Grain Filling Mechanisms in Spring Wheat*

II. Growth, accumulation and translocation in the daytime and at night during the four grain filling phases

Tadashi TAKAHASHI, Naoyuki TSUCHIHASHI and Kimio NAKASEKO

(Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan)

Received January 20, 1993

Abstract : The dry weights and water soluble sugar contents (WSC) in grain, ear, culm and leaves of the field grown spring wheat (cv. Haruyutaka) were measured every morning and evening throughout the grain filling period to determine the physiological and morphological mechanisms of grain filling. Many of these plant organs increased or decreased linearly during each grain filling phase, divided on the last report : (1) initial grain filling phase, (2) early grain filling phase, (3) late grain filling phase and (4) final grain filling phase. They were, therefore, analyzed by linear regression against the days after anthesis to determine the difference in dry matter accumulation among these four phases and the difference between daytime and night. The regression slope, increasing rate, in total biomass declined with growth and was almost zero during the final phase when canopy did not photosynthesize. The slope in grain dry weight peaked during the late phase ($60 \text{ mg pl}^{-1} \text{ day}^{-1}$). The WSC in the culm increased during the initial and early phase (22 and $11 \text{ mg pl}^{-1} \text{ day}^{-1}$, respectively), while it decreased during the late and final phases (-15 and $-19 \text{ mg pl}^{-1} \text{ day}^{-1}$). In addition, the grain increased similarly between daytime and night during the initial, early and final phases, although its increasing rate in the daytime ($45 \text{ mg pl}^{-1} \text{ day}^{-1}$) was threefold greater than at night ($16 \text{ mg pl}^{-1} \text{ day}^{-1}$). During the early phase, the WSC in the culm increased during the daytime ($18 \text{ mg pl}^{-1} \text{ day}^{-1}$), but decreased during the night ($-8 \text{ mg pl}^{-1} \text{ day}^{-1}$). Therefore, this suggests that the WSC is accumulated in culm during the daytime and that it is translocated into the grains at night.

Key words : Daytime and night time, Grain filling phase, Quantitative assessment of growth, Regression analysis, Spring wheat.

春播コムギの登熟機構の解明 第2報 登熟相別にみた昼夜の器官形成と同化産物分配: 高橋 肇・土橋直之・中世古公男 (北海道大学農学部)

要 旨 : 春播コムギの登熟機構を生理的・形態的に解明することを目的として、登熟期間におけるコムギの子実、穂、稈および葉身の乾物重および糖分含有量 (WSC) の推移を、圃場条件下で栽培した品種ハルユタカの群落において、連日の早朝と夕方に調査した。前報で分類した4つの登熟相; (1) 登熟初期, (2) 登熟前期, (3) 登熟後期および(4) 登熟末期において、調査した器官乾物重の多くが直線的に増加あるいは減少したことから、その速度を開花後日数に対する乾物重の直線回帰式の係数で表し、登熟相の違い、さらに日中と夜間との違いについて検討した。その結果、全乾物重の増加速度は登熟相の進行にともない低下し、光合成の停止する登熟末期ではほぼ0を示した。子実重の増加速度は登熟後期で最も高い値を示し ($60 \text{ mg pl}^{-1} \text{ day}^{-1}$)、稈の可溶性糖分は登熟初期 ($22 \text{ mg pl}^{-1} \text{ day}^{-1}$) および前期 ($11 \text{ mg pl}^{-1} \text{ day}^{-1}$) で増加し、後期 ($-15 \text{ mg pl}^{-1} \text{ day}^{-1}$) および末期 ($-19 \text{ mg pl}^{-1} \text{ day}^{-1}$) で減少することが数値により示された。さらに、子実重は登熟初期、前期および末期では、日中と夜間ともに同じ速度で増加するのに対して、登熟後期では日中の増加速度が夜間の3倍になることが明かとなった (日中: $45 \text{ mg pl}^{-1} \text{ day}^{-1}$, 夜間: $16 \text{ mg pl}^{-1} \text{ day}^{-1}$)。一方、稈のWSCは登熟前期において日中に増加して稈に蓄積するものの、夜間は減少して子実へと転流することが明かとなった (日中: $18 \text{ mg pl}^{-1} \text{ day}^{-1}$, 夜間: $-8 \text{ mg pl}^{-1} \text{ day}^{-1}$)。

