RELATIONSHIP OF MIXED-LINK BETA-GLUCAN ACCUMULATION TO ACCUMULATION OF FREE SUGARS AND OTHER GLUCANS IN THE DEVELOPING BARLEY ENDOSPERM

by

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Accumulation of free sugar, starch, mixed-link beta-glucan and hemicellulose in developing barley endosperms was investigated using a sequential enzymatic procedure. Bomi grown in a growth cabinet, the three cultivars Minerva, Lami and Triumph, grown in the field, and Minerva and Triumph grown under a range of water supply regimes in the phytotron were studied.

The first experiments showed that initiation of synthesis occurred in the order hemicellulose, starch, mixed-link beta-glucan 10 days, 11 days, and 17 days after fertilization, respectively. The second experiment indicated two separate accumulation patterns for mixed-link beta-glucan, one shared by Lami and Triumph, the other followed by Minerva. When moisture content declined to 40–50 %, the accumulation rate increased sharply in Lami and Triumph, but not in Minerva. A similar sharp increase in the rate of accumulation of hemicellulose was observed in Minerva and Triumph.

The moisture stress experiment showed that water supply and humidity are the major factors mediating the response of beta-glucan content to environment, but that Triumph, a low-content genotype, was always lower in beta-glucan content than Minerva.

Some implications of the results for elucidation of the control of endosperm development are discussed.

1. INTRODUCTION

A good deal of attention has been paid to the accumulation of reserve substances in cereals (2, 3, 7, 8, 18, 19, 20, 21, 23, 24, 25, 26, 27, 28, 30, 31). Among the carbohydrates, however,

only free sugars and starch have been examined in any depth. The existence of two peaks of free sugar content, at the beginning of drymatter accumulation is well documented, (2, 3, 8, 18, 19, 25, 26, 27, 31), but results for initiation,

termination, and rate of accumulation of starch, and initial and final contents vary substantially from author to author (3, 8, 19, 20, 23, 25, 26, 28, 31, 38).

In light of this it can be seen that a definitive method for following the accumulation of glucosan fractions in barley needed to be found. Little investigation of non-starch glucan accumulation has been made, except as pentosan and »crude fibre« by CERNING and GUILBOT (8) and MACGREGOR et al. (28). However, beta-glucans play an important structural role in the endosperm, comprising 75% of total cell wall composition (12, 13, 14), but rather less in the aleurone layer, where arabino-xylan is the dominant polysaccharide. Moreover, in both brewing (11, 14, 32, 33, 36) and animal feeding (16, 37) they are of considerable importance.

Cell-free synthesis of beta-glucan has been achieved with *Lolium* spp endosperm tissue culture preparations (34) and with many vegetative tissues (9). Screening methods for flour beta-glucan content, based on the viscosity it induces in aqueous extracts, have been devised by BENDELOW (5) and MORGAN (32), and used to study the inheritance of barley flour extract viscosity (1). However, the *in vivo* synthesis of beta-glucans has not previously been studied in barley, so a method of assaying specific glucan fractions sequentially has been devised and used to study four commercial barley cultivars with different malting properties. By this means data were obtained to describe the relationship between accumulation curves of free sugar, starch, beta-1,3:1,4-glucan and other beta-glucans in a single cultivar. Then comparisons were made for each of the glucosans, and finally the accumulation process for beta-1,3:1,4-glucan was studied in two of the cultivars (Minerva and Triumph) under a range of water supply regimes in a phytotron.

2. MATERIALS AND METHODS

2.1. Materials

Amyloglucosidase was a standard preparation from Boehringer Mannheim (Batch numbers 1308407, 1079407) having approx. 14 U·mg⁻¹. Incubation of a solution of pure beta-glucan from barley, supplied by the Department of Brewing Chemistry, Carlsberg Research Center,

with 1 mg·g⁻¹ of amyloglucosidase at pH 4.5, 65 °C for 24 hours produced no glucose-containing oligosaccharides, indicating that the preparations were beta-glucanase-free.

