Phytochemistry, 1980, Vol. 19, pp. 2299–2303. © Pergamon Press Ltd. Printed in England. 0031-9422/80/1101-2299 $02.00/0

THE RELATIONSHIP OF STARCH METABOLISM TO GRAIN SIZE IN WHEAT*

R. KUMAR and R. SINGH

Department of Chemistry and Biochemistry, Haryana Agricultural University, Hissar, India

(*Revised received* 28 *January* 1980)

Key Word Index *Triticum aestivum*; Gramineae; wheat; invertase; sucrose-UDP-glucosyltransferase; UDP-glucose pyrophosphorylase; ADP-glucose pyrophosphorylase; amylase; grain size; starch content.

Abstract—The present investigation was aimed at determining the levels of important enzymes of starch metabolism at different stages of grain development in wheats differing in final grain size and starch content per grain, to ascertain whether these enzymes have some relationship with grain size and/or starch content. Active starch synthesis in these varieties started from 14 days onward and continued till 35 days after anthesis. Invertase was active only during initial stages of grain development. Sucrose-UDP-glucosyltransferase had maximum activity at the 14 and/or 21 day stage and was present throughout the period of grain development. UDP- and ADP-glucose pyrophosphorylases and amylase were most active during the period of active starch synthesis and at the same time tended to parallel grain size and starch content at different stages of grain development.

INTRODUCTION

The rate of accumulation of starch in the cereal grain is one of the determinants of grain size and therefore of yield. Sucrose is the principal form in which carbohydrates are transported from photosynthetic tissue to grain via phloem and starch is synthesized from it in grains. The direct relationship between the concentration of sucrose in the cells of the endosperm and the rate of starch synthesis, not only in isolated endosperm but also in detached ears [1], suggested that synthesis of starch is regulated by the concentration of sucrose. Termination of the accumulation of starch as the grain matures could, therefore, be conceivably attributed to either the loss of synthetic capacity of the endosperm, or to cessation of the supply of assimilates to the grain, or both. However, recent investigations [2–4] have indicated that the normal pattern of accumulation of dry matter in the endosperm is determined within the endosperm itself, rather than through the provision of precursors from the rest of the plant.

The biochemistry of the sucrose–starch conversion in the cereal grains is well established now and the changes in the activities of various enzymes involved in this process have been studied during grain development in corn [5,6], barley [7–9], rice [10] and wheat [11]. Ghosh and Preiss [12, 13] proposed that the levels of ADPG, the production of which is controlled by the activity of ADP-glucose pyrophosphorylase, regulate the synthesis of starch. Tsai and Nelson [14] found the maize mutant Shrunken-2 to synthesize only 25–35% as much starch as normal maize. This mutant lacked ADP-glucose pyrophosphorylase. Similarly, Dickinson and Preiss [15] and Singh *et al.* [16] also suggested that the ADP (UDP)-glucose pyrophosphorylase step is rate-limiting in starch accumulation in developing cereal grains. However, according to Murata [17], starch accumulation is limited by sucrose-UDP(ADP)-glucosyltransferase activity. Amylase activity has been shown to be inversely related to grain density [18] which in turn was significantly correlated with the index of shrivelling and starch content of grain.

The present communication reports the levels of invertase, sucrose-UDP-glucosyltransferase, UDP- and ADP-glucose pyrophosphorylases and amylase at various stages of grain development in wheats differing in final grain size and starch content/grain. The activities of the above enzymes have further been correlated with grain size and starch content.

RESULTS AND DISCUSSION

Starch

Only very little starch was present at the 7 day stage (Fig. 1). Subsequently it increased rapidly from 0.2–0.4 mg per grain at the 7 day stage to 30–39 mg per grain at the 42 day stage. The maximum per cent increase in starch content was found in the intervals from 14 to 21 days. C-306, except at 28 days, had the maximum starch content per grain throughout the course of the study. HD-2009 had the minimum value for starch up to the 28 day stage and thereafter it crossed C-591. Until 21 days after anthesis, there was no significant difference in starch content of WH-157 and C-591. Thereafter at the 28 day stage, C-591 had a higher starch content than WH-157. While starch synthesis was still active in WH-157, HD-2009 and C-306, it showed only slight increase in C-591 after the 28 day stage, resulting in the lower content of starch at the final stage. Turner [11] while working on developing wheat

* The work presented here forms a part of the thesis submitted by R. K. to the Haryana Agricultural University, Hissar, in partial fulfillment of the requirements for the M.Sc. degree.

