Japan. Jour. Crop Sci. 59(2) : 327—333 (1990)

Changes in Cellular and Water Soluble Sugar Contents during Grain Filling Period in Three Parts of Main Culm of Spring Wheats*

Tadashi TAKAHASHI, Keiichi SENDA** and Kimio NAKASEKO
(*Faculty of Agriculture, Hokkaido University, Sapporo 060, Japan*)
Received July 19, 1989

Abstract: Changes in culm contents, cell wall constituents, net cellular matter and water soluble sugar content (WSC) were determined from anthesis to maturity in three parts of the main culm, peduncle, second and lower internodes, for three spring wheat varieties, tall Haruhikari, semi-dwarf Haruyutaka and tall late Selpek.

After anthesis cell wall constituent and net cellular matter increased in peduncle and the second internode, associated with the elongation, but a little in the lower internodes, of which elongation has almost ceased at anthesis (Fig. 2). Contrary to this, WSC as a storage material increased in each part until milk ripening, decreasing to almost zero at maturity, presumably due to translocation to the ear. WSC has been rapidly accumulated before anthesis in the lower internode, but started to store at about anthesis in peduncle and the second internode, and attained the maximum at about a week before milk ripening in the lower internodes, just at the milk ripe stage in the second internode and several days after milk ripening in peduncle.

For all varieties the percent WSC was higher in the second internode and the amount of WSC in the lower internodes was more than in peduncle and the second internode at milk ripe stage, suggesting that the second and lower internodes were main storage organs. In addition the percent and amount of WSC were larger for semi-dwarf Haruyutaka than for tall Haruhikari and Selpek.
Key words: Cell wall constituent, Lower internodes, Net cellular matter, Peduncle, Second internode, Spring wheat, Storage material, WSC.

春播コムギの登熟期間における主稈3部位の細胞内容物質および可溶性糖の消長：高橋　肇・千田圭一・中世古公男（北海道大学農学部）

要　旨：春播コムギ3品種（長稈ハルヒカリ，半矮性ハルユタカおよび長稈•晩生 Selpek）の主稈3部位（穂首節間，第2節間および下位節間）における構成物質（細胞壁構成物質，純細胞内容物質および可溶性糖）の推移を開花期から成熟期まで調査した．

開花後，細胞壁構成物質と純細胞内容物質は穂首節間と第2節間で伸長生長に伴い増加したものの，開花時に伸長の停止している下位節間ではほとんど増加しなかった（第2図）．これに対し，貯蔵物質と考えられている糖は，各節間とも乳熟期まで増加した後穂への転流とともに減少し，成熟期にはほぼ0の値を示した．糖は，下位節間では開花前にかなりの量が蓄積していたのに対し，穂首節間と第2節間では開花期に蓄積し始め，下位節間では乳熟期の1週間ほど前に，第2節間では乳熟期に，穂首節間では乳熟期の数日後に最大値に達した．

乳熟期の糖の含有率は全品種とも第2節間で高く，さらに含有量は下位節間で高いため，第2節間と下位節間が主要な貯蔵器官であると考えられた．一方，糖の含有率，含有量ともに半矮性のハルユタカで長稈のハルヒカリ，Selpek よりも高かった．
キーワード：下位節間，可溶性糖，細胞壁構成物質，純細胞内容物質，第2節間，貯蔵物質，春播コムギ，穂首節間．

Generally in wheat the reserve materials stored in the culm contribute to the grain growth during grain filling period. Lopatecki et al.[2] showed that oligosaccharide formed the dominant carbohydrate stored in culms prior to ripening of the grain. Thome and Kühbauch[15] reported the distinct increase of fructosan concentration during the period from anthesis to milk ripening. The reserve materials increase generally until milk ripening and then decrease, as shown with soluble carbohydrate by Spiertz and van de Haar[10], and with cellular contents by Takahashi et al.[13] Shimono[7] showed that the sugar content in second internode is the most of all at the two weeks after anthesis (milk ripening). On the other hand the content of reserve material

* This work was presented in the 186th (October, 1988) meeting of Crop Science Society of Japan.

** Present address : Hokkaido Prefectural Konsen Agricultural Experiment Station, Nakashibestu, Hokkaido 086-11, Japan.

was different in varieties, particularly, between tall and semi-dwarf types as reported by many researchers[1,10,12,16].