Lichenase (E.C. 3.2.1.73) was the kind gift of Dr. B. S. ENEVOLDSEN, of the Department of Brewing Chemistry, and it had an activity of 0.47 U·ml⁻¹ when diluted ready for use.

Cellulases from *Aspergillus* spp (1 U·mg⁻¹) and *Trichoderma viride* (0.5 U·mg⁻¹) were obtained from Koch-Light laboratories, Colnbrooke, England and Boehringer Mannheim, respectively.

Glucose oxidase assays of glucose content were made using the God-Perid system from Boehringer Mannheim.

All other chemicals used were analytical grade, glass-distilled water was used throughout, and standard 96% ethanol was used for preparing solvents. During all incubations two drops of toluene were added to inhibit bacterial growth.

2.2. Growth room study

2.2.1. Plants

Seeds of *Hordeum vulgare* cv. Bomi were sown in individual pots in a standard potting mixture. Once the coleoptiles appeared the plants were placed on fibrous matting kept moist with a capillary watering system. The plants were grown in a growth room with a 16 hour day at 12 °C and an 8 hour night at 6 °C. Relative humidity was maintained at approx. 70%. Light intensity at the bottom of the heat filter was 5.3 × 10³ lux. A light infection of powdery mildew (*Erysiphe graminis*) was present on senescing leaves, too late to have any real effect on assimilate contribution. Some sterility occurred in the tips of all ears, and occasionally scattered randomly along the ear.

2.2.2. Selection and harvest of material

Spikes on the point of flowering were labelled and noted. Since the number of plants was limited each had to supply a number of spikes, and these flowered over a period of approx. 6 days. By harvesting the last-flowering spikes first, and vice versa, it was possible to compress the harvest period considerably. For the purpo-

ses of this experiment spikes were required at two-day intervals from 7–33 days after flowering, and 4 were harvested at each interval.

2.3. Field study

2.3.1. Plants

Hordeum vulgare cvv. Minerva, Lami and Triumph were grown in adjacent 15 m² plots on a highly-fertile, heavy clay soil. Phosphorus and potassium were supplied to the soil in the previous autumn (66 kg per ha P₂O₅ and 88 kg per ha K₂O) and nitrogen supplied as Ca(NO₃)₂ at 90 kg per ha just before sowing. Mildew attacked less severely than usual, while yellow rust and barley rust (*Puccinia glumarum* and *P. hordei*, respectively) were worse than usual (J. LARSEN pers. commun.).

Despite a somewhat dry spring, flowering occurred at the usual time, but the period thereafter was cold and wet, delaying harvest by several weeks.

2.3.2. Selection and harvest of material

As in the growth room study spikes on the point of flowering were labelled, but only primary spikes were used and all were labelled on the same day. All spikes were chosen from the centre of the plots. From 10 days after flowering three spikes were collected from each plot twice weekly, immediately frozen in liquid nitrogen and stored at –70 °C until needed for analysis. Sampling continued until harvest-ripeness.

2.3.3. Moisture content

The moisture content of all freshly-thawed Lami samples was determined by freeze-drying then oven-drying to constant weight at 130 °C.

2.3.4. Micromalting

Micromalting and malt analysis were carried out in the Central Laboratories of the Carlsberg Breweries according to the methods of GJERTSEN et al. (15).

2.4. Phytotron study

2.4.1. Plants

Hordeum vulgare cvv. Minerva and Triumph were grown as single plants in pots on trolleys in the phytotron of the Royal College of Forestry in Stockholm (1, 39). The plants were grown on a sterile substrate of fine stones, sand and perlite, nutrient being supplied by watering with dilute Hoagland solution until the plants water requirements were satisfied. Until flowering the plants were kept in continuous light with 16 hours at 15 °C followed by 8 hours at 10 °C. All spikes were date-labelled as they emerged, and the date of fertilisation determined. At this time the plants were moved to a room maintained continuously at 20 °C and new watering regimes commenced as follows. Each treatment was given to seven plants of each cultivar.

1. »Rain« treatment. Water was given to the plants 12 hours in every 24 by an overhead spray (1) giving 2 sec bursts of rain followed by pauses of 45 ± 11 secs. 200 ml of Hoaglands solution were supplied to each pot at the end of each 12 hour rain period until the last week of the experiment, when only two additions were made, 4 days apart.