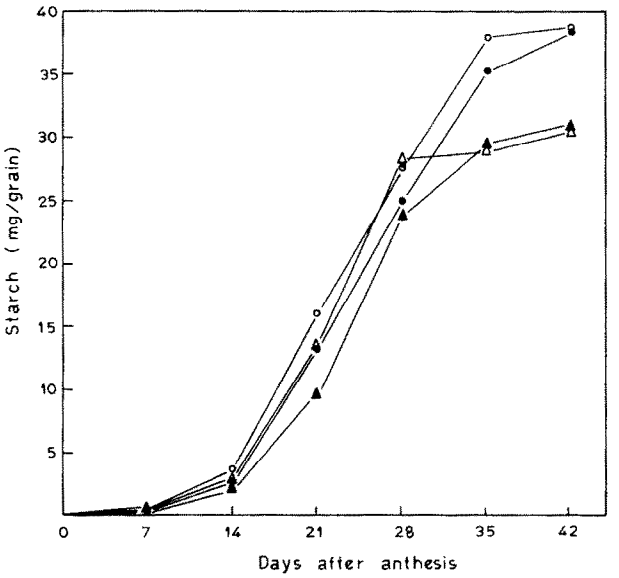

Fig. 1. Starch (mg/grain) in grains of WH-157 (●—●), C-306 (○—○), HD-2009 (▲—▲) and C-591 (△—△) after anthesis.

grains found the starch content to increase up to 38 days after anthesis.

Invertase

Fig. 2 shows invertase activity in grains at different times after anthesis. The enzyme showed maximum activity 7 days after anthesis in all wheats except HD-2009, where maximum activity was observed at 14 days. Subsequently, the activity decreased in all cases and disappeared at the 21–28 day stage. Maximum activity at the 7 day stage was obtained in C-591, followed by C-306 and WH-157.

Activity of this enzyme when compared to that of sucrose-UDP-glucosyltransferase (Fig. 3) was low indicating that this enzyme could not account for all of the metabolism of sucrose transported from leaves. At the same time, since the rate of starch synthesis increased with maturity and attained maximum rate only after 14 days from anthesis (Fig. 1) by which time invertase activity was almost over, it could not be implicated in starch synthesis in developing grains. However, its role in the initial stages of grain development cannot be ruled out and may be co-ordinating with sucrose-UDP-glucosyltransferase during the early periods of grain development. Alternatively, it may provide the substrates for respiration during the early phase of grain growth. A similar pattern of activity has been reported in rice [10, 19], corn [5, 20, 21] and barley [8].

Sucrose-UDP-glucosyltransferase

Activity could be detected in the very first sampling (7 day stage) with a subsequent increase until 14 days in C-306 and WH-157 and 21 days in C-591 and HD-2009, followed by a decline until maturity in all wheats (Fig. 3). However, the enzyme showed its activity until the last sampling in all cases. Higher levels of this enzyme from 14 days onwards co-incided with the maximum rate of starch synthesis, though with no parallelism with grain size and/or starch content, as at a number of stages the wheats WH-157, C-306 with larger grains and more starch content per grain had lower activity than that of wheats HD-2009, C-591 with smaller grain size and low starch content per grain. However, its activity was more than that of invertase at all stages.