In this experiment the changes of cellular contents and water soluble sugar content (WSC) were examined after anthesis for the three internodes of the main culm with three varieties which are tall, semi-dwarf and tall late type.

Materials and Methods

1. Crops

Measurements were made in the field of Agricultural Experiment Farm of Hokkaido University in 1987. Seeds of three spring wheat varieties, tall Haruhikari, semi-dwarf Haruyutaka and tall late Selpek were sown on 10 May by using the seeder tapes (Nihon Plant Seeder Co.) buried a seed at 5 cm intervals. Two seeder tapes were set at 5 cm intervals in each plot of which size was 2 m × 7.5 m. The plots were arranged in a randomized design with three replications. When the two to three leaves expanded, the seedlings were thinned to a single plant, arrange the 5 cm equi-distant square pattern (400 pls./m^2). A combination of N : P_2O_5 : K_2O as a fertilizer was applied at the ratio of 90, 120 and 90 kg/ha, respectively, just before sowing.

2. Sampling

The plants were sampled 10 times from heading to maturity. Fifty uniform plants were selected from each sampling area (50 cm × 50 cm, 100 plants), and leaf blades, ear and tillers were removed. The main culms were then divided into the three parts of internode, the peduncle (from the neck node to the flag leaf node), the second internode (from the flag leaf node to the second leaf node) and the lower internodes (from the second leaf node to the basal node). Each of these three parts of the internode was dried at 80°C for 48h, and then weighed and milled for the analysis of cellular contents and water soluble sugar content (WSC).

3. Estimation of cellular contents and cell wall constituent

The cell wall constituents and cellular contents were estimated by the neutral-detergent method[9]: the dried sample was weighed and 0.5～1.0 g, was added to a solution containing 100 m*l* neutral-detergent solution, 2 m*l* decahydoronaphthalene, and 0.5 g sodium sulfite, and boiled for 60 min and adjusted to even level and reflex. The solution was filterd through a tared nylon filter with vacuum. The filtered sample was rinsed with hot (80～90°C) water. It was then washed with acetone and dried at 100°C for more than 8h. Thereafter it was cooled in a desicator and weighed. The weight of cell wall constituents was calculated by subtracting the weight from the sample plus filter weight. The cell wall constituent contains cellulose, hemicellulose, lignin, SiO_2 and a part of protein. The weight of cellular contents was calculated by subtracting the weight of the cell wall constituent from the weight of the dried sample. The neutral-detergent solution consisted of 30 g sodium lauryl sulfate, 18.61 g disodium dihydrogen ethylene-diaminetetraacetic dihydrate, 6.81 g sodium borate decahydrate, 4.56 g disodium hydrogen phosphate, and 10 m*l* 2-ethoxyethanol in 1 *l* distilled water.

4. Estimation of water soluble sugar content (WSC)

WSC were estimated by the anthrone method[19]. For the extraction of WSC, the sample was weighed, 0.1～0.2 g was added to 50 m*l* water, and extracted in water at 20°C by shaking for 1 hour. Thereafter, the WSC solution was separated from solid material by a centrifuge. For reaction, 200 μl of extracted solution was added to 10 m*l* anthrone reagent, mixed and heated in a boiling water bath for 10 min. It was then cooled using ice water. The WSC of the reacted samples were measured using a photoelectric colorimeter with a 620 nm wavelength which shows linear regression with a standard sugar solution. The anthrone reagent was made by dissolving 0.2 g of anthrone in 100 m*l* of H_2SO_4 ; the latter prepared by adding 500 m*l* of conc. acid to 200 m*l* of water.

Table 1 shows the percent WSC as determined by the anthrone method and its relation to the percents of glucose, fructose, sucrose and fructosan as estimated by enzymatic determination (HK/G6P-DH method)[8,14]. WSC might contain glucose, fructose, sucrose and fructosan.

Result

1. Changes in culm dry weight

Fig. 1 shows the changes with time in the dry weight of three parts of the main culm for three varieties after anthesis. The total culm weight greatly increased from the anthesis to milk ripe stage, mainly due to the increase in peduncle and second internode, and was associated with elongation after anthesis. Dry weight of the lower internodes slightly increased for Haruhikari and Haruyutaka, but decreased for tall late Selpek of which culm elongated rapidly before the heading stage. Selpek had a significantly heavier dry weight in the lower internodes than the other two varieties. After the milk ripe stage, the dry weight of each part decreased gradually until maturity.