2. »Root« treatment. Water was supplied to the plants in this treatment by a capillary tube at such a rate that the amount passing *through* the pots in any 24 hour period was the same as in treatment 1.

3. »Control« treatment. 200 ml of Hoaglands solution were supplied to each pot morning and evening for 31 days, and the amount of water passing through all the pots was measured for each 24 hour period. In the last week of the experiment the watering was reduced to once daily, with Hoaglands solution being replaced by deionised water except on the 2nd and 6th days.

4. »Reduced water« treatment. 200 ml of Hoaglands solution were given to each pot each morning for nine days. For the following 22 days 200 ml were given every second day. In the final week of the experiment 200 ml of Hoaglands solution were given on the 2nd and 6th days, and 200 ml of deionised water on the 4th day.

5. »Control-reduced« treatment. As for treatment 3 for 20 days, then as treatment 4 for the rest of the experiment.

6. »Reduced-control« treatment. As for treat-

ment 4 for 20 days, then as for treatment 3 for the rest of the experiment.

2.4.2. *Harvesting*

Samples of five spikes were harvested from each cultivar at 14, 18, 22, 26, 30, 35 and 40 days after fertilisation. From each sample 10 kernels were removed (2 from the middle of each spike), weighed and placed in a stoppered tube, which was then placed in the freezer in a plastic bag with the remainder of the sample.

2.4.3. *Moisture content*

The moisture content of the kernels separated as above was determined as in section 2.3.3.

2.5. Analysis

2.5.1. *Preparation of samples for analysis*

When harvesting was completed, the developing grains were removed from their lemmae and paleae. The embryo, and as much of the pericarp as possible, was removed. The prepared grains are referred to as endosperms in the text.

In some treatments the embryos of some Triumph kernels continued to grow into plantlets without a pause i.e. viviparously (6); these seeds were excluded from the samples when they were found. Each sample was freeze-dried and weighed. The endosperms were stored in airtight containers at -20°C until required for analysis, at which time they were ground, where possible, in a Tecator CycloTec sample mill (Tecator Inc., Boulder, Colorado, U.S.A.) and a sample drawn for dryweight determination (section 2.3.3). The equilibrium moisture content was thus found and used to convert all subsequent data to a drymatter basis. Duplicate samples of between 100 and 200 mg of freeze-dried tissue were weighed into 30 ml stoppered centrifuge tubes.

2.5.2. *Extractions*

2.5.2.1. *Free sugar*

In this class is included all the carbohydrate soluble in 80% ethanol (v/v) (35). To each sample 25 ml of 80% ethanol was added and the material thoroughly suspended. In the cases where it proved impossible to grind the tissue the

whole endosperms were weighed out, covered with 10 ml of solvent and dispersed with a Polytron tissue homogeniser (Kinematica GmbH, Lucerne, Switzerland). The homogeniser was carefully washed with a further 15 ml of solvent, which was added to the homogenate. Thereafter these samples were treated exactly as the ground tissue.

The samples were occasionally resuspended over a period of an hour, then sedimented in a bench centrifuge at 1500 g for 10 min, and the supernatants transferred to round-bottomed flasks. The pellets were extracted a further three times.

The combined supernatants were concentrated on a rotary evaporator until most of the ethanol was removed, then frozen and freeze-dried. When quite dry all water-soluble material was redissolved in 40 ml of water, leaving a precipitate of lipids and protein, which was removed by centrifugation. The resultant supernatants were stored at -20°C until needed for analysis.

2.5.2.2. *Starch*

The free-sugar-free pellets were resuspended in 8 ml of water and heated to 90°C in a waterbath for an hour. Upon cooling 2 ml of 0.1 M-sodium acetate buffer (pH 4.5) were added and the pH adjusted if necessary. Amyloglucosidase was added at the rate of $2\text{ mg}\cdot\text{g}^{-1}$ of sample and an incubation carried out at 65°C for 16 hours. The incubation was stopped by the addition of 2.5 volumes of 96% ethanol and the tubes stood in the cold. Precipitates were pelleted in the bench centrifuge and washed as in section 2.5.2.1.

