Changes in α-Amylase Enzymes During Germination[1,2]

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ABSTRACT

The development of α-amylase enzymes was studied in Conquest, Betzes, and Klages barleys during germination in the presence and absence of added gibberellic acid (GA). Ion-exchange chromatography and isoelectric focusing were used to follow changes in individual α-amylase components. Although each barley contained the same three main groups of α-amylases there were varietal differences within the groups. GA had no effect on the α-amylase patterns but did accelerate the formation of α-amylase components in Betzes and Klages.

Key words: *α-Amylase, Barley, Germination, Gibberellic acid.*

An adequate level of α-amylase activity is a desired property of a good-quality barley malt. The general time course of α-amylase development during barley germination was established many years ago (11). It is now widely accepted that the large increase in α-amylase activity found during germination is caused by *de novo* synthesis of the enzyme (3,22). Other studies have shown that enzyme synthesis can be accelerated by the addition of gibberellic acid (GA) to germinating kernels (6,19). In addition, deembryonated kernels and isolated aleurone layers will synthesize α-amylase only in the presence of GA (22). These results suggest that during germination GA moves from the embryo to the aleurone layer and there activates the mechanism for α-amylase synthesis.

The use of new separation techniques such as disc electrophoresis and isoelectric focusing has shown that germinated barley α-amylase is not a single entity but is a complex mixture of enzymes. The number of components in this mixture is in dispute and values from 1 to 7 have been reported (1, 5, 8, 13, 16, 17, 20, 21).

Despite numerous investigations, the effect of GA on the germination pattern of different barley cultivars has not been widely studied (7,18). There is even less information available on the effect of GA on the rate of synthesis of individual α-amylase enzymes (8). Therefore, this study was initiated to obtain information on the formation of individual α-amylase components during germination in the malting barley cultivars Conquest, Betzes, and Klages. The effect of externally added GA on α-amylase synthesis in these cultivars was studied also.

EXPERIMENTAL

Germination

All kernels were soaked for 20 min in sodium hypochlorite solution (1.5%) and rinsed thoroughly with deionized, sterile water. Germinations were carried out at 18°C in sterile petri dishes, each containing three pieces of Whatman No. 1 filter paper, 50 kernels, and 4 ml of either water or GA solution (0.0001 *M*). All solutions contained penicillin (2.5 mg/500 ml) to inhibit bacterial growth. Samples (30 kernels) were removed after 2–6 days of germination and, after removal of shoots and rootlets, were deep-frozen until analyzed. Replicate germinations were carried out and the results obtained within each cultivar were similar.

Enzyme Extraction

Thirty kernels were ground in a mortar with fine sand and 15 ml of sodium acetate buffer (0.01 *M*, pH 4.75, 0.001 *M* thioglycerol, 0.01 *M* calcium chloride), centrifuged (25,000 × *g*, 10 min), and filtered through Whatman No. 1 filter paper. Portions of the clear extract were used for α-amylase assays, isoelectric focusing analysis and ion-exchange chromatography.

α-Amylase Activity

This was determined as described previously (2,14).

Isoelectric Focusing

This was carried out as described previously (12). α-Amylase enzymes were detected using the amylopectin β-limit dextrin plate technique described previously (15). This is specific for α-amylases.

Ion Exchange Chromatography

Small columns (6 × 1.5 cm) were prepared containing carboxymethyl cellulose (CMC) and were equilibrated with 0.01 *M* sodium acetate buffer (pH 4.75, 0.001 *M* thioglycerol, 0.01 *M* calcium chloride). A portion (3 ml) of the enzyme extract was placed on the column, washed in with a portion of the sodium acetate buffer, and eluted with a linear gradient formed with 250 ml of 0.01 *M* sodium acetate buffer (pH 4.75, 0.001 *M* thioglycerol, 0.01 *M* calcium chloride) and 250 ml of 0.3 *M* sodium acetate buffer (pH 4.75, 0.001 *M* thioglycerol, 0.01 *M* calcium chloride). The column flow-rate was set at 120 ml/hr and fractions were collected at 10-min intervals.