2. Changes in cell wall constituent, cellular contents and WSC

The contents of dry matter in culm can be divided first into two parts, that is, the cell wall constituent and cellular contents. The cellular contents into net cellular matter and WSC as a temporary storage material. Since the time trend of three components in each part was almost the same during the grain filling period for each variety, the patterns of Haruyutaka are shown in Fig. 2.

Cell wall constituent increased greatly from the anthesis to milk ripe stage in the peduncle and second internode, which elongated continuously after anthesis, but only a little in the lower internodes of which elongation was almost finished at about anthesis. After milk ripening, however, it changed very little until maturity in each part. The percentage of cell wall constituent at the milk ripe stage was the lowest in the second internode for each variety and semi-dwarf Haruyutaka had the lower values than any of the other tall varieties except the peduncle (Table 2). Contrary to this, cellular contents in each part increased from the anthesis to around milk ripening, then decreased until maturity, mainly reflecting the change in WSC which increased

Table 1. Percentages (dry weight basis) of WSC determined by anthrone method and those of glucose (glu), fructose (fru), sucrose (suc) and fructosan (frn) determined by emzymatic sugar determination (HK/G6P-DH method).

Parts of main culm	Anthrone method	HK/G6P-DH method				
	WSC	glu	fru	suc	frn	total
Peduncle	13.92	2.83	2.72	5.27	2.26	13.08
Second internode	24.69	2.76	1.99	14.54	5.25	24.53
Third internode	33.15	2.83	2.76	18.07	9.05	32.70
Lower internodes	26.78	2.08	3.22	14.36	7.52	27.18

The data were measured with spring wheat, cv Haruyutaka, at a week after anthesis (July 4, 1989).

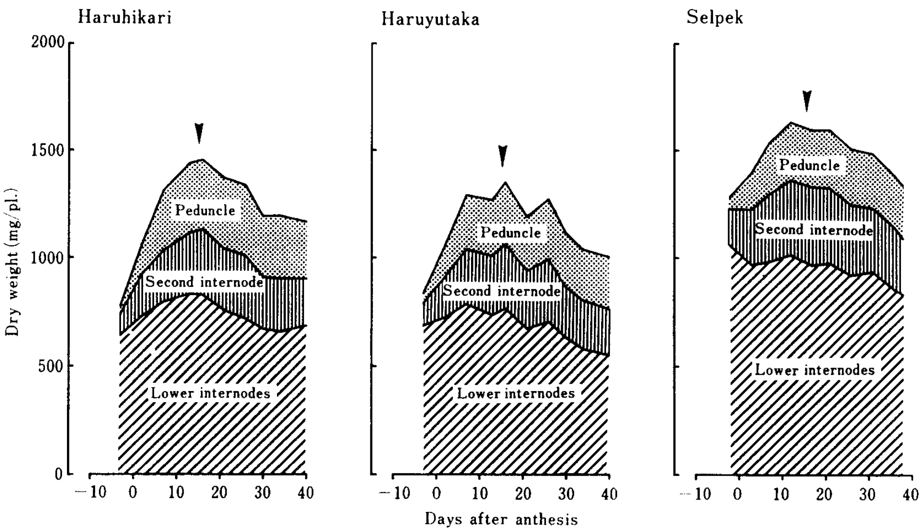

Fig. 1. Changes with time in the dry weights of three parts of the main culm for three varieties (from 10 days before anthesis to maturity). Arrows show the milk ripe stage.