2.5.2.3. *Mixed-link beta-glucan*

The extraction and analytical methods were derived from ANDERSON et al. (4), with modifications to take account of the small samples and the sequential nature of the determinations.

The starch-free pellets from section 2.5.2.2. were resuspended in 10 ml of 0.05 M-sodium maleate buffer (pH 6.5) and warmed in a waterbath to 90°C for 10 min. After cooling to room temperature 0.100 ml of lichenase solution were added to each tube and incubation carried

Figure 1.

- Flowchart representing the temporal relationship between the processes outlined in part 2.5.2.
- Outline of the procedure used to stop incubations and precipitate unhydrolysed polysaccharide.
- Outline of the procedure used to extract liberated oligosaccharide.
- Outline of the procedure used to prepare extracts for analysis.

out at 40 °C for 16 hours. Soluble sugars released by this procedure were extracted as above and prepared for further analysis.

2.5.2.4. »Hemicellulose«

This class included all beta-linked glucosans not released from the samples by previous treatments. Since the majority of these molecules are long chains of beta-1,4-linked glucose residues the term hemicellulose will be used, for convenience, if not strict accuracy. The pellets from the preceding procedure were resuspended in 9 ml of 0.1 M-sodium acetate buffer (pH 5.0) and 1 ml of the buffer, containing 5 mg each of the cellulases described in section 2.1, were added to each. The incubations were carried out at 40 °C for 24 hours and the released sugars extracted as described in section 2.5.2.1. The procedures outlined in section 2.5.2. are summarised in Figure 1.

2.5.3. Estimation of released sugar

2.5.3.1. Free sugars

Estimation was by the method of Dubois et al. (10), modified for use on a smaller scale. To 0.5 ml of a solution containing 0.020–0.100 mg of sugar per ml, 0.050 ml of a mixture of 4 parts of phenol to 1 part of water (w/w) was added, and to this mixture, 1.5 ml of 97% H₂SO₄ were added and mixed immediately, to ensure the most sensitive colour reaction (10). After 30 min a further 1.5 ml of H₂SO₄ were added and the absorbance measured in a Jasco Uvidec-1 spectrophotometer, against a blank containing no sugar, at 490 nm. Quadruplicate samples were prepared to guard against error due to contamination and insufficient mixing. It was found necessary to mix the samples thoroughly in the cuvette to obtain stable absorbance values.

Provided these precautions were taken standard deviations between replicates of less than 2% were obtained. Absorbance values were converted to sucrose equivalents by comparison to sucrose standards and a standard curve.

2.5.3.2. Glucose-containing hydrolysates

The starch extract could be assayed directly, but others had first to be hydrolysed to glucose. 1.5 ml of the aqueous extract were placed in a 4 ml glass tube, and 0.5 ml of 2 M-H₂SO₄ added. The tube was placed in a larger tube which was sealed and heated to 100 °C for 4 hours. This treatment breaks glycosidic linkages without breaking internal bonds of glucose (8). At the conclusion of the treatment the tube was opened and the contents of the smaller tube neutralised with 2 ml of 1 M-NaOH.

Hydrolysates were analysed for glucose content using the glucose oxidase method (section 2.1) with some modifications, in that only 4 ml of test solution were used, and that triplicates were analysed. Standards were run at the beginning of each batch, since generally more than the recommended number of samples were run at a time. Standard deviation of test results was typically less than 1%. Results were calculated as glucose released and converted to polysaccharide hydrolysed using a factor of 0.9 (formula weight glucoside residue divided by molecular weight of glucose).

2.5.4. Protein content

Protein content of samples from the phytotron experiment was measured using a semi-automated Kjeldahl method (Tecator Instruments AB, Helsingborg, Sweden) with a selenium catalyst and converted to protein using the factor $6.25 \times N$.