By reversing the reaction for sucrose synthesis, this enzyme catalyses the formation of UDP-glucose from UDP and sucrose. The fructose released could be phosphorylated by hexokinase to fructose-6-phosphate and with the help of phosphoglucoisomerase and

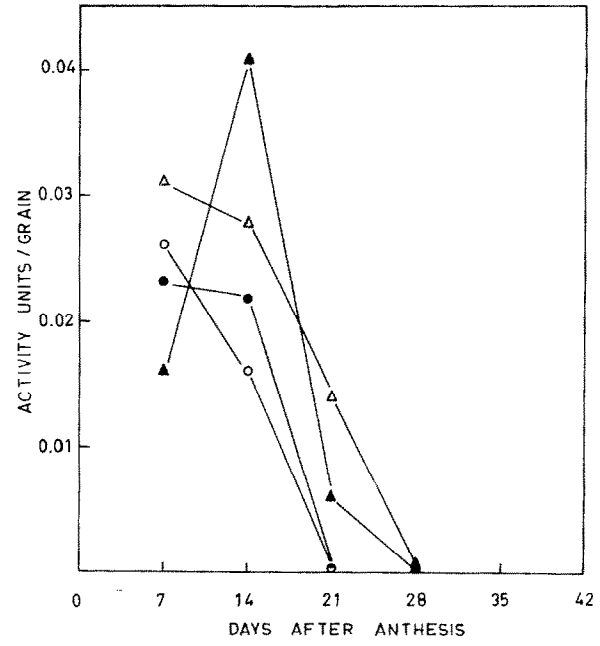

Fig. 2. Invertase activity in grains of WH-157 (●—●), C-306 (○—○), HD-2009 (▲—▲) and C-591 (△—△) after anthesis.

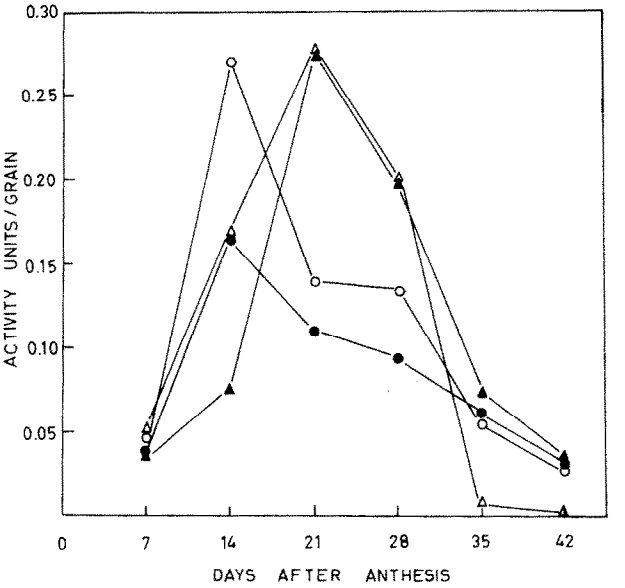

Fig. 3. Sucrose-UDP-glucosyltransferase activity in grains of WH-157 (●—●), C-306 (○—○), HD-2009 (▲—▲) and C-591 (△—△) after anthesis.

phosphoglucomutase it could be converted to glucose-1-phosphate [8, 10, 11, 22, 23] which can then enter the ADP-glucose pool by ADP-glucose pyrophosphorylase [11]. UDP-glucose is acted upon by UDP-glucose pyrophosphorylase to give glucose-1-phosphate and UTP. The glucose-1-phosphate formed by this route is also utilized by ADP-glucose pyrophosphorylase. The reversible phosphate transfer between ATP and UTP may be brought about by nucleoside diphosphokinase, which is present in wheat [24] and other cereals [8, 10]. Sucrose-UDP-glucosyltransferase and UDP-glucose pyrophosphorylase are, therefore, the enzymes responsible for bringing sucrose into grain metabolism.

UDP-glucose pyrophosphorylase

Fig. 4 shows the changes in UDP-glucose pyrophosphorylase activity of different wheats at various stages after anthesis. The activity was very low during the earlier stages of grain development but increased steadily after 7 days from anthesis to reach a maximum at 28 days and then declined until the final sampling. C-306 had the highest activity of this enzyme throughout the course of the study and the minimum level was observed in HD-2009 until 28 days. After this stage, HD-2009 crossed C-591 and at the final stage, the activity was found to be minimum in C-591. The level was comparable in the case of WH-157 and C-591 up to 28 days, after which there was a very sharp decline in C-591, with the result that the level of this enzyme in all other varieties at the 35 day stage was 2 to 3-fold higher than that of C-591.

