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Grain Filling Mechanisms in Spring Wheat*

I. Grain filling phases according to the development of plant organs

Tadashi TAKAHASHI, Naoyuki TSUCHIHASHI and Kimio NAKASEKO
(*Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan*)

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Abstract : To define the growth phases for analyzing the physiological and morphological mechanisms of grain filling in a spring wheat canopy, the dry weight of the grains and the water soluble sugar content (WSC) and structural weight of the culm were measured for the variety Haruyutaka everyday during the grain-filling period. The grain-filling period was divided into four phases ; (1) Initial grain filling (from anthesis to cessation of culm elongation) where assimilate is used mainly for culm elongation and the dry weight of the grains increase slightly ; (2) Early grain filling (from cessation of culm elongation to the milk ripe stage) where assimilate is used for both grain growth and culm reserve. (3) Late grain filling (from the milk ripe stage to termination of photosynthesis) where assimilate is used entirely for grain growth and the culm reserve is also translocated to the grain ; and, (4) Final grain filling (from the end of photosynthesis to maturity) where grain growth is supported only by translocation of the culm reserve because assimiltate is no longer being actively produced.

In the early and late grain-filling phases, the increase rate of the WSC in the culm was related linearly to radiation ($r=0.616^{***}$), while grain weight increased linearly with time ($r=0.992^{***}$), independent of radiation.

Key words : Assimilate, Culm elongation, Culm storage, Grain filling phase, Grain growth, Photosynthesis, Spring wheat, Water soluble sugar content.

春播コムギの登熟機構の解明　第1報 器官生長からみた登熟相の分類：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•形態的に解析することを目的として，解析の基礎となる登熟相の分類を試みた．本試験では，品種ハルユタカの群落について連日，子実および稈の乾物重と糖含有率を測定して，これらの動向から登熟期間を以下に示す4つの登熟相に分類した．(1) 登熟初期（開花期から稈伸長停止期まで）：同化産物は主として稈の伸長に利用され，子実重はゆるやかに増加する．(2) 登熟前期（稈伸長停止期から乳熟期まで）：稈の伸長が停止し，同化産物は子実成長と稈での一時貯蔵とに利用される．(3) 登熟後期（乳熟期から光合成停止期まで）：同化産物はすべて子実生長に利用され，稈の一時貯蔵物質も補足的に子実へと転流する．登熟末期（光合成停止期から成熟期まで）：新たな同化産物が生産されず，子実は稈からの転流物質によってのみ生長する．

なお，登熟前期および登熟後期では，稈の貯蔵養分の増加速度と日射量との間に正の相関関係が認められ，子実重は日射量の変動にかかわらず開花後日数の経過にともない直線的に増加した．

キーワード：可溶性糖分，稈の伸長，稈の貯蔵，光合成，子実生長，登熟相，同化産物，春播コムギ．

Endosperm cells in the wheat grain increase in number after pollination. A sink then is formed in those cells and starch accumulates there ; later, the endosperm cells lose their water centent and mature. Elsewhere, wheat culms accumulate reserve sugar then translocate it into the developing grains after the milk ripe stage. Based on these developmental parameters, we can divide the grain-filling period into two phases, one before and one after the milk ripe stage13).

To evaluate earlier phase, we measured the dry matter partitioning to grains, which ranged from 0% at anthesis to 100% at the milk ripe stage, by using a linear regression equation against days after emergence14). However, we found that the regression lines did not fit the observations in some cases, because solar radiation did not influence the grain growth rate, although it related closely to the increased accumulation of reserve in culm15). Furthermore, these grain growth and culm accumulation patterns are supported by the assimilation ability of the crop canopy.

We must investigate the physiological and the morphological mechanisms of grain-filling from canopy level throughout cell level in detail to estimate the grain growth and yield precisely. Our purpose in these studies is to assess the influences of environment and

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genotype on grain-filling mechanisms for each grain-filling phase properly defined. For the first step of the studies, we divide the grain growth period from anthesis to maturity by analyzing assimilation activity, organ morphogenesis and dry matter partitioning in the plant canopy.

