Starch Granules of Developing Wheat Kernels[1]

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ABSTRACT

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A Coulter counter was used to determine size distributions of starch granules during kernel development in bread wheats; the amounts of starch were also determined chemically. Results are presented as mass of starch per kernel, number of granules per gram of starch, number of granules per kernel, average granule mass, and limiting granule volumes for various mass fractions of the total. The results are consistent with the hypothesis that at least two populations of granules have distinct initiations and growth. Growth of the granules may be a dynamically fluctuating situation in which both gain and loss of material produce an overall gain. The development pattern was similar for three cultivars in one season but very different for three seasons of one cultivar, which suggests a seasonal effect on size of starch granules.

Previous work (Jenkins et al 1975) described the increase of starch mass in the wheat kernel during growth. Because wheat starch is composed of granules, this growth can be a result of proliferation of granules, an increase in individual granule mass, or a combination.

To find when and how these processes occur during the growing stage we used a Coulter counter to examine starches from three serially harvested cultivars of the same year and examined further data from one of these cultivars in two additional years. We compared starch mass per kernel, granule number per gram of starch, and granule number per kernel. In this way we estimated which periods of growth are marked by increase in granule number and which by increase in granule mass. We also estimated functions of granule volumes relative to time.

MATERIALS AND METHODS

Serially harvested wheat samples were previously described (Jenkins et al 1974). Time is expressed in days after emergence of the ear from the leaf sheath; ripeness, judged by ear moisture content, (Meredith and Jenkins 1975) occurs between 60 and 65 days. We examined grains ranging in age from 23 to 70 days. The samples were frozen, freeze-dried, and held at −20°C.

Dry grains were ground (Regent Maskin, Sweden) to pass a 40-mesh sieve. To inactivate amylases (Meredith 1970), the ground grain was hydrated in dilute acid (7 ml of $0.05N$ HCl per gram of grain), neutralized with NaOH after 20 min, and soaked overnight at 2°C. Starch granules were liberated by repeatedly grinding the sample in excess water with a mortar and pestle until all starch

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passed through 40-mesh and 200-mesh wire sieves. The starches were recovered by centrifugation and then were repeatedly suspended and centrifuged. Gummy material was removed from the surface of the starch cake. The washed starches were finally suspended in 0.1 m*M* thiomersal before dilution in Isoton (isotonic saline) for counting. Particles were sized and counted in a Coulter counter Model D equipped with a 140-μm orifice tube.

The amount of starch per kernel was chemically determined as previously described (Jenkins et al 1974).

Calculations

The Coulter counter gives as raw information the number of granules above a set volume, which pass through the orifice, in a known volume of liquid. The set volume is proportional to the product of the threshold setting, the attenuation setting, and the electric current passing through the orifice. Of these three factors, the current is the least precise; it is available to only two significant figures without ancillary measuring equipment. Hence, the granule volumes we measured and the masses and mass fractions we deduced from them are no more precise than two significant figures.

The calculations presented here are by integration of best-fit equations. Such procedures are not absolutely necessary because the calculations could be made by using Simpson's rule for obtaining the area under any curve. The integration method poses several problems concerning, among other things, the intersections of the curves. However, the overall averages approach the averages estimated by calculation with Simpson's rule.

Cumulative Mass Fraction Curve. The raw data was processed according to the complete technique given in the Coulter counter manual. For this purpose we prepared a calculator program that gave direct read-out of the fraction of starch mass made up by granules smaller than a given volume (or sphere-equivalent diameter). The volumes were corrected by a factor of 0.59 (Dengate et al 1978) so that they represented the volume of starch in each granule, not the volume of starch plus water.

The accumulated mass fractions for granules less than a given volume were tabulated with their respective volumes and graphed for each serially harvested sample. Figure 1 represents the general form of each curve relating mass fraction (M) and granule volume (V). The curves were divided into intervals (AB, BC, etc.) in such a manner that at least one of the equations

$$M = a + bV$$

or

$$M = a + b \ln V$$

or

$$M = aV^{b}$$

where a and b are constants, could be fitted to each interval with regression coefficient greater than 0.9 on the basis of at least four points. The best-fit curve was selected for each arbitrary interval. Calculations were performed by a standard curve-fitting program.