Figure 2. Accumulation of glucosans in Bomi barley.

a. Accumulation of free sugar. (●) free sugar as a percentage of endosperm dryweight. (■) free sugar content, mg per endosperm. b. Accumulation of drymatter (●) and starch (■) in mg per endosperm. c. Accumulation of mixed-link beta-glucan (■) and hemicellulose (●) in mg per endosperm. Horizontal axes: days after fertilisation.

3. RESULTS

3.1. Growth room study

Results are given in graphical form in Figure 2. Figure 2a shows how free sugar content fluctuated in the developing endosperm, in both

percentage terms and in terms of mg per endosperm. Percentage free sugar content follows a well-established path, increasing to a peak of about 50% at 10–12 days after flowering, then declining until maximum drymatter content is reached, when a second small peak may be observed. The curves found for Bomi in this experiment and in the field in 1976 by KREIS (26) are very similar in shape. The discontinuity between day 13 and day 15 arose because day 15 was the first day it was possible to extract an endosperm from the pericarp. Previously the pericarp was included in the samples, but only the curve of mg free sugar per endosperm was affected. Measurement of free sugar content of kernels with and without pericarp at 15 days gave similar results. Starch content is low until

day 13, but begins to increase rapidly between days 13 and 15, in tandem with endosperm dryweight, and reaches a maximum at day 31, at which time dryweight also peaks.

Hemicellulose and mixed-link beta-glucan accumulation curves are given in Figure 2c. First evidence of synthesis is found at 11 days for hemicellulose, and at 17 days for mixed-link beta-glucan, but synthesis lags behind endosperm dryweight increase until 15 days and 21–23 days, respectively.

3.2. Field study

The results of this investigation are presented in Table I and Figures 3 and 4.

Table I

Analysis of malt prepared from the lines of Minerva, Lami and Triumph used in the field experiment (section 3.2)

	Minerva	Lami	Triumph
Kernel protein, %	13,1	11,6	12,2
Wort Viscosity, cP	1,80	1,71	1,61
Extract, %	76,9	77,6	81,2
Soluble N mg·100 ml ⁻¹	80	80	98

Table I indicates the malting quality of the lines of the three cultivars that were investigated. The data obtained rank the cultivars Triumph – Lami – Minerva in descending order of malting quality by virtue of the decrease in extract and increase in wort viscosity found when listing the cultivars in this order. This is the result normally obtained when comparing these cultivars.

Figure 3a gives the results of dryweight determinations on endosperms of the three cultivars at each sampling date, and the curve of moisture content decline in Lami over the sampling period. After an initial period of very high moisture content the decline from 65 % at day 14 to 43 % at day 38 is linear. Thereafter the decline is steeper.

The curves of endosperm drymatter accumulation coincide closely with one another until day 45, after which Triumph stops. The final outcome was that Triumph had a somewhat lower final endosperm dryweight than Minerva or Lami.

Free sugar curves are presented in Figure 3b and can be used to relate the time scale of this experiment to that of the growth room experiment (section 3.1). The two peaks of free sugar content occur at days 10 and 35 in this experiment, and the comparable peaks are found in Bomi at days 13 and 29, implying that

Figure 3. Accumulation of glucosans in Minerva (O), Lami (▽) and Triumph (□) as a function of time.

a. Accumulation of drymatter (ascending curves). The descending curve gives the moisture content of Lami endosperms during the growth period.

b. Free sugar content of Lami endosperms during the growth period. (Solid triangles: % of dryweight. Open triangles: mg per endosperm). Horizontal axes: days after fertilisation.

Figure 4. Accumulation of glucosans as a function of endosperm dryweight (Symbols as for Figure 3)
 a. Accumulation of starch. b. Accumulation of mixed-link beta-glucan. c. Accumulation of hemicellulose.

development was 9 days slower in the field than in the growth room.

In order that the results obtained from the three cultivars might be compared directly, data were plotted against endosperm dryweight. Figure 4a shows that, as expected, starch content and dryweight were highly correlated for all three cultivars, but that Lami had a higher starch

content than Minerva or Triumph in the final stages of kernel growth.

