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Grain Filling Mechanisms in Spring Wheat

VI. Cultivar variation in nitrogen metabolism and changes in assimilate shortages

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Abstract : Improvement of wheat flour quality, an increase in grain nitrogen content accompanied by an increase in grain yield, is required through cultivation techniques and breeding program. Changes in nitrogen content in grains and vegetative organs were investigated to clarify nitrogen metabolism in four grain filling phases in spring wheat. Three cultivars including semi-dwarf Haruyutaka with early maturity bred in Hokkaido in Japan, semi-dwarf Norin 61 with early maturity bred in Kyushu in Japan, and tall Selpek with late maturity bred in Germany were selected for the present experiments. Plants from each cultivar were sampled at intervals of a few days throughout the grain filling period in 1993. In 1992, Haruyutaka canopy was covered with 95% shading cloths for three grain filling phases ; the initial phase from two days before anthesis to seven days after anthesis, the early phase from seven to 14 days after anthesis, and the late phase from 14 to 21 days after anthesis. Haruyutaka required more nitrogen for grain filling during its earlier phases than Norin 61 and Selpek. This fact resulted in a higher remobilization of nitrogen from vegetative organs into grains in Haruyutaka (73%) than in Norin 61 (49%) and Selpek (47%). Shading treatments did not affect the nitrogen content in grains until a week before maturity. However, the nitrogen in vegetative organs for each shading was translocated into grains rapidly during the last week of the final phase, with the result that the nitrogen content in each shading grain was higher than no shading. As strategies to achieve both high yield and high nitrogen percentage in grain, it appeared that much nitrogen must be accumulated in vegetative organs and allocated to more active photosynthetic organs until anthesis, and that more nitrogen must be absorbed from soil during earlier grain filling phases, then completely translocated into grains during the final grain filling phase.
Key words : Grain filling phases, Nitrogen metabolism, Nitrogen percentage in grain, Shading treatment, Spring wheat varieties.

春播コムギの登熟機構の解明　第6報　窒素代謝とその品種間差異および同化産物不足の影響：高橋　肇・永尾浩司・板垣　洋・高久俊宏・土橋直之・中世古公男（北海道大学農学部）

要　旨：コムギの収量を低下させずに子実中の窒素含有率を高めることは，粉の品質を高めるために栽培および育種における必要条件である．本研究では，春播コムギの窒素代謝を明らかにするため，子実および栄養器官における窒素含有量の変化を4つの登熟相をもとに調査した．1993年の試験では，北海道で育成された早生・半矮性品種ハルユタカ，九州で育成された早生・半矮性品種農林61号およびドイツで育成された晩生・長稈品種Selpekの3品種を用いて登熟期間中2～3日に一度の割合でサンプリングを行った．1992年の試験では，品種ハルユタカについて3つの遮光処理を設け（初期処理：開花前2日目から開花後7日目まで群落上面を95%遮光布で遮光，前期処理：開花後7日目から14日目まで95%遮光および後期処理：開花後14日目から21日目まで95%遮光），1週間に一度の割合でサンプリングを行った．子実における窒素要求は，登熟前半（登熟初期，前期および後期）においてハルユタカが農林61号およびSelpekよりも高かった．このため，子実中窒素に対する栄養器官からの転流による割合は，登熟期間を通じてハルユタカで73%と高く，農林61号およびSelpekでそれぞれ49%および47%と低かった．子実の窒素含有率は，成熟期の一週間前に至るまで遮光処理区と無処理区との間に差異がみられなかったが，栄養器官中の窒素が各処理区とも登熟末期の最後の一週間に子実へと急速に転流したため，収穫時では遮光処理区が無処理区よりも高くなった．高収量と高子実窒素含有率とを併せて達成するための戦略として，開花期までに栄養器官，特に光合成活性の高い器官に多量の窒素を蓄積し，登熟初期においてもさらに多くの窒素を土壌より吸収すること，登熟末期においてはこれらの窒素を子実へと完全に転流させることが必要と考えられた．
キーワード：子実中の窒素含有率，遮光処理，窒素代謝，登熟相，春播コムギ品種．

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It is necessary to increase protein content for improvement of flour quality in wheat. A total high protein yield have been achieved through cultivars with a high yield potential but a low protein concentration[3,15]. Inversely, a low grain yield was accompanied with a high protein content in grains[4]. Thus, it appears to be impossible to increase simultaneously grain yield and protein content in the grains through cultivation techniques and genetic manipulations.