Number of Granules per Gram and per Kernel. The ordinates of the curve are cumulative mass fractions of weight. Differentiation gives the mass fraction contained in an infinitesimal interval (dV). This differentiation can be performed by following the formulas for the respective equations,

$$dM = b\,dV$$

$$dM = b\,d \ln V$$

$$dM = ab\,V^{(b-1)}dV$$

The number of granules per gram (dN) contained in the interval (dV) surrounding the volume (V) is then

$$dN = dM/(\rho V)\ ,$$

where ρ is the density of the starch. Therefore the total number of granules between any two volumes (V_1 and V_2) can be estimated from one of the three corresponding integrals, depending on the section of curve being considered.

$$\int_{V_1}^{V_2} dN = \begin{array}{l} (b/\rho)\ \ln (V_2/V_1) \\ (b/\rho)\,[\,(1/V_1) - (1/V_2)] \\ {[ab/\rho(b-1)]}\,[V_2^{(b-1)} - V_1^{(b-1)}\,] \end{array}$$

If all integrals from the different sections of a curve are summed, then N, the number of granules per gram of sample, is obtained,

$$N = \sum_{AB}^{DE} \int_{V_1}^{V_2} dN$$

where AB is the interval at the extreme left of the curve and DE is the interval at the extreme right.

In the previous work (Jenkins et al 1974) on this material, we determined the mass of starch in a kernel of wheat. We multiplied the numbers of granules per gram by these figures to determine the number of granules per kernel.

Partition Particle Volumes. The variable M in the three equations used for curve fitting is the mass fraction below a given volume. Hence the value of M = 1.00 is associated with the maximum granule volume, and the values M = 0.85 and M = 0.50 are the values associated with 85 and 50% of the mass of the sample, respectively. Once the best-fit equation is established for the last section of the curve, M can be made equal to one of these figures. The curve-fitting program will then supply the corresponding value of V.

RESULTS

The mass of starch per kernel (Fig. 2A), the number of granules per gram of starch (Fig. 3A), and the number of granules per kernel (Fig. 4A) varied quite consistently through three cultivars in one season. However, there were great differences between seasons for a single cultivar (Figs. 2B, 3B, 4B).

In general, the mass of starch per kernel (Fig. 2) increased regularly to about 50 days, although Hilgendorf of 1972 showed delayed early growth. The number of granules per kernel (Fig. 4) increased after 30 days, although this increase was not marked in Hilgendorf until 40 days. The number of granules per gram (Fig. 3) decreased until about the 40th day and then increased at the same time that the number of granules per kernel increased. At about the

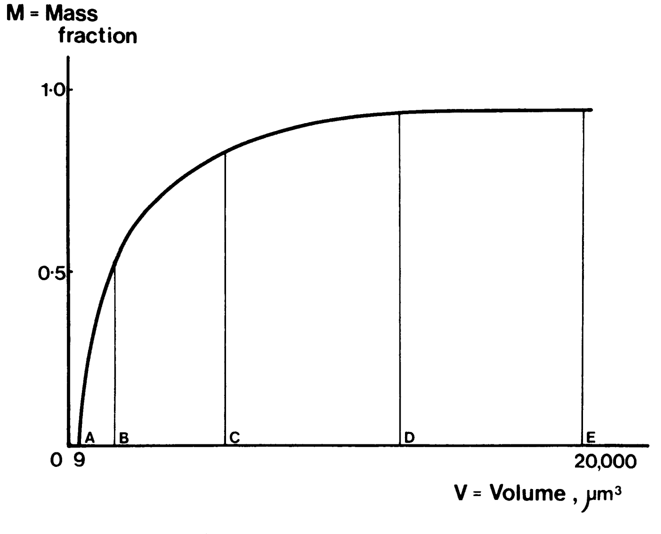

Fig. 1. General form of the cumulative curve of total mass of granules as a function of inidividual volumes. The minimum dry volume for the Coulter counter configuration was about 9 μm^3.