Starch content per grain was also highest in C-306 throughout the course of the investigation and minimum in HD-2009 until 28 days (Fig. 1). However, the values were

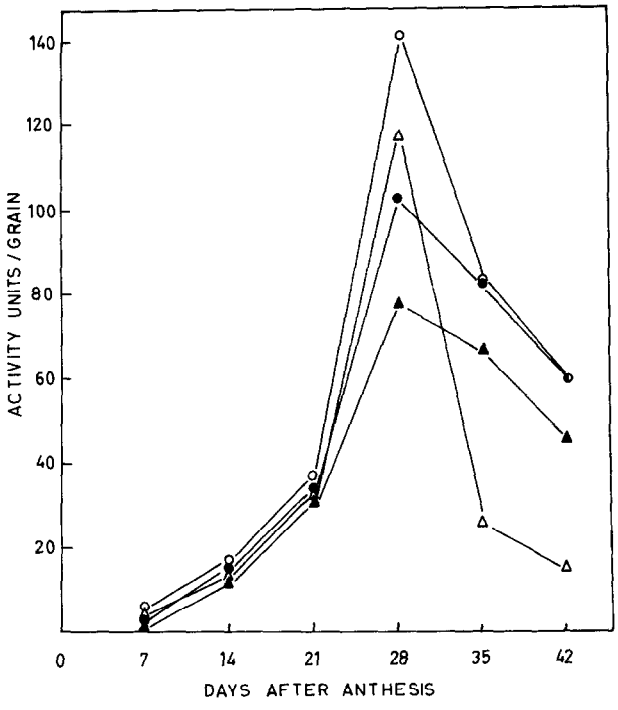

Fig. 4. UDP-glucose pyrophosphorylase activity in grains of WH-157 (●—●), C-306 (○—○), HD-2009 (▲—▲) and C-591 (△—△) after anthesis.

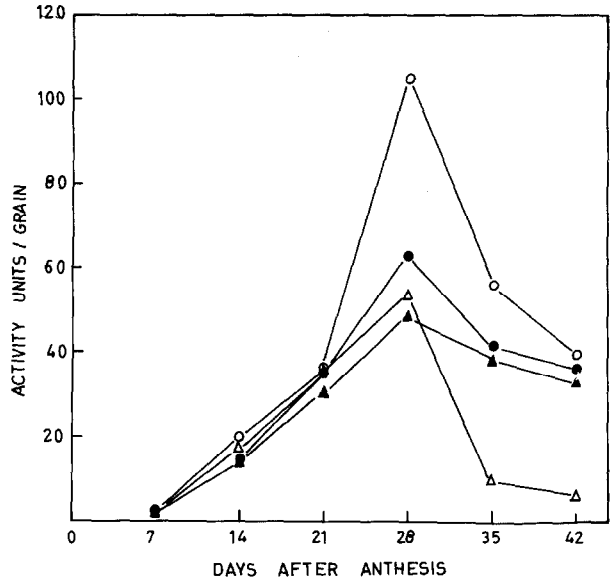

Fig. 5. ADP-glucose pyrophosphorylase activity in grains of WH-157 (●—●), C-306 (○—○), HD-2009 (▲—▲) and C-591 (△—△) after anthesis.

similar in C-591 and WH-157 up to 28 days. Thereafter, it increased significantly in WH-157 but did not show much increase in C-591. It could, therefore, account for the abrupt fall in activity of this enzyme from the 28th to the 35th day in C-591.

ADP-glucose pyrophosphorylase

The level of this enzyme was also very low at the first sampling but increased thereafter to attain a maximum value at 28 days and then declined (Fig. 5). The pattern of activities in the four wheats was identical to that of UDP-glucose pyrophosphorylase and tended to parallel grain size and starch content. However, the activity of UDP-glucose pyrophosphorylase was more than that of ADP-glucose pyrophosphorylase throughout the course of the experiment. This is in agreement with earlier reports [6, 10, 11]. However, in barley [8], the level of ADP-glucose pyrophosphorylase at 20 days was higher than that of the UDP-glucose pyrophosphorylase.