Competition concerning nitrogen distirbution occurres between vegetative organs relating to retainment of photosynthetic ability and grains of which protein content associates with grain quality. The nitrogen distribution pattern between the two organs is influenced by nitrogen availability[2,9].

We dealt with the following four grain filling phases to analyze the grain filling mechanisms[12] ; (1) initial grain filling phase : from anthesis to cessation of culm elongation ; (2) early grain filling phase : from cessation of culm elongation to milk ripe stage ; (3) late grain filling phase : from milk ripe stage to cessation of photosynthesis ; and (4) final grain filling phase : from cessation of photosynthesis to maturity. On the basis of these phases, influences of solar radiation shortage and varietal difference on grain growth and accumulation of WSC in culm were carried out to examine the mechanisms of carbohydrate metabolism in the grain filling period[13,14].

The present study is to understand the nitrogen metabolisms of wheat changing with growth and to develop strategies to achieve both high yield and high nitrogen content in grains. Changes in nitrogen content in grains and vegetative organs were investigated under different solar radiation using the cultivar Haruyutaka and among three wheat cultivars.

Materials and Methods

The experiments were conducted in 1992 and 1993 at the Experimental Farm of Hokkaido University.

1. Shading experiment in 1992

The spring wheat variety Haruyutaka was planted on April 11. Shading treatment was conducted using 95% shading cloth for each of the initial, early and late grain filling phases as in the previous report[13]. The shading periods were designated from two days before anthesis until seven days after anthesis as the initial shading ; from seven days until 14 days after anthesis as the early shading, and from 14 days until 21 days after anthesis as the late shading. The shading cloth was positioned 30 cm above the canopy.

2. Experiment in 1993 using three cultivars

In 1993, three spring wheat varieties Haruyutaka, Norin 61 and Selpek were planted on May 7. Haruyutaka is an early semi-dwarf variety, bred in Hokkaido Japan. Norin 61 is also an early semi-dwarf, bred in Kyushu Japan. Selpek is a late tall variety, bred in Germany.

3. Crops

In both years, seeds were set on seeder tapes (Nihon Plant Seeder) at every 5 cm and the tapes were planted 2 cm deep. Two tapes were placed in each row and thinned to a single plant after two or three leaves expanded. The final density was an equi-distant square pattern with 400 plants m^{-2}. The plots consisted of 50 cm by 50 cm sampling sub-plots with three replications. Fertilizer was applied prior to sowing : N, 90 kg ha^{-1} ; P_2O_5, 150 kg ; and K_2O, 75 kg. Fungicides and insecticides were applied a few times to control powdery mildew, rust, flies, army worms and aphids.

4. Sampling

Twenty standard plants were sampled from each plot at 5 am weekly and several-day intervals in 1992 and in 1993, respectively. Each plant was separated into grains and residual vegetative organs above ground, then heated for 30min at 105°C and dried for 48h at 70°C. The dried samples were weighed and milled for determination of nitrogen content by Kjeldahl method.

Results

1. Changes in nitrogen contents in grains and vegetative organs throughout the grain filling period

The amount of nitrogen in whole plant increased linearly from anthesis until cessation of photosynthesis, then did not change significantly until maturity for all three cultivars (Fig. 1). Nitrogen content in vegetative organs decreased from anthesis to maturity, suggesting translocation into grains. Nitrogen content decreased continuously just after anthesis in Haruyutaka, although was kept quite high until cessation of photosynthesis in Norin 61 and Selpek. Nitrogen content decreased rapidly from cessation of photosynthesis until maturity in these two cultivars. At harvest, grain nitrogen yields were 11.3, 9.0 and 12.6 gm^{-2} in Haruyutaka, Norin 61 and Selpek, respectively. Nitrogen content in grains were 1.92, 2.03 and 2.14%, respectively.

Table 1 shows the contributions of the nitrogen absorbed from soil and translocated from vegetative organs to nitrogen accumulation in grain for two phases, the initial plus early plus late phases (I+E+L), from anthesis (An) to cessation of photosynthesis (CP), and the final phase (F), from cessation of photosynthesis to maturity (Ma). These contributions were calculated by following four equations (also see Fig. 1) :

1. The contribution of nitrogen absorbed from soil during I+E+L :

$$=\frac{\text{Increase in nitrogen of whole plant from An to CP}}{\text{Nitrogen content in grain at maturity}}$$

2. The contribution of nitrogen translocated from vegetative organs during I+E+L phase :

$$=\frac{\text{Decrease in nitrogen of vegetative organs from An to CP}}{\text{Nitrogen content in grain at maturity}}$$