50th day all the curves reached a plateau and then in most cases showed a slight decrease.

We have indicated in Figs. 2–7 five stages that provide a hypothesis on which to base discussion of the generally-accepted concept that there are two types of granules, large and small, or A and B (Buttrose 1963, Evers et al 1974, Hughes and Briarty 1976, Kulp 1973, Reichert 1913, Sandstedt 1946). Stage I, up to 27 days, is the stage of initiation of type A granules. In stage II, from 27 to 40 days, the type A granules grow with little further initiation. In stage III, from 40 to 53 days, initiation and growth of type B granules

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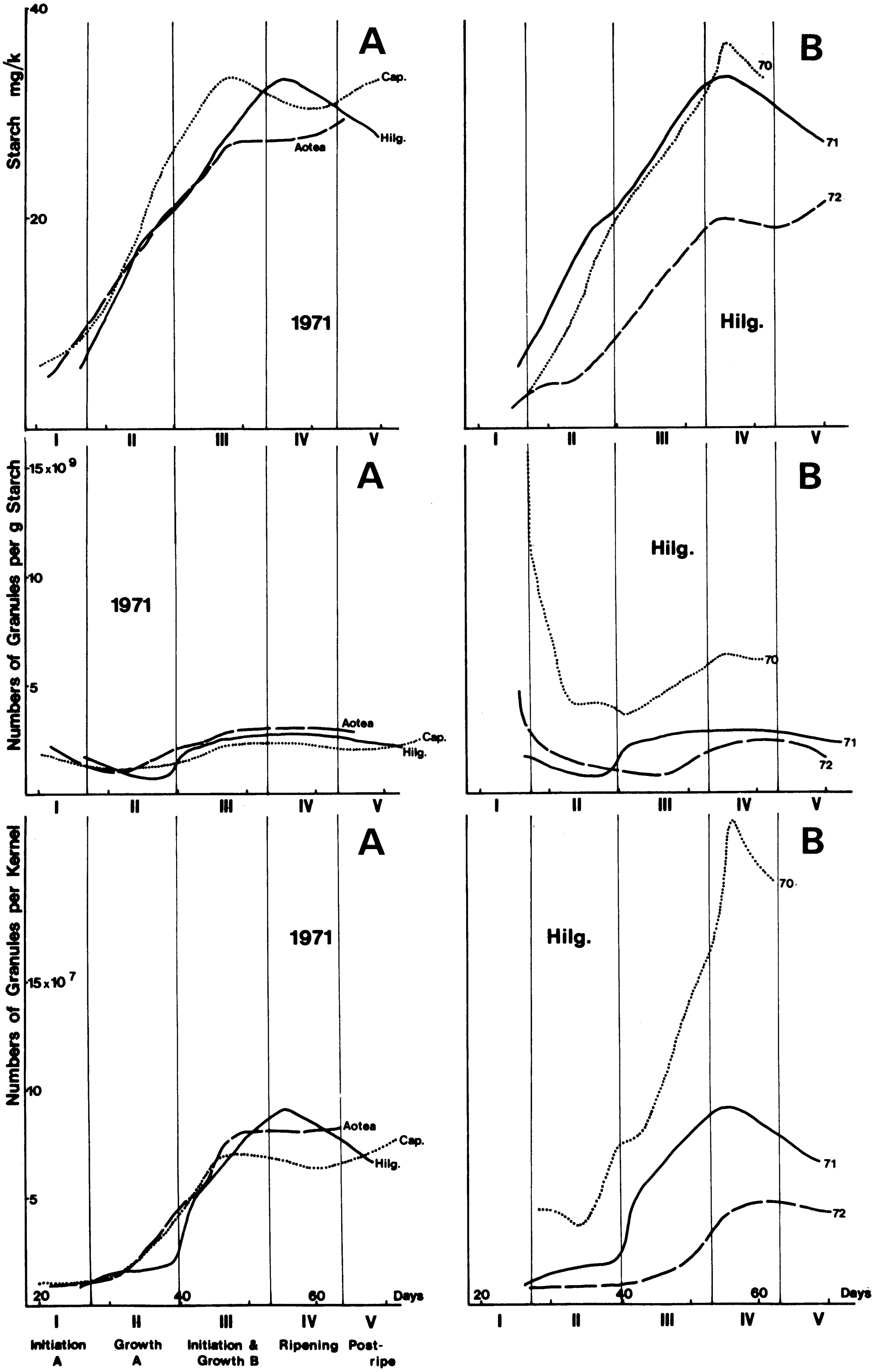