Both UDP-glucose pyrophosphorylase and ADP-glucose pyrophosphorylase increased markedly during the phase of active starch synthesis. There was an almost 7 to 8-fold increase in UDP-glucose pyrophosphorylase and 3 to 5-fold increase in ADP-glucose pyrophosphorylase activity per grain from 14 to 28 days after anthesis. This period co-incided with the onset of the phase of active starch synthesis. Starch synthesis ceased 35 days after anthesis and during this period the activity of both these enzymes decreased rapidly. This confirms the earlier findings of Turner [11], who observed that activities of both these enzymes increased during the phase of active starch synthesis in developing wheat grain. Jenner [25] studied the composition of the soluble nucleotides of the developing wheat grain and reported that the amounts of UDP-glucose and ADP-glucose more than doubled between 10 and 20 days after anthesis and this was the period when active starch synthesis commenced. He further concluded that the level of ADP-glucose is closely

related to the onset of starch synthesis in the endosperm. All these findings, therefore, indicate that UDP-glucose pyrophosphorylase and ADP-glucose pyrophosphorylase reactions allow rapid conversion of sucrose to starch. The mechanism for the above is already well documented [8, 10, 11].

Amylase

Activity in all cases increased steadily from 7 days onwards to attain a maximum value at 35 days (Fig. 6). HD-2009 had minimum activity until 28 days. At 35 days, maximum activity was observed in C-306 followed by WH-157, HD-2009 and C-591. Here also the enzyme level was comparatively higher in wheats having larger grains and more starch content per grain.

High amylase activity during the period of starch synthesis has also been reported by others in leaves and developing grains [26–31]. If the amylase had been responsible for the breakdown of starch in the endosperm, there would have been a decline in starch content and an increase in reducing sugar content of the grains. But such a relationship was not observed in this laboratory [4]; on the contrary, there had been a continuous increase in the accumulation of starch during the period of maximum amylase activity. The above facts do not allow amylase activity to be related to starch degradation. Positive correlations between amylase activity and starch synthesis in different wheats throughout the course of the experiment indicate that this enzyme functions at a site other than that of starch synthesis. During the early stages of wheat grain development, the amylase activity was reported to be localized almost entirely in the pericarp fraction of the grain and the amount in the endosperm was negligible [29, 31]. There may be some amylase functioning in the endosperm which may have the role of increasing the number of primer molecules by degrading starch. The magnitude of such degradation may be so small that during the period of active starch accumulation, it is not observable in this kind of study. However, the formation of primer molecules from starch in rice has been ascribed to phosphorylase [28].

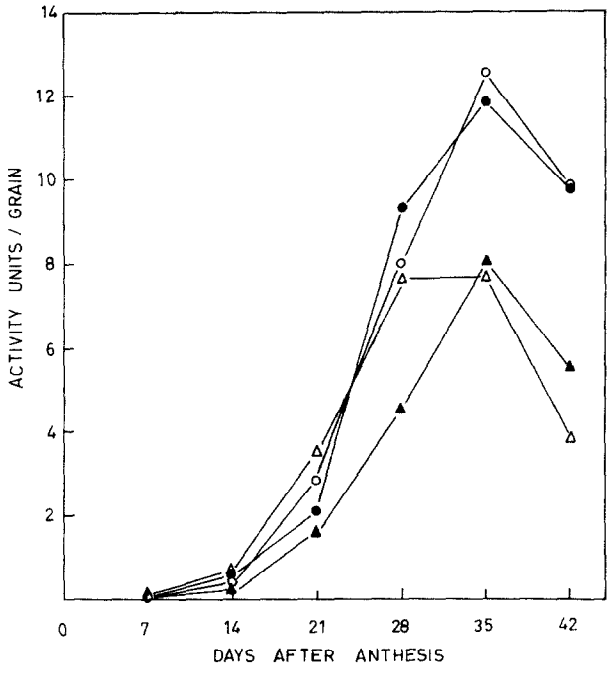

Fig. 6. Amylase activity in grains of WH-157 (●—●), C-306 (○—○), HD-2009 (▲—▲) and C-591 (△—△) after anthesis.