3. The contribution of nitrogen absorbed from soil during F phase :

$$=\frac{\text{Increase in nitrogen of whole plant from CP to Ma}}{\text{Nitrogen content in grain at maturity}}$$

4. The contribution of nitrogen translocated from vegetative organs during F phase :

$$=\frac{\text{Decrease in nitrogen of vegetative organs from CP to Ma}}{\text{Nitrogen content in grain at maturity}}$$

During the initial plus early plus late phase (I+E+L), the nitrogen amount absorbed from soil was almost the same as the nitrogen translocated from vegetative organs in Haruyutaka, although the nitrogen absorbed from soil was more than that translocated from vegetative organs in Norin 61 and Selpek. During the final phase (Final), nitrogen translocated from vegetative organs showed a high value (30-40%) in all three varieties. So that, throughout grain filling period, Haruyutaka showed that 73% of grain nitrogen is translocated from vegetative organs due to much translocation during I+E+L phase. However, in Norin 61 and Selpek, less than 50% of grain nitrogen was found to be translocated from vegetative organs.

2. Effect of lack of irradiation on absorption and translocation of nitrogen

Fig. 2 shows the effects of shading treatment on nitrogen distribution between grains and vegetative organs. The amount of nitrogen in whole plant (above ground) in initial shading increased until three weeks after anthesis as in the same as in no shading. The whole nitrogen in early and late shadings was slightly lower than that in no shading after the shading periods. The nitrogen in grains in each shading was less than no shading at one week

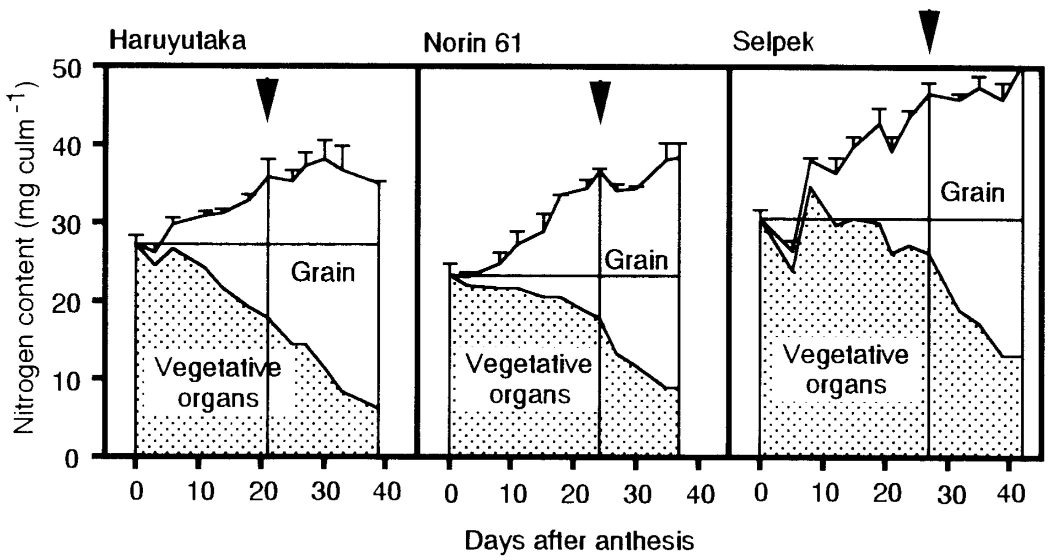

Fig. 1. Nitrogen distribution between grains and vegetative organs in three spring wheat varieties. Arrows indicate the cessation of photosynthesis.
The T-shape bars indicate standard errors.

before maturity, while grain weight for each shading was also less than no shading. At maturity, however, the nitrogen content in grains in each shading was almost the same as in no shading, while grain weight in each shading was still less than no shading. Thus, the nitrogen content in ripened grain were higher in all three shadings than no shading (2.42%, 2.46% and 2.40% in initial, early and late shadings, respectively, against 2.27% in no shading). On the other hand, the nitrogen in vegetative organs in each shading was almost the same as in no shading just after treatment (one, two and three weeks after anthesis for initial, early and late shadings, respectively). However, it was more than in no

Table 1. Contriutions of nitrogen absorbed from soil and translocated from vegetative organs to harvested nitrogen in grain in two grain filling phases, the initial plus early plus late phases (I+E+L), and the final phase (Final).