Fig. 2. Mass of starch per kernel, chemically determined, as a function of time during development, for **A,** cultivars Aotea, Cappelle Desprez (Cap.), Hilgendorf (Hilg.), in 1971 season; and for **B,** cultivar Hilgendorf in the 1970, 1971, and 1972 seasons. **Fig. 3.** Total numbers of granules per gram of starch, determined by Coulter counter between limits 9 μm^3 and 20,000 μm^3, as a function of time during development. **Fig. 4.** Total numbers of granules per kernel during development, derived from data of Figs. 2 and 3.

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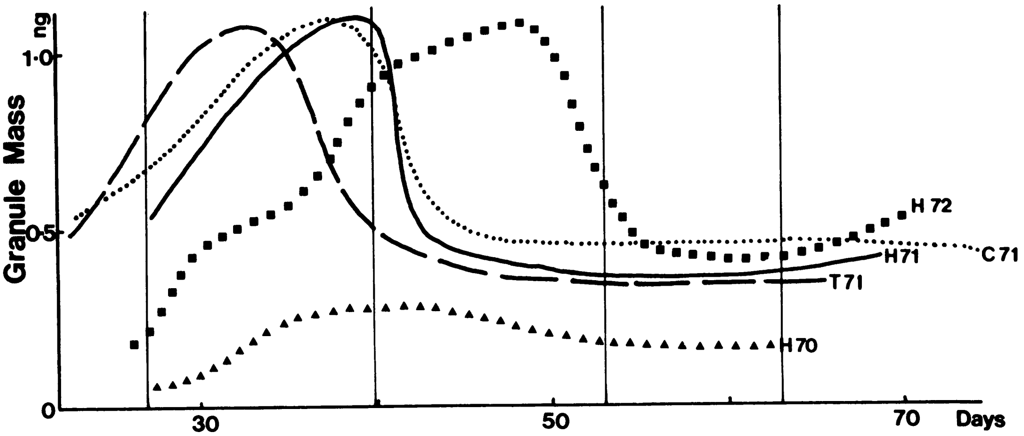

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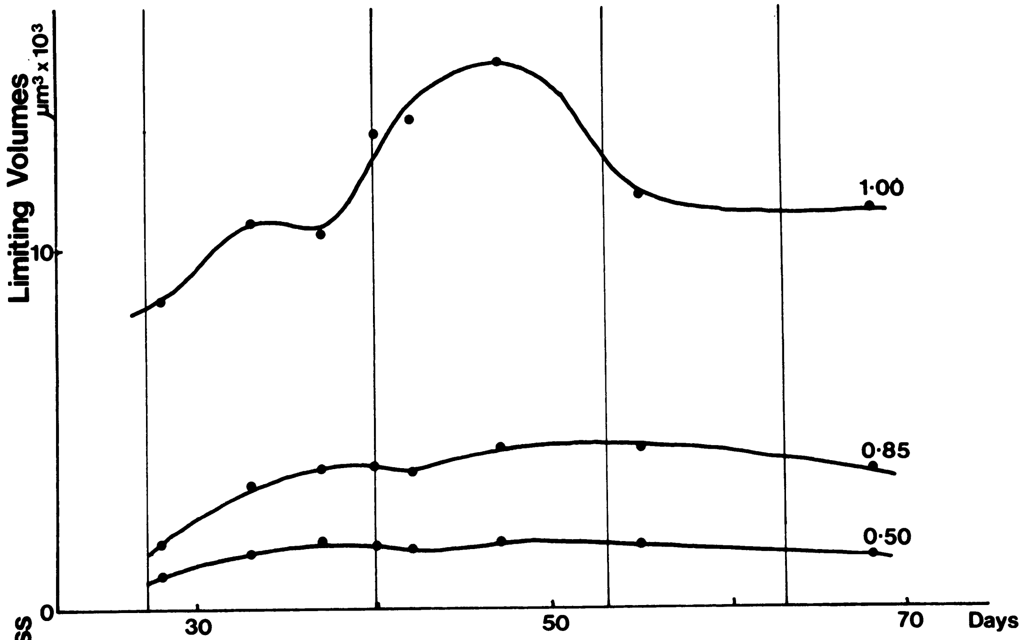