Figure 4b compares the accumulation curves of mixed-link beta-glucan for the three cultivars in the experiment. Two different curve shapes are obtained. Minerva accumulates mixed-link beta-glucan at a rate that is relatively high initially, and that increases smoothly as endo-

sperm weight increases. Lami and Triumph, on the other hand, commence with a relatively low rate of accumulation, which, in the case of Lami, increases to that of Minerva when the endosperm weight increases to 18 mg. When endosperm weight reaches 32 mg Triumph shows a sudden large increase in accumulation rate, a phenomenon also observed in Lami at 37 mg dryweight. This endosperm weight corresponds to the time that endosperm moisture content falls below 43 %, and moisture loss becomes uncontrolled.

The effect of this spurt is to bring mixed-link beta-glucan content to 85 % of that of Minerva. The accumulation rate then falls, so that by the time that maximum dryweight is obtained the mixed-link beta-glucan content is only 75 % of that of Minerva at the same kernel weight. As noted above, this phenomenon also occurs in Lami, in a more exaggerated way. The mixed-link beta-glucan: dryweight ratio *exceeds* that of Minerva, before finally declining due to a *negative* rate of accumulation, so that when final drymatter content is achieved, the content of mixed-link beta-glucan is only 75 % of that in Minerva at the same weight.

Figure 4c compares hemicellulose accumulation curves for the three cultivars. Features of the curves are the very similar patterns observed initially, and the sudden increase in rate in Triumph and Minerva when endosperm dryweight reaches 35 mg. This effect, which is not observed in Lami, is connected with the fall of endosperm moisture content below 43 %.

3.3. Phytotron study

Free sugar curves were similar to each other for Triumph and Minerva, and for all treatments, and indicated the same rate of development as found in Bomi in the growth room. The picture obtained for endosperm development was much as expected. Figure 5a gives the curves of drymatter accumulation for endosperms from treatments 1 (rain) and 2 (root-watering) for both Minerva and Triumph, and it can be seen from this that provision of water as rain leads to lower endosperm weights at all stages, except in Minerva at day 35. The effect of the rain is greater on Triumph than on Minerva.

Figures 5c and 5e give the curves of drymatter accumulation in treatments 3–6 for Triumph and Minerva respectively. In Triumph it can be seen that any imposition of drought (treatments 4, 5 or 6) leads to a check in dryweight increase. It is particularly interesting to observe that dryweight increases relatively rapidly at the end of the period of observation in treatment 6, where an initial stress period is supplanted by normal watering at 20 days after flowering. The increase commences after about day 26 (Figure 5c) at which time, nevertheless, this phenomenon only represents a process of catching up after an initial depression of dryweight. The same phenomenon may be observed in Minerva (Figure 5e), but in this case the depression of dryweight accumulation that was observed in Triumph when late drought stress was applied, did not occur. Consequently, the final drymatter yield in this cultivar was determined by the

Figure 5. Comparison of dryweight and mixed-link beta-glucan accumulation with time after flowering in the phytotron experiments.

The legend inscribed on a curve denotes the watering regime that led to the curve.

5a. Drymatter accumulation for rain (treatment 1) and root watering (treatment 2) M = Minerva, T = Triumph.

5b. Mixed-linked beta-glucan accumulation for rain (treatment 1) and root watering (treatment 2). M = Minerva, T = Triumph.

5c. Drymatter accumulation in treatments 3–6 for Triumph.

5d. Mixed-link beta-glucan accumulation in treatments 3–6 for Triumph.

5e. Drymatter accumulation in treatments 3–6 for Minerva.

5f. Mixed-link beta-glucan accumulation in treatments 3–6 for Minerva. For clarity's sake treatment numbers are included in the legends.

imposition of drought at the beginning of the grain growth period, in contrast to Triumph.

The curves of mixed-link beta-glucan accumulation presented in Figure 5b for both Minerva and Triumph clearly show that provision of water in the form of rain gives lower levels of mixed-link beta-glucan content than if similar quantities are supplied directly to the roots. This effect is quite clear in Triumph, even when the difference in the drymatter curves is taken into account. The data obtained thus confirm the results reported by AASTRUP (1).