Grain filling phase	Contribution to grain (%)	
	Absorption from soil	Translocation from veg. organs
Haruyutake		
I+E+L	30	33
Final	−3	40
Total	27	73
Norin 61		
I+E+L	45	18
Final	6	31
Total	51	49
Selpek		
I+E+L	43	12
Final	10	35
Total	53	47

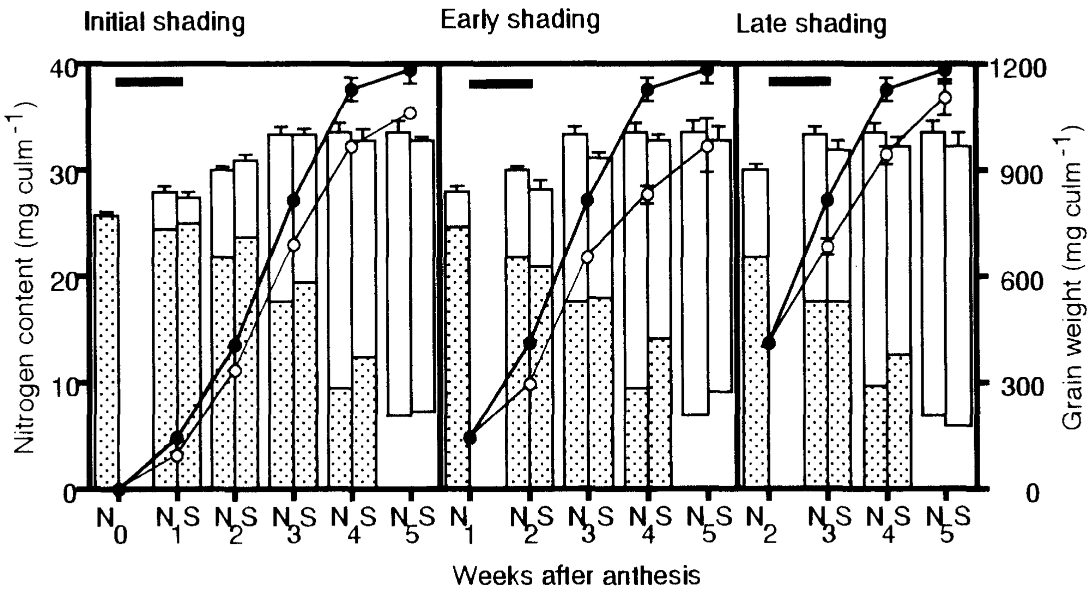

Fig. 2. Effects of shading treatments on nitrogen distribution between grains and vegetative organs
□ : nitrogen in grains ; ⁘ : nitrogen in vegetative organs.
●—● : grain dry weight in no shading ; ○—○ : grain dry weight in shading.
The capital letters, "N" means non shading, "S" means shading.
The horizontal bold bars indicate the period of shading treatment.
The vertical bars indicate standard error for grain weights and total nitrogen contents.
0 and 5 weeks after anthesis indicate anthesis and maturity, respectively.

Table 2. Effect (%)* of shading treatment on nitrogen content of each organ above ground in winter wheat at the date just after treatment, a week before maturity and maturity.

Shading period	Grain	Chaff	Culm	Flag leaf blade	Second leaf blade	Third leaf blade
At the date removed shading cloth						
Initial shading	68+5**	105±2	106±3	98±3	95±2	84±1
Early shading	88±2	104±3	97±5	95±4	88±3	72±4
Late shading	91±3	106±7	103±5	99±5	91±5	84±9
At a weel before maturity						
Initial shading	84+2	128±7	130±9	143±11	144±7	99±5
Early shading	78±1	149±8	154±10	148±11	131±5	106±5
Late shading	82±2	132±8	130±11	150±13	140±8	105±7
At maturity						
Initial shading	96±1	——	——	——	——	——
Early shading	88±5	——	——	——	——	——
Late shading	99±4	——	——	——	——	——

* the effect is shown as the percentages of nitrogen content (mg culm^{-1}) for each shading treatment against no shading.

** ±s.e.: standard error.

shading at four weeks after anthesis.

The nitrogen content in grains and each part of vegetative organs for initial, early and late shadings is evaluated on the basis of nitrogen contents in no shading at the date when shading cloth was removed and at a week before maturity (Table 2). Just after the shading period, nitrogen in grain showed a decrease of 9-32% by shading treatments. Nitrogen in chaff and culm increased slightly, while in leaf blade decreased. At a week before maturity, nitrogen in grains was still lower in shadings, but all vegetative organs excluding the third leaf blade maintained a high nitrogen content in shadings. These high nitrogen contents in vegetative organs seemed to be translocated into grains during only one week until maturity, bacause the nitrogen content in grains in all three shadings was almost the same as in no shading at maturity.