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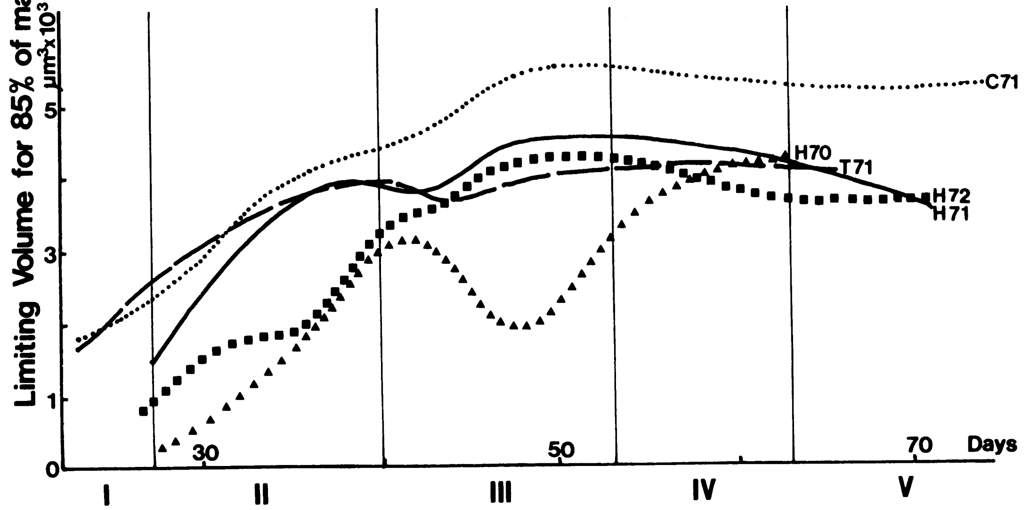

Fig. 5. Average granule mass (number-average) as a function of time during kernel development ■ = Hilgendorf 1972, ··· = Cappelle Desprez 1971, — = Hilgendorf 1971, — — = Aotea 1971, ▲ = Hilgendorf 1970. **Fig. 6.** Growth contours of limiting granule volumes containing specified proportions of the total granule mass, as a function of time during kernel development, for cultivar Hilgendorf of 1971 season. **1.00**, maximum volume of any granule; **0.85**, maximum volume that 85% of the mass attains; **0.50**, maximum volume that 50% of the mass attains. The sampling times indicated in these curves are those used throughout. **Fig. 7.** Growth contours of limiting granule volumes for 0.85 of total granule mass, as a function of time during kernel development.

occur and type A granules probably continue to grow also. Stage IV, from 53 to 63 days, is the period of ripening; there is little addition or loss of starch polymer, although there is a possibility (suggested by unpublished work) that the structures of the granules are changing, particularly near their surfaces. Stage V, after 63 days, we have called the postripe period; some cultivars may be stable but in others, starch content may change markedly (Jenkins et al 1974). Loss of starch from kernels in the final development stages is apparent in some of the recent results of Donovan et al (1977).

