

Grain Filling Mechanisms in Spring Wheat

IV. Effects of shadings on number and size of spikes, grains, endosperm cells and starch granules in wheat

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Abstract : Changes in the number and size of spikes, grains, endosperm cells and starch granules was examined in relation to the limited assimilates caused by shading treatments. Spring wheat canopy (*Triticum aestivum* L. cv. Haruyutaka) was covered with a 95% shading cloth so as to cause failure in the assimilation at the initial, early and late grain filling phases ; initial shading, from two days before anthesis until seven days after anthesis ; early shading, from seven days until 14 days after anthesis ; and late shading, from 14 days until 21 days after anthesis. The grain yield decreased (11% as compared to no shading) due to a decrease in the number of grains (17%), especially decreasing (62%) at the third floret position in the initial shading, while a decrease of one grain weight at all floret positions resulted in 20% and 16% decreases in the grain yield in early and late shadings, respectively. The reduction of grain weight was not due to a decrease in the number of endosperm cells, but to a decrease in the size of large starch granules. Differences in the effects of shading on changes in sink characters could be explained in terms of the physiological process of grain formation.

Key words : Endosperm cell, Floret position in spikelet, Grain filling phase, Grain yield and yield component, Shading, Sink formation, Spring wheat, Starch granule.

春播コムギの登熟機構の解明 第4報 遮光処理がコムギの穂数、子実粒数と粒重、胚乳細胞数およびデンプン粒数に及ぼす影響：高橋 肇*・金澤俊成**（北海道大学農学部・**岩手大学教育学部）

要 旨：穂数、子実粒数と粒重、胚乳細胞数およびデンプン粒数を測定することで、遮光処理による光合成産物の不足がコムギのシンク形成に及ぼす影響を調査した。遮光処理は、登熟期間中の3つの登熟相において春播コムギ品種ハルユタカの群落上面を95%遮光布で覆い、群落での光合成生産をほぼ停止させた。開花前2日目から開花後7日目までの登熟初期における処理を初期処理、開花後7日目から14日目までの登熟前期における処理を前期処理、さらに開花後14日目から21日目までの登熟後期における処理を後期処理とした。子実収量は、初期処理によって11%減少した。これは、粒数が17%減少したためであり、特に、第3小花位置での粒数が62%減少したためである。前期処理および後期処理ではそれぞれ20%および16%減少し、すべての小花位置における粒重がそれぞれ減少したことがその原因となった。粒重の減少は、胚乳細胞数の減少によるものではなく、胚乳内の大型デンプン粒が小さくなったことに起因していた。各登熟相における遮光の影響は、それぞれの子実形成の生理的事象によって説明することができた。

キーワード：子実収量と収量構成要素、遮光処理、小穂内の小花位置、シンク形成、デンプン粒、登熟相、胚乳細胞、春播コムギ。

The number of endosperm cells increases after pollination in wheat grains, and then cell volume increases through accumulation of starches and further other ingredients^{8,10}. The culm elongates, then stores reserve materials in itself during reproductive stages. The reserve materials translocate into developing grains. Furthermore, most of the reserve materials in vegetative organs are also translocated into the grains, when canopy photosynthesis is restricted as senescence.

In the previous paper¹³), heavy shading

treatments (95% shading) were conducted to the canopy that still was able to produce assimilate. Hence, the reserved material in culm was utilized for grain filling instead of assimilates newly produce. Moreover, the rate of grain growth was kept to about half that for no shading, although grains grew under lack of assimilates.

The decrease of the rate of grain growth directly results in a decrease of yield. However, the effect on sink components may differ among shading periods. Grabau et al.⁶) showed that the yield reductions due to early shading were related to depressed floret fertil-

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ity, while those due to mid shading resulted from decrease of kernel weight. These differences may influence the quality of wheat flour.

Our purpose in this study is to realize the effect of the reduction of grain growth rate due to lack of photosynthates on formation of wheat sink components. We investigate the number and size of spikes, grains, endosperm cells and starch granules for shading experiments as reported previously¹³⁾.

Materials and Methods

1. Crop

The spring wheat cultivar, Haruyutaka, was planted on April 11, 1992 at the Experimental Farm of 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. Fertilizer was applied prior to sowing in the rate of N : P₂O₅ : K₂O = 90 : 150 : 75 kg ha⁻¹. Fungicides and insecticides were periodically applied to control powdery mildew, rust, flies, army worms and aphids.