Materials and Methods

The spring wheat variety Haruyutaka was planted on April 24, 1991 at the Experimental Farm of Hokkaido University. Seeds were set on seeder tapes (Nihon Plant Seeder), every 5cm and the tapes were planted at a depth of 2cm. Two tapes were placed in each row and thinned to a single plant when two to three leaves expanded. The final density was an equi-distant square pattern with 400 plants m^{-2}. The plot size was 2m × 24m with three replications that consisted of 50cm × 50cm sampling areas. Fertilizer was applied just before sowing : N, 90kg $hectare^{-1}$; P_2O_5, 150kg ; and K_2O, 75kg. Fungicides and insecticides were applied a few times during the growing season to prevent powdery mildew, rust, flies, army worms and aphids.

Twenty standard plants were removed from each sampling area every day at 5 : 00am from two days before anthesis until 37 days after anthesis (at maturity). Tillers and roots were taken off ; main culms were further separated into ears, culms including the leaf sheath, and leaf blades. These plant parts were heated for 30 minutes at 105°C and dried for 48 hours at 70°C, then cooled in a desiccator and weighed. All of the grains were removed from the ears with tweezers and weighed. The culms were milled and their water soluble sugar content (WSC) was determined by the anthrone method[20].

CER was measured in the flag leaf, second leaf and third leaf of intact plants in the field on clear days. Each plant was isolated to allow high PAR irradiance (>1500 micromoles m^{-2} s^{-1}) and measured using a portable photosynthesis measurement system (Shimazu, ADC system SPB-H2).

Results

In general, changes in dry weight of the spring wheat canopy are due mainly to grain growth, nutrient accumulation in culm and culm elongation during grain filling period. Figure 1 shows the change in dry weight of these three characters : grain, WSC and structural material in the culm. The culm structural meterial increased linearly until six days after anthesis, then did not change significantly. The WSC in the culm did not increase during the first six days following anthesis, but after the culm ceased elongation, it swang up and down attaining a maximum value 14 days after anthesis (milk ripe stage). It then decreased to almost zero at 37 days after anthesis (maturity). Grain increased slowly for the first six days after anthesis, then increased rapidly with the increase in total biomass. Twenty-seven days after anthesis, the grain growth slowed, then ceased at 34 days after anthesis.

Figure 2 shows the changes in photosynthetic rate in the flag leaf, second leaf and third leaf. All three leaves showed continued high photosynthetic rates for the first 17 days following anthesis, then the rates declined rapidly to zero by 26 days after anthesis.

We divided the period from anthesis to maturity into three periods : (1) from 2 days before anthesis to 6 days after anthesis when culm elongates, (2) from 7 days to 26 days after anthesis, and (3) from 27 days to maturity when photosynthetic activity in canopy ceases. Figure 3 shows the relationship between solar radiation and rate of accumulation of WSC in the culm for each of these three periods. There is a positive linear relationship for only the second from 7 days to 26 days after anthesis ($r=0.616^{**}$) ; there is not a linear relationship for the other two periods. Using regression analysis, we estimated that the culm accumulated WSC at a higher radiation than 16.6 $MJm^{-2}day^{-1}$ and translocated it into the grain at a lower radiation than that for the second period.

Figure 4 shows the relationship between grain dry weight and days after anthesis for the second period. There is a signifcant linear relationship with a high correlation coefficient (>0.99). Grain weight increases linearly with days after anthesis and it is not influenced by fluctuations in radiation.

Discussion

We previously demonstrated that there is a positive linear relationship between daily radiation and the increased rate of accumulation reserve in the culm, while there is no significant relationship between daily radiation and grain growth[15]. In this paper, we investigated culm storage, grain growth and their relationship in detail. We found that the WSC in the culm increased and decreased with the fluctuation in radiation from 6 to 26 days after anthesis (Figure 3), while the grain growth rate remained constant regardless of radiation (Figure 4). We conclude that grain growth is dominant among growing organs and reserve material in the culm compliments this grain growth as an assimilate buffer during grain filling. The grain dry weight increased slowly for the first 6 days after anthesis; then it increased rapidly until the 26th day, when photosynthetic activity ceased, until it slowed again (Figure 1). These changes in grain dry weight with growth can be attributed to morphogenesis in the grains. Nuclei in the endosperm cells divide[4] and the number of endosperm cells increases during the first half of the growth period, while the volume of the endosperm cells increases during the second half of the growth period[5,10,11].