Discussion

Nitrogen absorption from soil was continued until the cessation of photosynthesis for all three varieties. Then the accumulation of nitrogen into grain was attributed to only translocation of nitrogen from vegetative organs during the final phase. Simpson et al.[7] found that all nitrogen imported into each organ in the transpiration stream was eventually translocated to the phloem for retranslocation from the organ. According to their theory, the absorbed nitrogen in whole plant might come through the xylem tube by transpiration at photosynthesis, and the accumulated nitrogen in grain might come through the phloem tube connected to each vegetative organ. Sarandōn and Gianibelli[6] suggested the greater nitrogen content in the grain was attributable to a rapid uptake of the nitrogen applied and not to a longer period of nitrogen accumulation. These findings lead one to hypothesize that for a high nitrogen content in harvested grain it is important to absorb much nitrogen through the xylem tube from soil with high transpiration until cessation of photosynthesis. The translocation of nitrogen into grain was continued until maturity, just as reported by Borghi et al.[1] and Sarandōn and Gianibelli[6]. Although the absorption of nitrogen through the xylem tube ceased with cessation of photosynthesis, the translocation of nitrogen through the phloem tube might continue until maturity with the translocation of carbohydrate.

The amount of nitrogen in vegetative organs decreased from anthesis to cessation of photosynthesis only for Haruyutaka. The nitrogen demand by grain might be higher for Haruyutaka than Norin 61 and Selpek, so the remobilization of nitrogen through phloem tube was greater than the absorption through the xylem tube for Haruyutaka. High protein yield is usually produced by cultivars with a high yield potential but a low grain protein concentration[3,15]. Moreover, a higher nitrogen remobilization was found in high grain protein genotypes, which was attributed to greater nitrogen demand by the grain ; this enhanced the senescence of tissue and resulted in a lower grain yield[5,8]. Haruyutaka can be thought of as a high grain protein genotype because of its higher nitrogen demand for earlier grain filling phase and its higher nitrogen harvest index (NHI) at maturity (82%), against 72 and 73% for Norin 61 and Selpek, respectively. However, it showed a higher grain yield (591 gm^{-2}) than Norin 61 and Selpek (445 and 586 gm^{-2}, respectively). During the earlier grain filling phase, the high nitrogen content in the upper organs, i.e., flag leaf or chaff, was maintained until the later phase for Haruyutaka. This makes Haruyutaka show a high grain yield and avoid earlier senesce.

All three shading treatments increased the percentages of nitrogen content in harvest grain. During the shading period, the amount of nitrogen in grain decreased when the grain dry weight decreased for all shadings. The absorption of nitrogen in whole plant was inhibited by restricted photosynthesis and transpiration. Until a week before maturity, the amount of nitrogen in grain for three shadings was less than for no shading in proportion to grain weight. At maturity, however, the amount of nitrogen in grain for each shading was the same as for no shading. This indicated that nitrogen in vegetative organs for shadings was translocated into grain rapidly during only one week in the last part of the final phase. Therefore, it is concluded that the nitrogen demand by the grain is determined by grain dry weight throughout the grain filling period so that a considerable amount of nitrogen remains in vegetative organs at one week before maturity. However, most of the remaining nitrogen is translocated into grain during several days at the end of grain filling.

Fifty to eighty percent of the nitrogen in the grain comes from storage in vegetative structures in preanthesis[4,9], while grain dry matter accumulated depends mainly on photosynthates produced after anthesis[10,11]. Our previous study showed that carbohydrate was accumulated in grain with high activity during the early and late grain filling phase when vegetative organs have high photosynthetic activity and sink capacity has already completed[12]. Contrary to this, the present study showed that nitrogen was translocated from vegetative organs and accumulated in grain considerably during the final phase when the vegetative organs had ceased their photosynthetic function. Therefore, the strategies to achieve both of high yield and high nitrogen percentages in grain were listed as follows : (1) Wheat plant must accumulate much nitrogen in vegetative organs by anthesis, (2) More nitrogen must be effectively distributed to more active photosynthetic organ, (3) More nitrogen must be absorbed from soil and be accumulated into each organ above ground during initial, early and late grain filling phases, (4) Most nitrogen in vegetative organs must be translocated into grain completely during final grain filling period.

Generally, early harvest is recommended in rainy region to avoid pre-sprouting. However, the nitrogen mobilization should be observed during several days just before maturity, so we should pay attention to the increase in nitrogen content in grain before we decide a harvesting date.

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