Fractions were assayed for α-amylase and those containing activity were pooled either as α-amylase 1 or as α-amylase 2. Each pool was then assayed quantitatively for α-amylase and analyzed

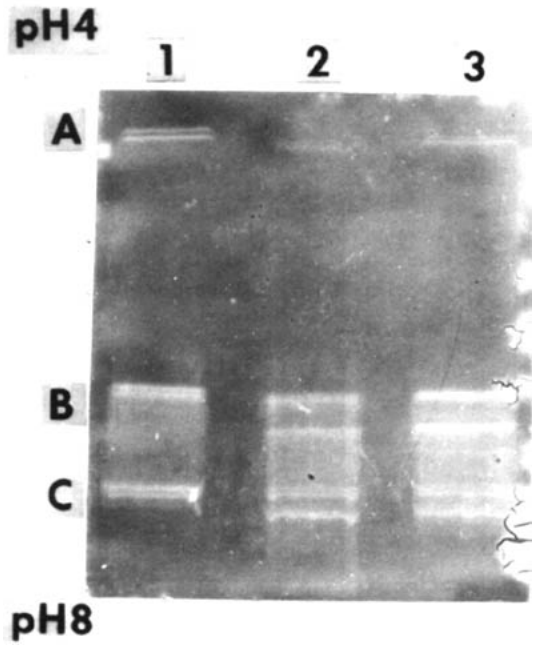

Fig. 1. Isoelectric focusing analysis of α-amylases from 4-day germinated barley: 1) Conquest; 2) Betzes; 3) Klages.

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[2]Presented at the 43rd Annual Meeting, St. Louis, May 1977.

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by isoelectric focusing. A reported result is the mean of two separate chromatographic runs.

RESULTS AND DISCUSSION

The α-amylase enzymes in germinated Conquest barley are shown in Fig. 1. There were three main groups of enzymes shown as A, B and C. Many barley varieties examined previously appeared to contain these three groups, but the enzyme pattern within each group sometimes varied with variety.[3] The A component, which had an isoelectric point of 5.1, has been designated α-amylase 1. It is a minor component and consisted of two closely-spaced bands of activity. There are many similarities between the properties of this enzyme and of the α-amylase present in immature barley. Components B and C have been combined in previous work (12) and called α-amylase 2. However, it is important when investigating α-amylase patterns by electrophoretic methods to distinguish between these two groups. Component B is the major enzyme not only in Conquest but also in all barley varieties studied so far. It represents at least 80% of the total activity of germinated barley kernels. In Conquest this component consisted of three closely-spaced major bands of activity and some minor bands. The major bands had an isoelectric point of 6.4–6.5.

[3]Unpublished results.

The third group C has been identified only tentatively as an α-amylase on the basis of its action on amylopection β-limit dextrin. It had an isoelectric point of 6.8–6.9 and again consisted essentially of two bands of activity.

All α-amylase components were present during early stages of germination and appeared to increase with time of germination.

Betzes had a similar α-amylase 1 pattern (Fig. 1). However, the α-amylase 2 group was different. Component B consisted of two well-spaced bands of activity and component C contained three bands.

Klages had a pattern similar (Fig. 1) to Betzes but there were extra, minor bands associated with both the B and C components.

Addition of GA to germinating barley kernels did not change the α-amylase pattern (Fig. 2). Only the results for Conquest are shown here but no changes were detected with the other two cultivars.

Because of its high sensitivity and resolving power, isoelectric focusing is a very useful technique for analyzing complex protein mixtures. However, the technique, as it is used in this study, does not give quantitative information about the various α-amylase enzymes. Ion-exchange chromatography on small columns of CMC was used for this purpose. A typical separation of α-amylases 1 and 2 is shown in Fig. 3. Complete separation of the two enzymes was confirmed by analyzing the pooled fractions in each peak by isoelectric focusing (Fig. 4).

The addition of GA to Conquest barley increased the rate of α-amylase formation but, with this particular barley sample, it did not significantly change the amount of enzyme present after 6 days of germination (Fig. 5). Maximum activity was obtained after 5 days in the presence of GA, compared to 6 days in untreated kernels. The rate of synthesis of α-amylase 2 was similar to that of the total enzyme. Synthesis of the minor component, α-amylase 1, followed a similar pattern but the activities were much smaller (Fig. 6). Maximum activity of this enzyme was achieved after 5 days in both treated and untreated kernels.