The data obtained in the drought stress experiment (treatments 3–6) are presented in Figures 5d and 5f. Curves of mixed-link beta-glucan content for normally-watered samples of Triumph (Figure 5d) and Minerva (Figure 5f) follow the drymatter accumulation pattern until day 26, but from then on total content declines, albeit to a much larger extent in Triumph. Another point of importance in evaluating this experiment is the severe effect continuous drought (treatment 4) had on the growth of endosperms. Kernel moisture content in both Minerva and Triumph declined below 20% at day 24 and gave rise to severe shrivelling. The effect of this can be observed in the mixed-link beta-glucan curves where, after day 24, no further accumulation took place. Consequently, kernels from this treatment ended up with the lowest mixed-link beta-glucan contents in absolute terms, but with percentage contents that were between those normally-watered and those stressed for only part of the grain growth period. The protein content figures presented in Table II bear out this analysis. When the final harvest was taken at day 35, total protein was similar in all treatments except in Triumph from the rain treatment, where the final content was reduced by 30%. However, percentage protein content shows a distinct trend towards higher values as drought stress increases. Treatment 4 is an exception to this generalisation, due once again to the disruption of accumulation by forced maturation.

Returning to the figures, it remains to discuss the results obtained when partial drought stress is applied. It may be recalled that in Triumph both late drought and early drought gave rise to broadly similar figures for dryweight increase, compared to normal watering. This uniformity

Table II

Percentage protein content in phytotron produced endosperms from treatments 1–6 (section 2.4.1) at day 35.

Treatment	Minerva	Triumph
1	14.6	12.2
2	14.1	14.2
3	16.4	14.2
4	17.4	16.9
5	17.6	16.0
6	20.4	18.3

of response extends to the results obtained from analysis of beta-glucan content, so that no matter when stress is applied, a similar final result is obtained. The picture obtained for Minerva in this context is apparently quite different, in that late drought stress leads to a much higher absolute content of mixed-link beta-glucan than early stress, but if Figure 5e is recalled it will be remembered that early stress also badly affects kernel dryweight accumulation. Consequently comparison of relative accumulation patterns shows that early and late stress have similar effects, and that both treatments cause increased mixed-link beta-glucan percentages, compared to normal watering.

4. DISCUSSION

As indicated in the introduction, this study was undertaken to clarify some points concerning the accumulation of glucosan in barley. It is of interest to review briefly some of the statements made previously by various authors before proceeding.

It is generally accepted that the accumulation of starch, in absolute terms, reflects the curve of dryweight increase. The time of initiation of accumulation is disputed, however. KREIS (26) shows it commencing seven days after flowering and continuing in a more-or-less linear fashion until 35 days after flowering, when his study stopped. CERNING and GUILBOT (8) agree with this picture, whereas MACGREGOR et al. (28), under Canadian conditions, show a slightly later commencement and a very rapid *sigmoid* increase, tailing off 30 days after flowering. JEN-

KINS *et al.* (21) present rather a different picture. They claim that 40% of grain drymatter at flowering is starch, found in the pericarp, and that the major activity is not *de novo* synthesis, but transfer of pre-existing material. Their data are confounded by the presence in their samples of non-reducing sugars, pentosan and beta-glucan, which they include in the starch fraction. Finally, in an ultrastructural study, WILLIAMS and DUFFUS (40) indicate that amyloplast growth (but not necessarily chemically measurable starch deposition) commences about two days after flowering.

A second point on which clarification was required is the relationship between the various physically-fractionated classes of so-called »barley beta-glucan«, »barley gum« *etc.* ANDERSON *et al.* (4) have detailed the results obtained by various workers for content of barley beta-1,3:1,4-glucan in mature barley, and have outlined an enzymatic method which, instead of relying on the solubility of a rather heterogeneous group of polymers (12, 13) in non-standard solvent systems, relies on the solubility of a well-defined group of oligomers in a standard solvent. With slight modifications, this method was adopted in the present investigation.