2. Shading treatment

Shading treatment was conducted using a 95% shading cloth for the initial, early and late grain filling phases, respectively¹²⁾ to inhibit source activity and to restrict assimilates. We assigned the shading period as follows; the initial shading from two days before anthesis to seven after anthesis, the early shading from seven to 14 days after anthesis, and the late shading from 14 to 21 days after anthesis. The shading cloth was positioned 30 cm above the canopy.

3. Sampling

Grains at the first floret position of the seventh spikelet from bottom were sampled using forceps. The sampling was conducted at 6 am. The grains were then stored in a fixative containing 75% ethanol and 25% acetic acid at 5°C.

4. Harvesting measurement

Plants per 0.5 m² were harvested in three replications at maturity. Number of spikes per m² and grains per spike, grain weight and yield were determined. Twenty standard spikes were selected, and the weight of individual grains were measured for three florets from

all the spikelets.

5. Number of endosperm cells

The cross sections of grains were made from those embedded in paraffin. Those were sampled after endosperm cell cease to divide. Number of endosperm cell and its layer were counted using microscope.

6. Number of starch granules

Three grains for maturity were homogenized and suspended in 50% glycerin solution. We counted the number of starch granules using hemocytometer, and three types of starch granules were identified according to definition by Bechtel et al.²⁾ The three types of starch granules were distinguishable by their diameter, i.e. A type is more than 16 μm, B type between 5 to 16 μm, and C type less than 5 μm.

Results

1. Effects of shadings on yield and yield components

Table 1 shows yield and yield components with four shading treatments. Grain yield decreased 11–20% for three shadings. Number of spikes did not change significantly between shadings. Grain number per spike decreased 17% in initial shading, while decreased only 8% in the early and late shading. On the contrary, grain weight decreased 16% and 10% in the early and late shading, respectively, although increase in the initial shading. Therefore, shadings decreased grain yield due to decrease in grain number per spike during the initial phase and further decrease in grain weight during the early and late phases.

2. Changes in weight and number of grain at three floret positions

Table 2 shows weight and number of grain at the first, second and third floret positions with no shading. Total grain weight per spike was similar in the first and second floret, but that in the third floret was only half that in the other two. Grain numbers per spike were between 12 and 13 in the first and second florets, against 8 and 9 in the third floret. The weight of one grain of the first and second floret was also similar, while that of the third floret was 80% of others.

Percentages of the total grain weight, grain number per spike and one grain weight of three shadings were calculated based on those for no shading at three floret positions (Table

Table 1. Yield and yield components for each shading treatment.

Shading period	Grain yield (gm ⁻²)	Spike number (m ⁻²)	Grain number per spike	Grain weight (mg)
No shading	594	669	27.5	32.2
Initial phase	529 (89%)	668 (100%)	22.9 (83%)	34.6 (107%)
Early phase	473 (80%)	686 (103%)	25.4 (92%)	27.2 (84%)
Late phase	500 (84%)	680 (102%)	25.3 (92%)	29.0 (90%)
LSD(0.05)	46	NS	NS	4.9

Percentages in parenthesis based on no shading.

Table 2. Weight and number of grains of three floret positions with no shading.

Floret position	Total grain weight (mg)	Grain number per spike*	One grain weight (mg)
First floret	455 ± 46**	12.7 ± 1.0	35.8 ± 3.0
Second floret	469 ± 56	12.5 ± 1.2	37.6 ± 3.8
Third floret	249 ± 52	8.5 ± 1.5	29.5 ± 3.2

* Each spike had fifteen spikelets.

** ± s.e.: standard error.

3). Total grain weights of initial shading were almost 100% in first and second florets, but was considerably low, 40%, in third floret. Total grain weights of early and late shadings were about 90% in all three florets, especially being 76% in the third floret of early shading. The grain number of initial shading was also considerably low at 38% in the third floret; but those of early and late shadings were almost 100% in the three florets. On the contrary, the weight of one grain of initial shading were more than 100% in the three florets, while those of early and late shadings were about 80% and 90%, respectively, in all three florets.

Thus, decrease in grain number in the third floret caused the decrease in yield in initial shading, but decrease in one grain weight of all florets caused the decrease in early and late shadings.

3. Number of cells and cell layers

Table 4 shows the number of cell and cell layers per cross section of a grain from four shading treatments. The number of cell in late shading was similar to that in no shading, but the number in initial and early shadings were fewer and about 70% of that in no shading. Furthermore, the number of cell layers in late

shading was also similar to that in no shading, while the numbers of layers in initial and early shadings were two or three fewer than that in no shading.