The change in reservation patterns in the culm can also be described by morphogenesis of the grain. In the first half of the grain filling period, the reserve material is stored in the

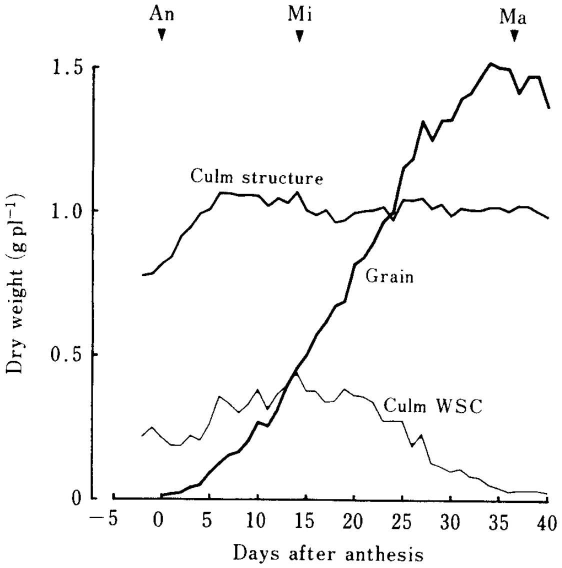

Fig. 1. Changes in the dry weight of grain (bold line), water solube sugar content (WSC) in culm (narrow line) and culm structural material (standard line) in Haruyutaka.
Arrows show main growth stages;
An: anthesis, Mi: milk ripe stage;
Ma: maturity.

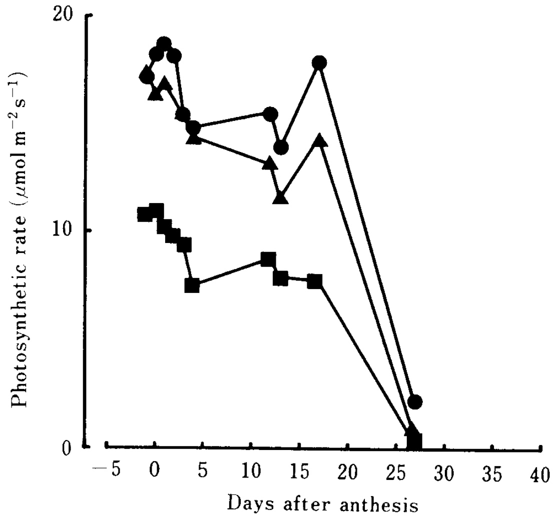

Fig. 2. Changes is photosynthetic rates measured under intensive PAR conditions (more than 1500 μmol m^{-2}s^{-1}) in the flag leaf (circle), second leaf (triangle) and third leaf (square) during the grain filling period in Haruyutaka.

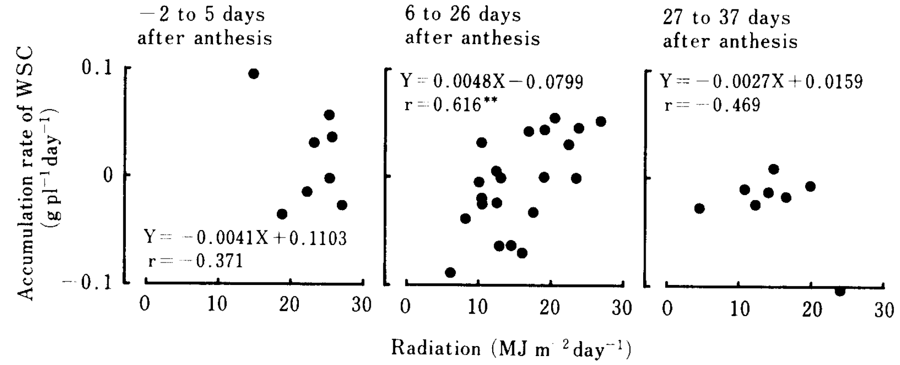

Fig. 3. Relationship between solar radiation and accumulation rate of water soluble sugar content (WSC) in the culm for three periods during grain filling.** : 1% of significance.