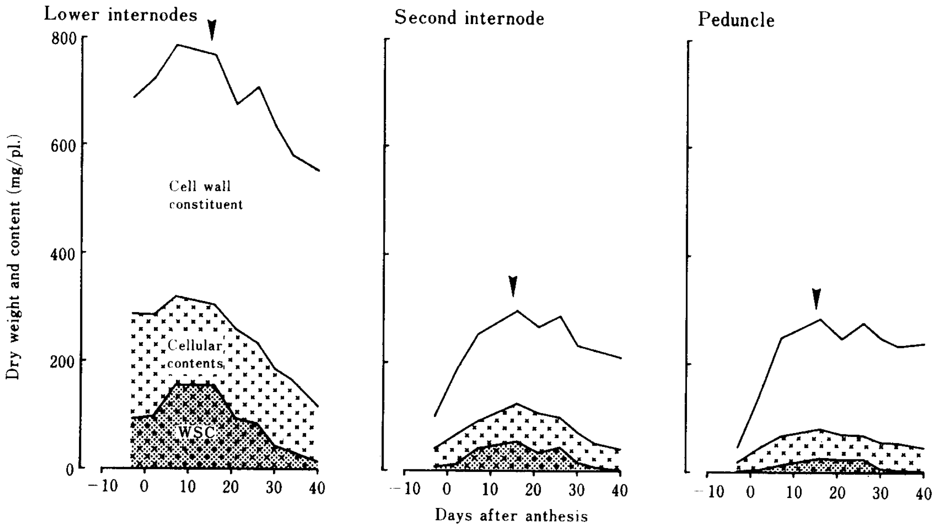

Fig. 2. Changes with time in cell wall constituents, cellular contents and WSC of the three parts of the main culm for Haruyutaka (from 10 days before anthesis to maturity). Arrows show the milk ripe stage.

Table 2. Percentages of cell wall constituent (dry weight basis) at the milk ripe stage.

	Peduncle	Second internode	Lower internodes	Total
Haruhikari	72.0	64.0	71.7	70.1
Haruyutaka	71.2	57.6	60.6	62.2
Selpek	74.3	63.8	68.3	68.3
Mean	72.5	61.8	66.9	66.9
Significance	NS	NS	**	**

** : 1% level of significance.
NS : not significance.

over milk ripening and almost disappeared at maturity. Net cellular matter (cellular contents minus WSC) also increased over milk ripening and then slightly decreased in peduncle and the second internode, However its variation was relatively small compared to that of WSC.

In the lower internodes WSC had already accumulated before anthesis, but started to store at about anthesis in the second internode and peduncle. The maximum value of WSC was obtained at about a week before milk ripening in the lower internodes, just at the milk ripe stage in the second internode and several days after milk ripening in peduncle. As shown in Fig. 3, such a time lag was also recognized in the time shift of percent WSC. The time trend and maximum level (about 20%) of percent sugar was relatively similar between the lower and second internodes, but shifted lower in the peduncle (about 10%) than those of the other two parts. The percentage of cellular contents including WSC declined from about 40% at anthesis to about 20% at maturity in each part, though stagnated around milk ripening, reflecting the increase in the amount of WSC.

3. Varietal difference in the increase and decrease of WSC

Table 3 shows the amount and percentage of WSC in three parts of the main culm at anthesis, milk ripe stage and maturity for the three varieties. At anthesis there were a little WSC in the peduncle and the second internode, but a considerable amount of WSC was accumulated in the lower internodes, especially for Haruyutaka and Selpek, indicating that the lower internodes play a role as storage organ for surplus photosynthate, before anthesis. The amount of accumulated WSC in the lower internodes by anthesis was 36 mg, 98 mg and 113 mg, equivalent to 75%, 84% and 91% of that of the total for Haruhikari, Haruyutaka and Selpek, respectively

Extending over milk ripening, WSC started to accumulate in the peduncle (12～22 mg), especially in the second internode (30～52 mg) for each variety and the percent WSC in the second internode reached 12.2%, 19.1%

and 16.2% at the milk ripe stage for Haruhikari, Haruyutaka and Selpek, respectively. In the lower internodes Haruhikari and Haruyutaka accumulated almost equivalent WSC to the amount accumulated in the peduncle and the second internodes, but Selpek decreased WSC. As the results, the amount of accumulated WSC in the main culm from the anthesis to milk ripe stage was the highest for semi-dwarf Haruyutaka and lowest for tall late Selpek. After the milk ripe stage the accumulated WSC disappeared gradually in each part and percent WSC decreased to about 1% level at maturity.

Discussion

Dry weight of the main culm generally increase from the anthesis to milk ripening, then decreased until maturity[5,13]. The increase up to around milk ripening was mainly dependent on the increase in net cellular matter and cell wall constituent associated with the growth of upper internodes, and was also, at least partially, due to WSC as a reserve material in the culm after anthesis. The decrease after milk ripening, however, was attributed only to a decrease in WSC, since net cellular matter and cell wall constituent changed little until maturity.

After anthesis, the flag leaf is the main supplier of the assimilate for grain growth[3,4,6] and the velocity of movement through the top internode was about double that down the leaf sheath[15]. In addition, the reserve material stored in the culm has also been reported to contribute to grain growth[6,18]. The general pattern of WSC as a storage material obtained in this experiment suggests that WSC continuously accumulates in each internode of the main culm until milk ripening and then translocates to other plant organs, presumably to grain, finally near zero at maturity. However, Selpek decreased the WSC in the lower internodes from the anthesis to milk ripening, indicating that the pattern of accumulation and translocation of WSC differ among varieties.