キーワード : 回帰分析, 生長の量的評価, 登熟相, 日中と夜間, 春播コムギ。

As a first step in studying the physiological and morphological mechanisms of grain filling in spring wheat, we divided the grain filling period into four phases based on photosynthetic activity, organ morphogenesis, and dry matter partitioning in the canopy⁹⁾. The four phases are : (1) **Initial grain filling phase**

from anthesis to cessation of culm elongation where assimilate is used primarily for culm elongation ; (2) **Early grain filling phase** from cessation of culm elongation to the milk ripe stage where assimilate is used for both grain growth and stored in the culm ; (3) **Late grain filling phase** from the milk ripe stage to cessation of photosynthesis where assimilate is used entirely for grain growth and

* This work was presented at the 194th (October, 1992) meeting of the Crop Science Society of Japan.

stored material in the culm is also translocated to the grains; and, (4) **Final grain filling phase** from cessation of photosynthesis to maturity where grain growth is supported solely by translocation of reserve material in the culm.

Our objective in this paper was to quantitatively assess growth, accumulation, and translocation for each organ in a spring wheat canopy. We measured the dry weight and WSC in grain, ear, culm and leaves to evaluate not only the seasonal changes but also the differences between daytime and night.

Materials and Methods

The spring wheat variety, Haruyutaka, was planted on 24 April 1991 at the Experimental Farm at Hokkaido University. Seeds were set on seeder tapes (Nihon Plant Seeder) 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 equidistant square pattern with 400 plants m^{-2} . The plot size was 2 m by 24 m with three replications, consisting of 50 cm by 50 cm samples. Fertilizer was applied prior to sowing: N, 90 kg hectare^{-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, armyworms and aphids.

Twenty standard plants were removed from each sampling area at 5 am and 6 pm every-day from anthesis to maturity. Tillers and roots were discarded and the main culms were separated into ears, culms including the leaf sheath, and leaf blades. These plant parts were initially heated for 30 minutes at 105°C, then dried for 48 hours at 70°C, and finally desiccated and weighed. All of the grains were removed from the ears with tweezers and they were weighed. The ears (without grains) and the culms were milled and their water soluble sugar content (WSC) was determined using the anthrone method¹¹⁾.

Results

1. Increasing rates in plant organs during grain filling phases

When the changes in weights of total biomass, grain, structural material and WSC in culm and ear, and leaves were followed during every grain filling phases, divided on

the last report⁹⁾, many of them increased or decreased linearly. So these weights (Y) were fitted to linear regressions ($Y = aX + b$) against the days after anthesis (X), and 19 of 28 regressions were significant (Table 1). The regression of total biomass weight during final phase was not significant when canopy photosynthesis ceased. Those of structural material weight in culm during early, late and final phases were not significant, either, when culm elongation ceased. A coefficient (a) of the linear regression, which means the slope, is equal to the increasing rate in each part weight. LSDs (>0.01) for the regression coefficients (a), converted by the equation $(Y - b)/X$, were calculated to find the differences among grain filling phases.