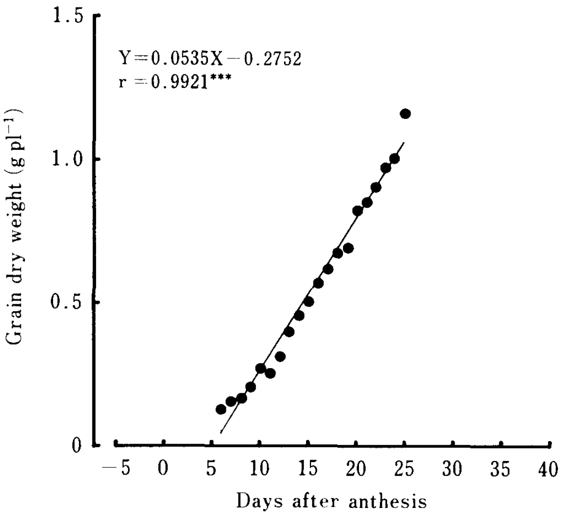

Fig. 4. Relationship of days after anthesis to grain dry weight from 6 to 26 days after anthesis in Haruyutaka. *** : 0.1% of significance.

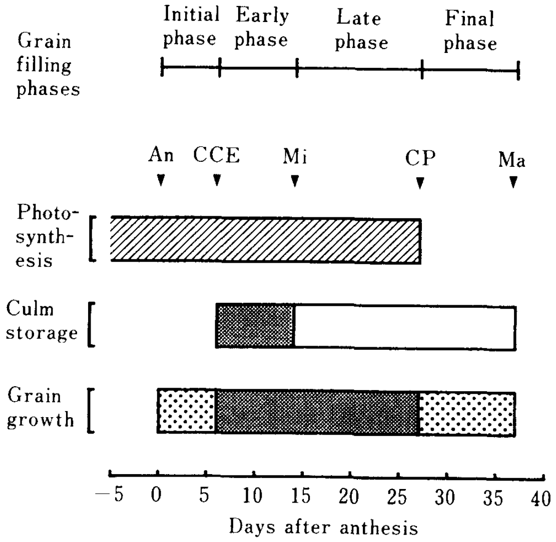

Fig. 5. Four growth phases difined with patterns in grain growth and culm storage rates and photosynthetic activity.
Arrows indicate critical stages for defining these phases ; An : anthesis, CCE : cessation of culm elongation, Mi : milk ripe stage, CP : cessation of photosynthesis, Ma : maturity.
▨ : activity, ▩ : increase,
⁙ : slight increase, □ : decrease.

culm as surplus assimilate because the endosperm cells are dividing and there is not enough sink capacity to accumulate starch directly in the endosperm. In the second half of the grain filling period, sugar is translocated from the culm to the grains to supplement grain growth because the endosperm cells have completed division and are now enlarging thereby forming a sink capacity large enough to accumulate assimilate. This theory is supported by a pervious experiment which shows that the effect of defoliation on grain growth appeared 4-10 days after treatment[7].

Now we can divide the grain filling period into four phases (Figure 5) ;

(1) **Ininitial grain filling phase** from anthesis to cessation of culm elongation where assimilate is used primarily for culm elongation and grain dry weight increases little ;

(2) **Early grain filling phase** from cessation of culm elongation to the milk ripe stage where assimilate is used for 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 reserve material in the culm is also translocated to the grain ; 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.

The grain growth rate is not always constant over different varieties or environments. Many researchers report that the divisions in endosperm nuclei and endosperm cells are accelerated by high temperatures during the grain filling period[4] and grain growth is also accelerated by high temperature[12,16,18]. Tashiro and Wardlaw[17] have found that high temperatures induced sterility and other grain damage. Briarty et al.[1] showed that there are differnces in the starch granule growth rate in different cell layers of the endosperm. Jenner and Rathjen[6] concluded that the flow of sucrose is limited by the capacity of the process of transporting sugar during the final stages of its passage into the grain.

Several anatomical studies document the vascular transportaion passages ; Wingwiri et al.[19] observed the vascular system in the rachis. Hanif and Langer[3] studied the spikelet, and Lingle and Chevalier[8] studied the development of the vascular tissue of grain caryopses. Evans et al.[2] found that the bundle number and phloem area at the top of the main stem can vary over a considerable range in proportion to spikelet number and grain yield. In future we must investigate the relationship between grain growth and reserved assimilate and their interaction with the environment and the influence of the limitations of the transportation passages. Next, the second step, to assess these dynamics in the grain growth and its relational organs growth numerically, we will analyze the changes in dry matter by using the regression analysis.

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* In Japanese with English abstract.