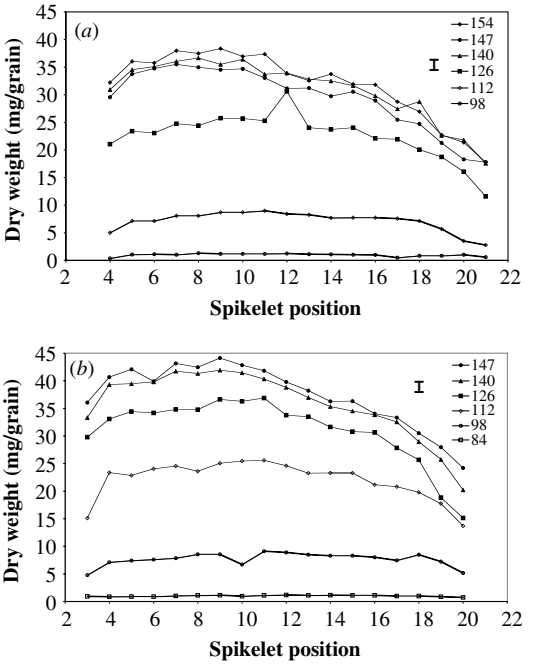


Fig. 1. Dry weight in grains/spikelet (mg/grain). Spikelet positions numbered from the base of the spike. Symbols and text refer to days after planting. Vertical bars represent S.E. (D.F. = 72 for Sport and D.F. = 90 for WL). (*a*) Sport, (*b*) WL.

the mean grain was greatest in spikelet positions 5–10 (Fig. 1*a*, *b*). Cultivar Sport had a lower number of grains/spikelet in the uppermost and lowermost parts of the spike than cv. WL (Fig. 2). Cultivar Sport had $1{\cdot}58 \pm 0{\cdot}04$ tiller spikes/plant and cv. WL had $1{\cdot}33 \pm 0{\cdot}08$ tiller spikes/plant at 140 DAP and later (results not shown). On average, cv. Sport had $21{\cdot}98 \pm 0{\cdot}12$ spikelets/spike (range 15–25) and cv. WL $20{\cdot}37 \pm 0{\cdot}08$ spikelets/spike (range 16–23).

Cultivar Sport had lower grain yield than cv. WL. The grain filling in tillers was later than in main shoots (Table 4).

Nitrogen concentration

From 112 DAP, N concentration of grain varied depending on spikelet position for cv. Sport (Table 2a). The N concentration was negatively correlated with spikelet position for cv. Sport, but not for cv. WL except at 98 and 147 DAP (Table 2b).

In the beginning of the grain filling, grain N concentration was high. From 126 DAP in cv. Sport and from 112 DAP in WL, there were no significant differences in one cultivar between sampling times for the same spikelet position.

Nitrogen amount

At maturity, the total amount of N (mg/spike) in main stem grains was significantly ($P < 0{\cdot}01$) greater

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Table 3. *Average excess ^{15}N amount in grains/spikelet of total excess ^{15}N amount in the whole plant, %, for spikelet regions 5–9 and 15–19, and standard error (S.E.) with corresponding degrees of freedom in parentheses in two cultivars at different sampling times, days after planting (DAP)*

Cultivar and spikelet position region	84 DAP	98 DAP	112 DAP	126 DAP	140 DAP	147 DAP	154 DAP
Sport 5–9		0·067	0·279	0·472	0·754	0·316	0·024
Sport 15–19		0·035	0·220	0·278	0·166	0·023	0·014
S.E.		0·0100 (18)	0·0166 (18)	0·0147 (18)	0·0282 (18)	0·0463 (8)	0·0073 (8)
WL 5–9	0·0520	0·267	0·386	0·441	0·641	0·215	
WL 15–19	0·0471	0·206	0·275	0·182	0·064	0·019	
S.E.	0·00418 (18)	0·0159 (14)	0·0166 (17)	0·0206 (18)	0·0291 (18)	0·0388 (8)	

Table 4. *Main shoot (MS) dry weight grain yield (mg/plant), tiller (T) dry weight grain yield (mg/plant), and N amount (mg/plant) in two spring wheat cultivars at different sampling times and standard error (S.E.) with corresponding D.F. in parentheses*

	Sport			WL		
Sampling time DAP	MS grain yield mg/plant	T grain yield mg/plant	Found N mg/plant	MS grain yield mg/plant	T grain yield mg/plant	Found N mg/plant
84	0	0	50·9	37	0	50·0
98	31	2	53·5	344	91	55·4
112	318	113	56·9	1051	471	59·1
126	865	574	58·6	1361	982	60·4
140	1173	947	59·8	1579	1299	59·8
147	1178	1214	60·9	1629	1399	64·4
154	1124	1214	59·5			
S.E.	20·3 (4)	20·3 (4)	1·11 (4)	51·7 (5)	28·8 (4)	1·00 (5)