A temporary storage of material in the culm was pointed out to occur in the lower part of the uppermost culm internode by Stoy[11], the second internode by Wardlaw and Porter[18], or the top and second internode by Rawson and Hofstra[6]. Shimono[7] also observed the great change of sugar content of the second internode during the grain filling period. Similarly, in this experiment, the percent WSC at the milk ripe stage was higher in the second internode than in the peduncle and lower internodes for each variety. However, after anthesis Haruhikari and Haruyutaka accumulated in the lower internodes an almost equivalent WSC to the amount accumulated in the peduncle and the second internode (Table 3), indicating that the lower internodes were also a main storage organ for these varieties. Generally, lower leaves supply assimilate to the base and roots of plants[11,18]. With Haruhikari and Haruyutaka, which have erect and small sized leaves, light was observed to penetrate deeper within the canopy when compared with Selpek (unpublished). From these observations, the difference in the increase and decrease of WSC from the anthesis to milk ripening might reflect the difference in the light conditions within the canopy.

The amount of WSC decreased from the

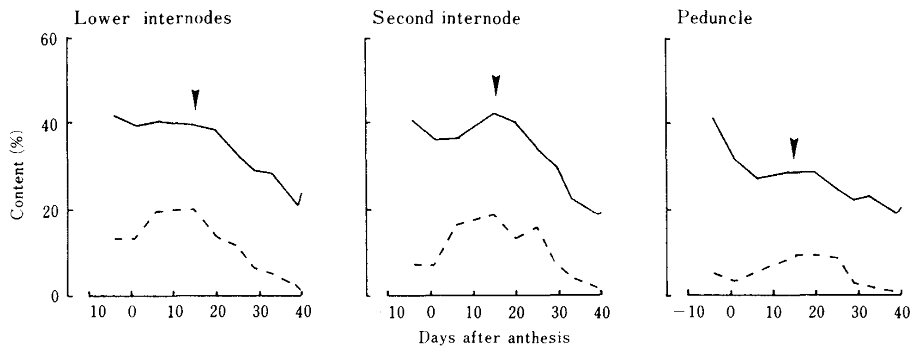

Fig. 3. Changes with time in the percentages of cellular contents (solid line) and WSC (broken line) for dry weights of three parts of main culm of Haruyutaka. Arrows show the milk ripe stage.

Table 3. The amount (mg/pl.) and percentage (parenthesis) of WSC in three parts of the main culm at anthesis, milk ripening and maturity for three varieties.

Variety and internode	Anthesis (An)	Milk ripe stage (Mi)	Maturity (Ma)	An-Mi	Mi-Ma
Haruhikari					
Peduncle	3.9 (2.8)	23.8 (7.2)	1.5 (0.6)	19.9	-22.3
Second internode	7.9 (4.1)	37.5 (12.2)	1.8 (0.8)	29.6	-35.7
Lower internodes	35.9 (4.9)	84.6 (10.2)	3.1 (0.5)	48.7	-81.5
Total	47.7 (4.5)	145.9 (10.0)	6.4 (0.6)	98.2	-139.5
Haruyutaka					
Peduncle	5.2 (3.6)	27.6 (9.7)	2.7 (1.1)	22.4	-24.9
Second internode	13.3 (7.2)	56.8 (19.1)	2.2 (1.0)	43.5	-54.6
Lower internodes	98.2 (13.5)	155.2 (20.2)	7.7 (1.4)	57.0	-147.5
Total	116.7 (11.1)	239.6 (17.7)	12.6 (1.2)	122.9	-227.0
Selpek					
Peduncle	4.2 (2.4)	15.8 (6.0)	1.2 (0.5)	11.6	-14.6
Second internode	6.8 (2.7)	58.7 (16.2)	3.5 (1.3)	51.9	-55.2
Lower internodes	113.3 (11.8)	83.1 (8.5)	9.7 (1.2)	-30.2	-73.4
Total	124.3 (8.9)	157.6 (9.9)	14.4 (1.1)	33.3	-143.2
Varietal difference					
Peduncle	NS (NS)	* (**)	NS (NS)		
Second internode	NS (NS)	NS (NS)	NS (NS)		
Lower internodes	** (*)	* (**)	NS (NS)		
Total	** (**)	** (**)	NS (NS)		

**, * : 1%, 5% level significance. NS : nonsignificance.

milk ripe stage to maturity, almost equivalent to the amount accumulated until milk ripening, and was about 60% larger for Haruyutaka than Haruhikari and Selpek, suggesting that semi-dwarf varieties have more reserve materials in culm than tall ones as reported by many workers[1,10,12,16]. It is interesting to note that semi-dwarf Haruyutaka accumulated twice the WSC in the lower internodes as that in the upper two internodes.