Total dry weight showed the largest regression coefficient (96 $\text{mg pl}^{-1} \text{day}^{-1}$) during the initial grain filling phase, which was double as much as during the early and late phases (50 and 44 $\text{mg pl}^{-1} \text{day}^{-1}$, respectively). The slope was almost zero during the final phase when canopy did not photosynthesize. Grain dry weight showed the small regression coefficient (18 $\text{mg pl}^{-1} \text{day}^{-1}$) during the initial phase; this indicates slow grain growth just after pollination. The slope increased during later phases; it peaked during the late phase (60 $\text{mg pl}^{-1} \text{day}^{-1}$) and decreased during the final phase (35 $\text{mg pl}^{-1} \text{day}^{-1}$). The WSC in culm showed positive regression coefficients during initial and early phases (22 and 11 $\text{mg pl}^{-1} \text{day}^{-1}$), while they were negative during the late and final phases (-15 and -19 $\text{mg pl}^{-1} \text{day}^{-1}$). This demonstrates that material is accumulated and stored in the culm until the milk ripe stage, then it is translocated to other plant parts. Structural material in the culm showed significant regression only during the initial phase (41 $\text{mg pl}^{-1} \text{day}^{-1}$), because the culm elongation ceases from the early phase. As these way, the regression coefficients could, therefore, assess the growth of plant organs quantitatively.

In addition, the WSC in ear showed positive regression coefficients during the initial and early phases (1.2 and 1.8 $\text{mg pl}^{-1} \text{day}^{-1}$), while they were negative during the late and final phases (-1.1 and -1.2 $\text{mg pl}^{-1} \text{day}^{-1}$), similarly to WSC in the culm. Leaves dry weight showed negative regression coefficients during the late and final phases.

Table 1. Regression equations in dry weights of total, grain, WSC and structural material in culm and ear, and leaves during four grain filling phases.

Grain filling phase	Regression equation	Correlation coef.	Measurement points	F value	Significance	LSD (0.01)
Total dry weight						
Initial	Y = 96.2X + 1521	r = 0.98	7	106.5	**	7.42
Early	Y = 50.5X + 1794	r = 0.88	9	23.3	**	
Late	Y = 44.2X + 1878	r = 0.94	13	78.5	**	
Final	Y = 0.8X + 3044	r = 0.03	8	0.0	NS	
Grain						
Initial	Y = 18.2X - 3	r = 0.86	7	37.5	**	2.92
Early	Y = 39.8X - 139	r = 0.97	9	115.9	**	
Late	Y = 60.7X - 414	r = 0.99	13	766.0	**	
Final	Y = 34.9X + 310	r = 0.93	8	39.0	**	
WSC in culm						
Initial	Y = 21.5X + 168	r = 0.77	7	7.2	*	5.11
Early	Y = 10.7X + 251	r = 0.65	9	5.2	NS	
Late	Y = -14.6X + 625	r = 0.87	13	34.6	**	
Final	Y = -18.8X + 685	r = 0.87	8	17.8	**	
Struct. in culm						
Initial	Y = 41.0X + 819	r = 0.99	7	282.6	**	4.33
Early	Y = -2.3X + 1076	r = 0.38	9	1.2	NS	
Late	Y = 0.8X + 993	r = 0.10	13	0.1	NS	
Final	Y = -5.8X + 1193	r = 0.70	8	5.9	NS	
WSC in ear						
Initial	Y = 1.2X + 17	r = 0.83	7	10.9	*	0.40
Early	Y = 1.8X + 13	r = 0.81	9	13.0	**	
Late	Y = -1.1X + 46	r = 0.74	13	13.0	**	
Final	Y = -2.2X + 78	r = 0.93	8	39.6	**	
Struct. in ear						
Initial	Y = 9.8X + 270	r = 0.98	7	133.9	**	1.33
Early	Y = 1.4X + 327	r = 0.56	9	3.2	NS	
Late	Y = 3.4X + 301	r = 0.80	13	19.7	**	
Final	Y = -2.3X + 453	r = 0.57	8	2.8	NS	
Leaves						
Initial	Y = 4.5X + 249	r = 0.62	7	3.1	NS	2.85
Early	Y = -0.9X + 266	r = 0.23	9	0.4	NS	
Late	Y = -4.9X + 327	r = 0.89	13	41.6	**	
Final	Y = -4.9X + 325	r = 0.78	8	9.3	*	

LSD show 1% level of significant difference among the regression coefficients.