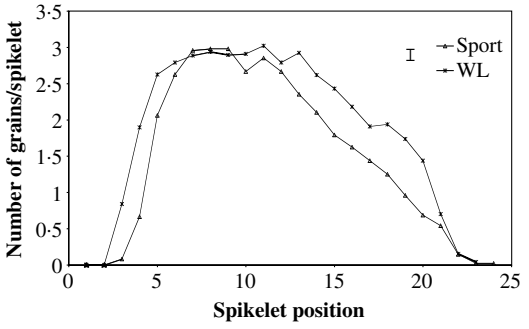


Fig. 2. Number of grains/spikelet in two spring wheat cultivars. Means from sampling time 140 days after planting (DAP) and later. Symbols and text refer to cultivars. Vertical bar represents S.E. (D.F. = 90).

in cv. WL than in cv. Sport (results not shown). The amount of N (mg/grain) for the average grain was greater in cv. Sport than in cv. WL ($P = 0{\cdot}052$). The amount of N in main stem grain for cv. WL increased from 84 DAP to 147 DAP and for cv. Sport from 98 DAP to 140 DAP (Fig. 3*a*, *b*).

During the period 112 to 126 DAP for Sport and 98 to 112 DAP for WL, the increment in N amount was greatest and was uniform for spikelet positions 5–18 for cv. Sport and 4–19 for cv. WL. The increase in N amount was low in the top of the spike, above position 18 for cv. Sport and 19 for cv. WL. After 126 DAP in cv. Sport, the greatest increase in N amount was for spikelet positions 4–11.

^{15}N concentration in the grains

During grain filling, the atom % excess ^{15}N in grains in spikelet position 8–19 was significantly negatively correlated with the spikelet position (Table 5). At the first sampling time after anthesis, 98 DAP for cv. Sport and 84 DAP for cv. WL, the atom % excess ^{15}N in spikelet position 5–10 was positively correlated with the spikelet position. A negative correlation was found with spikelet position at 140 DAP and later.

Generally, there was no significant correlation between atom % excess ^{15}N in spikelet position 5–10 and grain number/spikelet (Table 5).

A positive correlation between the grain atom % excess ^{15}N and the average grain weight was found for

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Table 5. *Pearson correlation coefficient between grain* ^{15}N *atom % excess and spikelet position* (*corr*) *in spikelet regions 5–10 and 8–19, and Pearson correlation between grain* ^{15}N *atom % excess and number of grains/spikelet* (*corrg*) *in spikelet region 5–10 in two spring wheat cultivars at different sampling times, days after planting* (*DAP*)

	In spikelet position region 5–10				In spikelet position region 8–19				In spikelet position region 5–10			
	Sport		WL		Sport		WL		Sport		WL	
Sampling time DAP	corr	*P*	corr	*P*	corr	*P*	corr	*P*	corr	*P*	corr	*P*
84			0·782	0·066			−0·565	0·055			0·435	0·389
98	0·757	0·082	−0·605	0·203	−0·885	0·000	−0·948	0·000	0·153	0·772	−0·657	0·156
112	0·730	0·100	−0·852	0·031	−0·884	0·000	−0·907	0·000	0·282	0·589	−0·563	0·245
126	−0·846	0·034	−0·469	0·348	−0·988	0·000	−0·987	0·000	−0·293	0·572	−0·083	0·875
140	−0·992	0·000	−0·991	0·000	−0·981	0·000	−0·967	0·000	−0·619	0·190	−0·849	0·032
147	−0·946	0·004	−0·965	0·002	−0·733	0·007	−0·607	0·037	−0·505	0·307	−0·675	0·141
154	−0·790	0·062			0·609	0·036			−0·955	0·003		