The number of granules per gram is the reciprocal of the average mass of the granules. Variations of the average granule mass are presented in Fig. 5. Volume (or mass) growth of any or all granules increases the average granule mass; for instance, in stage II the mass of starch per kernel increases rapidly but the number of granules per kernel is relatively static.

In stage III the number of granules per kernel and the number of granules per gram both increase, which suggests that new granules appear and are relatively small during this period.

Behavior in stage IV depends on the cultivar. For Hilgendorf, starch mass and number of granules per kernel diminish slightly, as noted previously (Jenkins et al 1974, 1975; Meredith and Jenkins 1970); for Aotea and Cappelle, the starch mass and number of granules per kernel continue to increase slightly. There is no clear pattern of changes in these parameters in stage V.

Although new small granules obviously form during stage III, we may still question whether the total mass increase is accounted for by these new granules or whether there is also a general increase in all granule sizes. Figure 6 is one example of similar curves for each cultivar and season; it shows growth contours of the limiting granule volumes that contain specified percentages of the total starch mass. The 1.00 line represents the maximum volume that any granule attains; the 0.85 line, the maximum volume that 85% of the mass of granules attains; and 0.50, the maximum volume for 50% of the mass of granules. The excursions of the 1.00 line may be interpreted as variations of a small population because granules of this size are scarce. On the other hand, these excursions quite possibly represent distinguishable alternate loss and gain of starch by the largest granules. Possibly such excursions of loss and gain take place in all the granules but are undetected. Small granules in the interstices between large granules may grow at the expense of the large granules. Conversely, large granules may grow at the expense of small ones, analogous to what happens in unstable foams or emulsions.

Figure 7 presents the 0.85 contours of limiting volumes for all the cultivars and seasons; this contour seems to represent the bulk of the material, unaffected by the extremely large granules. These curves are not affected by loss of fine granules in the preparation of the starches. The general trend is of a pattern of growth through stages I, II, and III. We think the dip in the curves early in stage III represents real loss of size of the original population of granules (type A), with subsequent continuation of their growth. This implies that granule growth may include brief periods when loss of material occurs. Indeed the slight downward slope of the curves through stages IV and V suggests shrinkage of the larger granules, an occurrence that also would be in accord with a hypothesis of fluctuating growth.

If we assume spheres of density of 1.6, the limiting volume 5×10^3 μm^3 of Fig. 7 corresponds to an anhydrous mass of 8 ng (21 μm diameter), demonstrating how much larger these granules are than the average ones of Fig. 5, where the peak average size is about 1 ng (11 μm diameter). The maximum of the 1.00 limiting curve in Fig. 6 is about 26 ng (31μm diameter).

CONCLUSIONS

We replaced the experimental curve of cumulative mass-volume by two or three continuous equations in separate regions of the curve. In this way we could approximate the overall average particle volume that can be calculated also by Simpson's rule. This method requires fewer experimental points. There is a further possible advantage in that intersections of equations may indicate distinct classes of particles. However, use of the equations is limited.

The curves of starch per kernel and of granules per gram were obtained from independent experiments on the same material, and the curves of granules per kernel were calculated from these two curves. The total picture given by the three curves suggests at least two populations of granules with distinct genesis and growth. We cannot, however, readily decide if these two populations are due to physiologically distinct genesis and continuous growth or to a more dynamically fluctuating situation of continuous genesis with varying growth rates that may even include periods of loss from the granules.

We are inclined to believe that the situation is one of fluctuating growth from discontinuous genesis. Our results to date suggest an interruption during starch growth in developing grain, possibly indicating a major change in the synthesis pathway or in the physiologic mechanism of deposition of the polymer.

Our major conclusion from the results is that the pattern of starch development and growth was very similar for three markedly different cultivars growing at different dates in one year, but that very different patterns were exhibited by one cultivar in three consecutive seasons. Thus a seasonal effect on size of starch granules as well as on grain yield and protein content is to be expected.

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