Relationship between enzyme levels and grain size and/or starch content

Varietal differences in respect of various enzyme activities at different stages of grain development were highly significant ($P < 0.01$). Although UDP-glucose pyrophosphorylase and ADP-glucose pyrophosphorylase had highly positive correlations with grain size and starch content at each stage of development, the r values were not significant except at the 14 day stage in the case of UDP-glucose pyrophosphorylase with grain size. Amylase was significantly ($P < 0.05$) correlated at the 35 and 42 day stages with starch content. Sucrose-UDP-glucosyltransferase was significantly ($P < 0.05$) correlated with both grain size and starch content only at the 14 day stage. Within each variety at different stages, amylase had highly significant ($P < 0.01$) positive correlations with starch content in all varieties. UDP-glucose pyrophosphorylase was also significantly ($P < 0.05$) correlated with starch content in all varieties except C-591. ADP-glucose pyrophosphorylase, however, had high positive r values but was significantly correlated only in HD-2009. Though amylase had highly significant correlations with starch content within varieties and even with grain size and starch content at some stages across varieties, no specific role could be assigned to this enzyme in starch synthesis. As UDP- and ADP-glucose pyrophosphorylases were most active during the period of maximum starch synthesis and at the same time tended to parallel grain size and starch content at different stages of grain development, the above may be considered rate-limiting steps in starch accumulation in developing wheat grains. In rice also, UDP-glucose pyrophosphorylase activity was shown to increase with increasing grain size [16].

Since the grain size across varieties did not have any effect on economic yield ($r = 0.02$), none of the parameters investigated here was limiting the economic yield. Rather the yield depended upon the sink size, as was evident from the significant ($P < 0.05$) positive correlations between economic yield and different yield components. It may, therefore, be proposed that the sink size in wheat may be increased to obtain increased grain production.

EXPERIMENTAL

Plant material. Wheat varieties, namely WH-157 and C-306 having larger grains (final dry wt 44.7 and 45.9 mg/grain, respectively) and more starch per grain (final starch content 38.4 and 38.6 mg/grain, respectively) and HD-2009 and C-591 having smaller grains (final dry wt, 37.4 and 36.0 mg/grain, respectively) and less starch per grain (final starch content, 31.0 and 30.8 mg/grain, respectively), were grown in the field following the recommended agronomic practices. About 600 earheads per replication (3 replications in each treatment) were tagged in all 4 wheats at the start and at the same stage of anthesis. Tagging was done in such a way that samples from 3 replications of the same wheat came on 1 day. First sampling was made 7 days after anthesis and then at weekly intervals until maturity of crops.

Estimation of starch. Starch in dried grains was estimated by the procedure of ref. [32].

Enzyme extraction. 10 earheads harvested randomly from each replication were brought to the laboratory in a polythene bag buried in an ice-bucket. 3 spikelets from the upper and 3 from the lower end of each earhead were discarded. 1 g of grains (whole grains, including both embryo and pericarp) removed randomly from earheads were hand-homogenized at 0° in a mortar and pestle with 0.1 M Tris–maleate buffer, pH 7. The homogenate was centrifuged at 10000 g for 30 min at 0° and the supernatant decanted. The residue was washed once with extracting buffer and contrifuged as before. The combined extract, made to a known vol., served as the enzyme prepn.

Enzyme assays. Invertase and sucrose-UDP-glucosyltransferase were assayed by the methods described in ref. [21]. 1 unit of enzyme in each case is defined as the amount of enzyme capable of utilizing 1 μmol of sucrose per min at 35°. UDP- and ADP-glucose pyrophosphorylases were assayed spectrophotometrically by coupling a product of the reaction to the G-6-P dehydrogenase reaction [33]. 1 unit of enzyme is the amount which catalyses the production of 1 nmol of G-1-P per min at 30°. Amylase was assayed according to ref. [34]. Maltose in the supernatant was estimated colorimetrically [35]. 1 enzyme unit is the amount required to produce 1 mg of maltose per min of incubation at 20°. Each enzyme was assayed in duplicate. Hence, the value at each stage in all cases is the average of 6 estimations.

Acknowledgements—The authors thank Shri D. R. Vasudeva for providing the seed material and Dr. D. S. Wagle, Professor and Head, Department of Chemistry and Biochemistry for providing the necessary facilities to carry out the present investigation. Dr. S. D. Dhiman is thanked for his help in raising the crop in the field.