References

1. Kim, N.I. and G.M. Paulsen 1986. Assimilation and partitioning of photosynthate and nitrogen in isogenic tall, semidwarf, and doubledwarf winter wheats. J. Agron. & Crop Sci. 156 : 73—80.
2. Lopatecki, L.E., E.L. Longair and C.W. Farstad 1957. Soluble carbohydrates of wheat stems and leaves. Can. J. Bot. 35 : 9—12.
3. Makunga, O.H.D., I. Pearman, S.M. Thomas and G.N. Thorne 1978. Distribution of photosynthate produced before and after anthesis in tall and semi-dwarf winter wheat, as affected by nitrogen fertiliser. Ann. Appl. Biol. 88 : 429—437.
4. Patrick, J.W. 1972. Distribution of assimilate during stem elongation in wheat. Aust. J. Biol. Sci. 25 : 455—467.
5. Rawson, H.M. and L.T. Evans 1971. The contribution of stem reserves to grain development in a range of wheat cultivars of different height. Aust. J. Agric. Res. 22 : 851—863.
6. —— and G. Hofstra 1969. Translocation and remobilization of ^{14}C assimilated at different stages by each leaf of the wheat plant. Aust. J. Biol. Sci. 22 : 321—331.
7. Shimono, K. 1986. Studies on nutritional physiological and nitrogen-fertilizer management of winter wheats. Rep. Hokkaido Prefect. Agric. Exp. Stn. 57 : 1—80*.
8. Smith, D., G.M. Paulsen and C.A. Raguse 1964. Extraction of total available carbohydrates from grass and legume tissue. Plant Physiol. 39 : 960—962.
9. Soest, P.J. Van and R.H. Wine 1967. Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell-wall constituents. J.A.O.A.C. 50 : 50—55.
10. Spiertz, J.H.J. and H. van de Harr 1978. Differences in grain growth, crop photosynthesis and distribution of assimilates between a semi-dwarf and a standard cultivar of winter wheat.

Neth. J. Agric. Sci. 26 : 233—249.
11. Stoy, V. 1963. The translocation of C^{14}-labelled photosynthetic products from the leaf to the ear in wheat. Physiol. Plant. 16 : 851—866.
12. Takahashi, T., K. Nakaseko and K. Gotoh 1988. Differences in productivity between short- and long-culm varieties and their relationships with cell wall and cellular components of culm in spring wheat. Japan. Jour. Crop Sci. 57 : 53—58*.
13. ————, ———— and ———— 1988. Dry matter partitionings of tall Haruhikari and semi-dwarf 2—47 in spring wheat. Japan. Jour. Crop Sci. 57 : 522—526*.
14. Takebe, M. and T. Murakami 1984. The enzymatic determination of sugars in sugar beet plant with hexokinase and glucose-6-phosphate dehydrogenase. Japan. Jour. Crop Sci. 53 : 113—114*.
15. Thome, U. and W. Kühbauch 1985. Change in the carbohydrate pattern in the cell content of wheat stems during grain-filling. J. Agron. Crop Sci. 155 : 253—260.
16. Thorne, G.N. 1982. Distribution between parts of the main shoot and the tillers of photosynthate produced before and after anthesis in the top three leaves of main shoots of Hobbit and Maris Huntsman. Ann. Appl. Biol. 101 : 553—559.
17. Wardlaw, I.F. 1965. The velocity and pattern of assimilate translocation in wheat plants during grain development. Aust. J. Biol. Sci. 18 : 269—281.
18. ———— and H.K. Porter 1967. The redistribution of stem sugars in wheat during grain development. Aust. J. Biol. Sci. 20 : 309—318.
19. Yemm, E.W. and A.J. Willis 1954. The estimation of carbohydrates in plant extracts by anthrone. Biochem. J. 57 : 508—514.

* In Japanese with English summary, tables and titles and notes of figures.