2. Differences in dry matter accumulation between daytimes and nights

Figure 1 shows the grain dry weight for morning and evening everyday during four

phases. The values for evening were almost higher than for morning throughout four phases. In particular, the tendency was remarkable during the late phase. This sug-

gests that the grain grew actively for daytime. Figure 2 shows the WSC in the culm for morning and evening everyday. The values for evening were higher than for morning during the initial and early phases, thus the WSC seems to be accumulated mainly for daytime. Moreover, much of the WSC seems to be translocated for night during the late and final phases, because the values for evening were similar to those for morning. Contrary to this, the WSC in the ear for evening were always higher than for morning during all four phases⁸⁾ (Figure 3). This emphasize previous findings that the ear functions not only as a storage organ, but also as an assimilation organ like leaf^{1,2,4,5,10)}. The assimilate photosynthesized by the ear is translocated into grains or other organs during the night. Therefore, the values for morning and evening converged by senescing during final phase.

The dry matter accumulation rates in WSC and structural material of total biomass, grains, culm, ear and leaves were calculated for daytimes and nights during four grain filling phases (Table 2). Regressions of these dry weights against the days after anthesis were used for the calculation. The grain increased similarly between daytime ($8.6 \text{ mg pl}^{-1} \text{ day}^{-1}$) and night ($9.6 \text{ mg pl}^{-1} \text{ day}^{-1}$) during the initial phase, when the endosperm cells are propagating. The WSC in the culm also increased similarly between daytime ($11 \text{ mg pl}^{-1} \text{ day}^{-1}$) and night ($11 \text{ mg pl}^{-1} \text{ day}^{-1}$), while the structural material increased highly, especially for daytime ($25 \text{ mg pl}^{-1} \text{ day}^{-1}$). On the other hand, the WSC in the ear and the dry weight in the leaves increased for daytime and decreased for night at the about same quantities. This indicates that the assimilate is accumulated there for daytime and it is translocated into the grains for night, as described before. Ultimately, total biomass increased mainly for daytime ($70 \text{ mg pl}^{-1} \text{ day}^{-1}$).

Then, the grain increased similarly between daytime ($18 \text{ mg pl}^{-1} \text{ day}^{-1}$) and night ($22 \text{ mg pl}^{-1} \text{ day}^{-1}$) during the early phase, too. The WSC in the culm increased for daytime ($18 \text{ mg pl}^{-1} \text{ day}^{-1}$), but it decreased for night ($-8 \text{ mg pl}^{-1} \text{ day}^{-1}$). This suggests that the WSC is accumulated in the culm as surplus assimilate for daytime and it is translocated in the grains as supplementary one for night. The WSC in the ear and the dry weight in the

Fig. 1. Relationship between days after anthesis and grain dry weight for four grain filling phases in spring wheat variety Haruyutaka. Opened circles and solid lines show the morning data, and closed circles and broken lines show the evening data.

Fig. 2. Relationship between days after anthesis and water soluble sugar content (WSC) in culm for four grain filling phases in spring wheat variety Haruyutaka. Opened circles and solid lines show the morning data, and closed circles and broken lines show the evening data.

leaves increased for daytime and decreased for night at same quantities, so that most of the daily increase in total biomass ($51 \text{ mg pl}^{-1} \text{ day}^{-1}$) produced for daytime ($50 \text{ mg pl}^{-1} \text{ day}^{-1}$).