REFERENCES

1. Jenner, C. F. (1970) *Aust. J. Biol. Sci.* **23**, 991.
2. Jenner, C. F. and Rathjen, A. J. (1977) *Aust. J. Plant Physiol.* **4**, 691.
3. Singh, R. and Juliano, B. O. (1977) *Plant Physiol.* **59**, 417.
4. Kumar, Rakesh (1979) M. Sc. Thesis, Haryana Agricultural University, Hissar.
5. Tsai, C. Y., Salamini, F. and Nelson, O. E. (1970) *Plant Physiol.* **46**, 229.
6. Ozbun, J. L., Hawker, J. S., Greenberg, E., Lammel, C., Preiss, J. and Lee, E. Y. (1970) *Plant Physiol.* **51**, 1.
7. Baxter, E. D. and Duffus, C. M. (1971) *Phytochemistry* **10**, 2641.
8. Baxter, E. D. and Duffus, C. M. (1973) *Phytochemistry* **12**, 1923.
9. Baxter, E. D. and Duffus, C. M. (1973) *Phytochemistry* **12**, 2321.
10. Perez, C. M., Perdon, A. A., Resurreccion, A. P., Villareal, R. M. and Juliano, B. O. (1975) *Plant Physiol.* **56**, 579.
11. Turner, J. F. (1969b) *Aust. J. Biol. Sci.* **22**, 1321.
12. Ghosh, H. P. and Preiss, J. (1965) *Biochemistry* **4**, 1354.
13. Ghosh, H. P. and Preiss, J. (1966) *J. Biol. Chem.* **241**, 4491.
14. Tsai, C. Y. and Nelson, O. E. (1966) *Science* **151**, 341.
15. Dickinson, D. B. and Preiss, J. (1969) *Plant Physiol.* **44**, 1058.
16. Singh, R., Perez, C. M., Pascual, C. G. and Juliane, B. O. (1978) *Phytochemistry* **17**, 1869.
17. Murata, T. (1972) *Jpn. Agric. Res. Q.* **8**, 127.
18. Klassen, A. J., Larter, E. N. and Hill, R. D. (1971) *Crop Sci.* **11**, 265.
19. Murata, T., Sugiyama, T., Minamikawa, T. and Akazawa, T. (1966) *Arch. Biochem. Biophys.* **113**, 34.
20. Jaynes, T. A. and Nelson, O. E. (1971) *Plant Physiol.* **47**, 623.
21. Shannon, J. C. and Dougherty, C. T. (1972) *Plant Physiol.* **49**, 203.
22. De Fekete, M. A. R. and Cardini, C. E. (1964) *Arch. Biochem. Biophys.* **104**, 173.
23. De Fekete, M. A. R. (1969) *Planta* **87**, 311.
24. Kirkland, R. J. A. and Turner, J. F. (1959) *Biochem. J.* **72**, 716.
25. Jenner, C. F. (1968) *Plant Physiol.* **43**, 41.
26. Gates, J. W. and Simpson, G. M. (1968) *Can. J. Botany* **46**, 1459.
27. Haapala, N. (1969) *Physiol. Plant.* **22**, 140.
28. Baun, L. C., Palmiano, E. P., Perez, C. M. and Juliano, B. O. (1970) *Plant Physiol.* **46**, 429.
29. Sandstedt, R. M. and Beckord, O. C. (1946) *Cereal Chem.* **23**, 548.
30. May, L. H. and Buttrose, M. S. (1959) *Aust. J. Biol. Sci.* **12**, 146.
31. Meredith, P. and Jenkins, L. D. (1973) *Cereal Chem.* **50**, 243.
32. Clegg, K. M. (1956) *J. Sci. Food Agric.* **7**, 40.
33. Turner, J. F. (1969a) *Aust. J. Biol. Sci.* **22**, 1145.
34. Noelting, G. and Bernfeld, P. (1948) *Helv. Chim. Acta* **31**, 286.
35. Summer, J. B. and Howell, S. F. (1935) *J. Biol. Chem.* **108**, 51.