4. Distribution of starch granules

Table 5 shows the number of starch granules for three size classes in a grain. The number of large type A granules decreased 20%, 40% and 30% of those of initial, early and late shadings, respectively. The number of middle type B granules increased as compared to type A for initial shading. The small type C granule distribution did not change significantly in initial and late shading, but increased in early shading.

Thus, decrease in size of large starch granules caused the decrease in one grain weight in early and late shadings. In contrast, in initial shading, increase in the number of medium-sized granules compensated for the decrease in the number of large granules, so that the one grain weight did not decrease.

Discussion

The previous paper¹³⁾ indicated that the three shadings inhibited grain growth through restricted supply of assimilates during the shading period, then after removing the shad-

Table 3. Effects (%) of three shading treatments on weight and number of grains for three floret positions.

Shading period	Total grain weight	Grain number per spike	One grain weight
First floret			
Initial phase	110±11*	103±7	107±9
Early phase	85±14	101±8	84±10
Late phase	92±11	102±8	90±9
Second floret			
Initial phase	98±12	90±14	108±10
Early phase	83±14	99±9	83±12
Late phase	93±11	102±7	91±10
Third floret			
Initial phase	40±26	38±24	107±15
Early phase	76±26	98±22	77±19
Late phase	96±17	106±12	90±12

* ± s.e.: standard error.

Table 4. Number of cells and cell layers per cross section in a spring wheat grain.

Shading treatment	Number of cells per cross section	Number of cell layers
Control	930	13.9
Initial	645	11.9
Early	670	10.5
Late	860	13.7
LSD (0.05)	138	2.5

Table 5. Number ($\times 10^6$) of starch granules for three size classes in a grain.

Shading period	Type A 16 μm <	Type B 5–16 μm	Type C 0–5 μm
No shading	6.2	9.6	145
Initial phase	5.5	11.4	147
Early phase	3.8	9.0	196
Late phase	4.4	7.4	131
LSD (0.05)	1.3	NS	36

NS: non significant.

ing cloth, the growth rate recovered to the same level as in no shading. Therefore, the shading treatment mainly appeared to affect morphogenesis such as the number of cells and cell layers in endosperm and starch granule size during only the shading period.

Studies on shading or leaf removing have indicated that the source limitation during the earlier stage of grain filling resulted in a decrease in grain number and during the later stage resulted in a decrease in grain weight^{1,3,5,6,9}. In the present experiment, the initial shading decreased the grain number per spike and the early and late shadings decreased the grain weight just as in these previous reports. In addition, decrease in grain number was attributed to the decrease in the number of third floret, and decreases in grain weight were due to the decreases in the weight of all floret positions. Stockman et al.¹¹⁾ demonstrated that shading treatments, in which applied to plants for an 8-day period ending 6 days before anthesis, had a significant effect on the number of kernels per spike.

Thus, the amount of available assimilates in the plant at anthesis (i.e., the earliest period of grain filling) must be important to determine the number of fertile grain.

Furthermore, differences in connection of vascular system among florets are also thought to be one of the important factors relating to inner morphogenesis of a grain. Hanif and Langer⁷⁾ observed the lower three florets were supplied by the principal vascular bundles of the rachilla. However, our results suggested that the first and second florets have a priority for assimilates supplied over the third floret. Further, Wingwiri et al.¹⁵⁾ found a difference in connection of central bundles among spikelets. However we did not find any significant differences in shading effects on grain filling among spikelets.

The numbers of cells and cell layers decreased in the initial and early shadings, because endosperm cells divide and their number increase during these initial and early phases^{8,10}. Wardlaw¹⁴⁾ found that low light

intensity following anthesis resulted in a reduction in the number of cells per endosperm, supporting our results. However, decrease in the number of endosperm cells did not cause the decrease in grain weight, because the number of endosperm cells decreased without any decrease in grain weight with initial shading; moreover, grain weight decrease occurred without any decrease in the number of endosperm cells with late shading.

The number of larger starch granules, type A, decrease significantly in the early and late shadings. These decreases directly caused a decrease in the grain weights of these shadings. Duffus⁴⁾ mentioned that the small or B-type granules had a greater amylopectin content than the large or A-type granules throughout endosperm development. Thus, flour quality may be improved with an increase in small starch granules due to source limitation like this shading treatment. In particular, it is important that the large A-type granules decreased and the middle B-type granules increased in the initial shading, although the grain weight was almost the same as in no shading.

In the present experiment, we shaded the plant canopies intensively but temporarily (95% but only one week of shadings). Otherwise, the radiation intensity would frequently fluctuate owing to weather conditions in the field environment. We might expect the radiation lack or surplus to affect not only the yield but also the quality of wheat depending on the duration or degree.

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