During the late phase, the grain increased daily $61 \text{ mg pl}^{-1} \text{ day}^{-1}$ which was higher than

total biomass ($44 \text{ mg pl}^{-1} \text{ day}^{-1}$), while the WSC in the culm decreased ($-15 \text{ mg pl}^{-1} \text{ day}^{-1}$). The increasing rate in the grain for daytime ($45 \text{ mg pl}^{-1} \text{ day}^{-1}$) was three times as much as for night ($16 \text{ mg pl}^{-1} \text{ day}^{-1}$), while the WSC in the culm decreased even for daytime ($-3.8 \text{ mg pl}^{-1} \text{ day}^{-1}$). We hypothesize that grain growth required the WSC in culm in addition to photosynthate even for daytime because the endosperm cells have already propagated and the sink capacity became very large after the milk ripe stage^{3,6,7}). Total biomass increased mainly for daytime ($35 \text{ mg pl}^{-1} \text{ day}^{-1}$), similarly to those during the initial and early phases.

The grain increased $16 \text{ mg pl}^{-1} \text{ day}^{-1}$ for daytime and $19 \text{ mg pl}^{-1} \text{ day}^{-1}$ for night during the final phase, although total biomass did not daily increase ($0.8 \text{ mg pl}^{-1} \text{ day}^{-1}$). Because stored material was translocated into the grains. About one half of the translocated material came from the WSC in the culm ($-19 \text{ mg pl}^{-1} \text{ day}^{-1}$) and the other half came from other organs. We are unsure of the meaning of the negative values during daytime and the positive values during night for

the structural material in the culm from the early to final phases.

Fig. 3. Relationship between days after anthesis and water soluble sugar content (WSC) in ear for four grain filling phases in spring wheat variety Haruyutaka. Opened circles and solid lines show the morning data, and closed circles and broken lines show the evening data.

Table 2. Dry matter accumulation rate ($\text{mg pl}^{-1} \text{ day}^{-1}$) in water soluble sugar content (WSC) and structural material of grains, culm, ear and leaves for four grain filling phases.

	Total	Grains	Culm		Ear		Leaves
			WSC	Struct.	WSC	Struct.	
Initial grain filling phase (anthesis to cessation of culm elongation)							
Day	96.2	18.2	21.5	41.0	1.2	9.8	4.5
Light hours	70.4	8.6	10.5	24.6	12.0	4.9	9.9
Night	25.9	9.6	11.0	16.4	−10.7	5.0	− 5.4
Early grain filling phase (cessation of culm elongation to milk ripe stage)							
Day	50.5	39.8	10.7	− 2.3	1.8	1.4	− 0.9
Light hours	50.3	17.5	18.3	−12.7	10.1	1.8	15.3
Night	0.2	22.3	− 7.6	10.4	− 8.3	−0.4	−16.2
Late grain filling phase (milk ripe stage to cessation of photosynthesis)							
Day	44.2	60.7	−14.6	0.8	− 1.1	3.4	− 4.9
Light hours	35.2	44.8	− 3.8	−21.7	8.3	5.8	1.8
Night	9.0	15.9	−10.8	22.5	− 9.4	−2.4	− 6.7
Final grain filling phase (cessation of photosynthesis to maturity)							
Day	0.8	34.9	−18.8	− 5.8	− 2.2	−2.3	− 4.9
Light hours	−12.4	16.4	− 6.4	−16.1	2.4	−2.8	− 6.0
Night	13.2	18.5	−12.5	10.3	− 4.6	0.5	1.1

Discussion

In this experiment, it was found that many of the weights in plant organs increased or decreased linearly, so these changes were expressed using linear regressions against days after anthesis. These regressions might help to explain the influence of environment and genotype on the grain filling mechanisms in wheat, later on.

Use of ^{14}C will also help us study such assimilation, translocation and partitioning more precisely. However, it is too difficult to use continuously in the field because environmental factors like radiation fluctuate everyday. Although we did not obtain information about specific assimilate, we could know how it is translocated and where it is stored in the entire canopy. In addition the differences in dry matter accumulation between daytime and night could be assessed by measurement at morning and evening everyday. It is necessary to find the relation between this assessment and morphogenesis, further. In future, this mechanisms should be assumed to apply to other situations; environment, genotype and so on. We can use this procedure to assess the effect of such a different on growth, accumulation and translocation in wheat.

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* In Japanese with English summary.