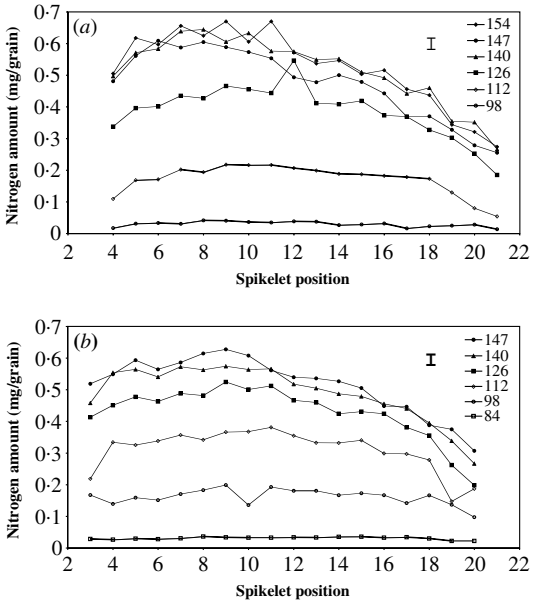


Fig. 3. Nitrogen amount in grains/spikelet (mg/grain). Spikelet positions numbered from base of the spike. Symbols and text refer to days after planting (DAP). Vertical bars represent S.E. (D.F. = 71 for Sport and D.F. = 83 for WL). (*a*) Sport, (*b*) WL.

the whole spike (Table 6). In spikelet position 8–19, this correlation was significant and positive during the whole grain filling period. In spikelet position 5–10 the correlation between the grain atom % excess ^{15}N and the average grain weight was positive at the first sampling time after anthesis, 98 DAP for cv. Sport and 84 DAP for cv. WL, but from 112 DAP in Sport and 98 DAP in WL the correlation was mostly not significant.

Relative ^{15}N *amount in the grains*

Six days after anthesis, at 84 DAP for cv. WL and at 98 DAP for cv. Sport, the grains in the spikelets had a very small accumulation of ^{15}N (Table 3). In the beginning of the grain-filling period, until 112 DAP for both cultivars, the relative amount of ^{15}N absorbed and distributed to grains was relatively even among the spikelet positions except for the uppermost and lowermost ones. At 126 DAP the relative amount of ^{15}N in grains/spikelet was greater in the lowermost half of the spike than in the uppermost half. At the end of the grain-filling period there was an increase in the relative amount of ^{15}N in grains/spikelet only in the lowermost fertile spikelets in the spike and the accumulation of ^{15}N proceeded 14 days longer in the lowermost part of the spike than in the uppermost part of the spike. Cultivar Sport had a lower increase in the relative amount of ^{15}N in the upper part of the spike than cv. WL.

DISCUSSION

The duration of N accumulation among main stem spikelets differed for two cultivars having different grain filling period, yield potential and grain N concentration. For cv. WL, the grain N concentration was similar among all spikelets during the entire grain filling period, while for cv. Sport differences in grain N concentrations were found to be dependent on spikelet positions. Cultivar Sport exhibited the general pattern for cultivars at full maturity, namely the grains in the uppermost spikelets have the lowest nitrogen concentration and the highest nitrogen concentration in the lowermost spikelets (Levi & Anderson 1950; Simmons & Moss 1978; Stoddard 1999; Bramble *et al*. 2002). If stable grain quality is desired, a constant grain N concentration among all spikelets could be a more favourable trait than

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Table 6. *Pearson correlation coefficient between grain* ^{15}N *atom % excess and average grain weight for the grains in a spikelet (corr) in different spikelet position regions in two cultivars at different sampling times, days after planting (DAP)*

	In spikelet position region 5–10				In spikelet position region 8–19				All spikelets			
	Sport		WL		Sport		WL		Sport		WL	
Sampling time DAP	corr	*P*	corr	*P*	corr	*P*	corr	*P*	corr	*P*	corr	*P*
84			0·938	0·006			0·735	0·006			0·654	0·002
98	0·752	0·084	0·067	0·899	0·678	0·015	0·429	0·164	0·886	0·000	0·472	0·041
112	0·571	0·237	−0·551	0·257	0·933	0·000	0·880	0·000	0·770	0·000	0·799	0·000
126	−0·823	0·044	−0·413	0·416	0·766	0·004	0·923	0·000	0·481	0·023	0·847	0·000
140	−0·713	0·112	−0·834	0·039	0·909	0·000	0·902	0·000	0·582	0·006	0·636	0·002
147	−0·254	0·627	−0·471	0·356	0·577	0·050	0·549	0·065	0·370	0·109	0·398	0·091
154	−0·766	0·076			−0·570	0·053			−0·097	0·685		

variation in grain N concentration. Low variation in N concentration among grains dependent on spikelet position, similar to that found in cv. WL, has seldom been reported but in the study by Simmons & Moss (1978) a high protein cultivar showed low variation in N concentration among grains. Cultivar WL is a low protein cultivar and therefore the difference in grain N concentration among spikelets is not likely to be related to the protein level of cultivars.