Significant features of the results of the individual experiments have been pointed out in the relevant sections. It therefore remains to investigate the relationship between the sets of results from different experiments. By comparing timescales for free sugar content it can be seen that starch accumulation in both the field and growth room experiments commences at 12–13 days after flowering. Final starch contents were similar, in Lami and Bomi, to those reported by TORP (38), but lower in Minerva and Triumph. TORP's method depends on the solubility of polysaccharides in dimethyl sulphoxide, a characteristic not restricted to starch, and the increase in hemicellulose in Minerva and Triumph accounts for the difference.

The interconnection between the two classes of beta-glucosan measured is obvious when Figures 4b and 4c are compared. In all three cultivars, sudden changes in the rate of accumulation of one or other of the classes can be seen. In Triumph this change can be observed for both mixed-link beta-glucan accumulation and for hemicellulose accumulation. Nevertheless, it

seems that Triumph has its low final mixed-link beta-glucan content as a result of favouring the synthesis of hemicellulose at this stage. Alternatively, synthesized mixed-link beta-glucan may be further modified to give polymers inaccessible to lichenase (by addition of sidechains, perhaps). The fact that Lami lost mixed-link beta-glucan near maturity while hemicellulose increased is evidence for the second alternative.

It is important to note that though the field experiment was conducted in a season and an environment considered ideal for the production of low-beta-glucan barley, Triumph maintained a substantial advantage over Lami and Minerva in this respect. The phytotron experiment was carried out to see if this advantage persisted under sub-optimal conditions. The experimental protocol outlined in section 2.4.1 encompassed the entire range of moisture supply in which it is possible to grow barley successfully.

The results presented confirm that Triumph has a lower content of mixed-link beta-glucan than Minerva under any of the conditions tested, and that over the range reported mixed-link beta-glucan content behaves as predicted. AASTRUP's results (1) are thus confirmed, and it has been possible to isolate drought stress and relative humidity as variables influencing the data reported by SMART (33) and show that they are decisive factors if not the only ones affecting the final content of mixed-link beta-glucan in the barley endosperm.

Reference has already been made to the possibility of using free sugar curves to relate the time scale of experiments conducted in different environments. By comparing results obtained in sections 3.1 and 3.2 with such a time scale transformation in mind, it is possible to see that initiation of synthesis of starch, of mixed-link beta-glucan and of hemicellulose is related to specific levels of free sugar content. As noted by JENNER and RATHJEN (23, 24), however, the regulation of starch-synthesizing ability over time, as opposed to the regulation of the instantaneous rate of starch synthesis is independent of sucrose concentration. This also applies to the other glucosans under consideration.

Cessation of starch synthesis is far more likely to be due to an internal signal (23) connected to the decline in kernel moisture content. Many authors (2, 3, 8, 19, 20, 23, 24, 25, 28, however

31) have demonstrated, either explicitly or implicitly, that the stage of kernel growth where moisture content is 40–45% is when starch accumulation declines almost to zero. This stage has been referred to as »physiological maturity« (7, 17, 26) often while pointing out that changes continue to occur thereafter.

By comparing moisture content of Lami with curves of drymatter accumulation, it may be seen that, at the stage when »physiological maturity« was reached, starch accumulation was nearly complete, whereas it was at this stage that the accumulation rate of other glucosans suddenly increased (Figures 4b and 4c). These data, the continued increase of dryweight after the moisture content declined below 40%, and results obtained by other workers for protein content (3, 7, 19, 31, however 28) and other substances (2, 3, 8, 17, 19, 21, 23, 24, 31) suggest that assimilate is available at a much later stage than was previously assumed. Furthermore, unpublished data of STOY and DERERA (V. STOY, pers. commun.) from wheat show that assimilate deposited in the stem is remobilised at a very late stage and laid down in the grain, implying the existence of a functional link between the grain and the plant up until harvest ripeness.

The data obtained in this study suggest that there is unequal competition for assimilate among the various glucosan-synthesizing mechanisms, the starch mechanism being the most successful in attracting assimilate, and that it is only when the starch-synthesizing machinery declines in activity at 40–45% moisture that sufficient assimilate becomes available for the other mechanisms to work at capacity. Further work will obviously be required to test this hypothesis.

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