The results from the present study exhibited dominance for grain growth and nitrogen accumulation of the lower mid and central spikelets in the beginning of the grain filling period. During the grain filling this priority was suppressed by a strong grain growth and nitrogen accumulation priority according to spikelet position from top to base. In the meantime N accumulated almost uniformly among the spikelets in the spike. The results for the beginning of the grain-filling period are consistent with previous findings (Langer & Hanif 1973; Kirby 1974; Simmons & Moss 1978), but the priority to the spikelets in the base of the spike during late grain filling is, to the best of our knowledge, new information, and is contradictory to previous findings (Herzog & Stamp 1983).

The spikelets in the spike are initiated between the double ridge and the terminal spikelet stage of the spike primordium. The spikelets just below the middle of the spike are formed first and the first formed spikelets achieve the highest grain number/spikelet and grain weight in the spike (Kirby 1974). A priority has also been observed within the spikelet, with lowest priority to the apical florets and the highest priority to the second floret from the base (Simmons & Moss 1978). In the present study the correlation between recently taken up N, expressed as atom % excess ^{15}N, and spikelet position was significantly negative. Thus, the lowest part of the spike, independent of spikelet grain number and grain weight in the spikelets, obtained the highest concentration of recently taken up N.

In the wild progenitors (wild einkorn *Triticum boeoticum*, wild emmer *T. dicoccoides* and *Aegilops tausschii* Coss) of the cultivated bread wheat *T. aestivum*, the rachis disarticulates into diaspores each consisting of a spikelet and a section of the rachis (Bor & Guest 1968; Kerber & Rowland 1974; van Slageren 1994; Salamini *et al.* 2002). The property observed here of maturing from top to base expressed as ceasing duration of N-accumulation seems to be a prerequisite to rachis disarticulation in the wild progenitors of bread wheat. In spite of the tough rachis, this property still exists in the cultivated bread wheat *T. aestivum*.

It has been shown in field conditions that nitrogen is taken up predominantly pre-anthesis (Austin *et al.* 1977; Heitholt *et al.* 1990; Palta *et al.* 1994; Fangmeier *et al.* 1999). However, late nitrogen applications during heading or later are used during conventional wheat production, e.g. for bread wheat production in northern Europe, to increase the protein concentration and improve the bread-making quality (Lütke Entrup & Oemichen 2000; Fogelfors 2001; Woolfolk *et al.* 2002). However, the influence of late nitrogen applications on specific grains, spikelets, spikes and protein compositions has rarely been studied.

During grain filling the pattern of N accumulation by developing grains shifted in favour of the lowermost fertile spikelet positions. It is possible that nitrogen absorbed pre-anthesis by the root and remobilized from the vegetative plant parts reaches the grains differently, both in position and time, than the nitrogen absorbed post-anthesis by the root. This might lead to differences in amounts and types of protein polymers, depending on the pre- or post-anthesis origin of the nitrogen for the grains.

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However, there is no evidence from the present study for such a difference.

According to bread-making quality recommendations, uniform as well as good quality is of importance (Johansson *et al*. 2001). For truly uniform quality, it is important to have similar quality from diverse environments (Johansson & Svensson 1998). The environmental variation in bread-making quality is caused by differences in protein polymer composition (Johansson *et al*. 2002). The variation in quality is also likely to be influenced by differences in maturation time of the wheat. Variation in the termination of N accumulation in different spikelets in the spike might cause variation in protein quality. Pre- and post-anthesis nitrogen and protein conversion time might also be of importance. Thus, influences of late nitrogen applications on protein quality in various parts of the spike require further analysis.

Grains from apical spikelets have lower dry weights and lower macro- and micronutrient concentrations than central and basal spikelets. Within the spikelet, the lowest concentrations of macro- and micronutrients have been found in grains more apical from the rachis (Calderini & Ortiz-Monasterio 2003). In the present study, the ending of N accumulation among spikelets in the spike N was in a basipetal order, resulting in a short duration of the accumulation of N to the grains in apical spikelets and a long duration of the accumulation of N to the basal spikelets. Just before complete senescence of the plants, the site in the spike for N accumulation was only the lowermost fertile spikelets with heavy grains and not the uppermost spikelets with light grains. Thus, nutrients are likely to reach light and heavy grains differently during the grain filling period. Differences in mineral concentrations between different grains in the spike and spikelet may be explained by the duration of accumulation in an individual grain. Light grains in uppermost spikelets might not be reached by the late flow and cannot thereby be influenced by late treatments or late uptake from the soil.

The conclusion from the current work is that grain accumulation of nitrogen ceases at different times depending on spikelet position. The cessation order is basipetal, i.e. from top to base. Uppermost spikelets contain small grains and have the shortest N accumulation period.

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