Changes in α-amylases during germination of Betzes barley are

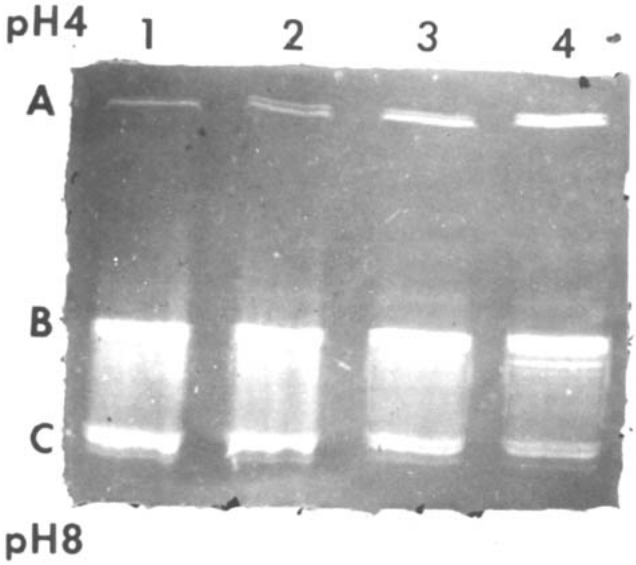

Fig. 2. Isoelectric focusing analysis of α-amylases from Conquest barley germinated in the presence and absence of exogenous GA. 1) 2 days; 2) 2 days + GA; 3) 6 days; 4) 6 days + GA.

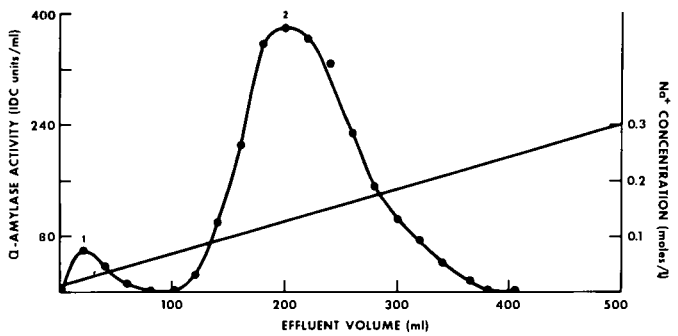

Fig. 3. Ion-exchange chromatography on CMC of 4-day germinated Klages barley. 1) α-amylase 1; 2) α-amylase 2.

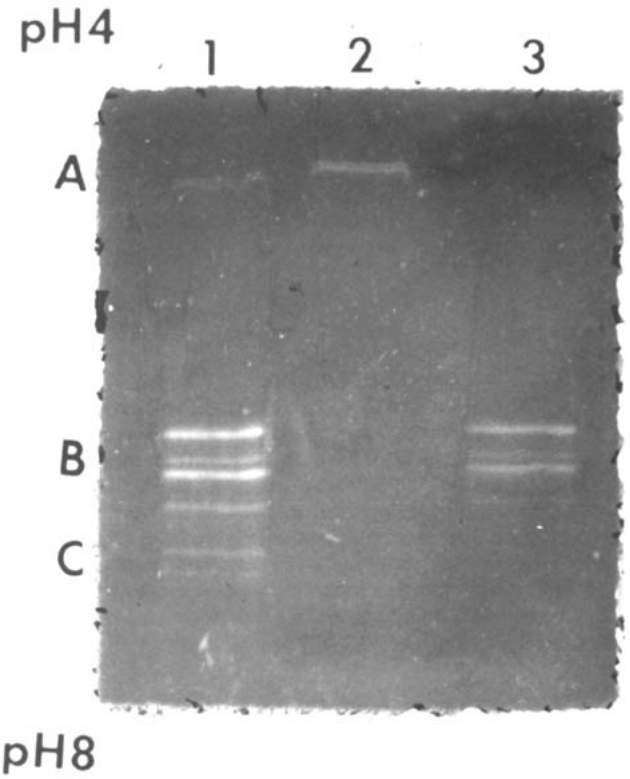

Fig. 4. Isoelectric focusing analysis of fractionation on CMC of α-amylases from 4-day germinated Klages. 1) 4-day germinated Klages; 2) α-amylase 1 from CMC column; 3) α-amylase 2 from CMC column.

shown in Fig. 7. In untreated kernels synthesis of α-amylase was very slow during the first 4 days, but the rate of synthesis did increase between 4 and 6 days of germination. The maximum activity achieved after 6 days was low—only 50% of that found in Conquest—but enzyme synthesis was still increasing rapidly at this stage. A much longer period of germination would be required to determine the maximum amount of amylase that could be produced by Betzes. In the presence of GA, α-amylase was synthesized much more rapidly and had reached a much higher level after 6 days than in the untreated seeds. Again, maximum levels of enzyme had not been attained after 6 days.

Synthesis of α-amylase 2 was similar to that of the total amylase except toward the end of the germination period of the treated seeds when the rate of α-amylase 2 synthesis decreased. During this period, α-amylase 1 was rapidly synthesized in GA-treated kernels and this enzyme represented an ever-increasing proportion of the total enzyme being produced (Fig. 8). In the absence of GA, synthesis of α-amylase 1 was very slow and not detectable until the 4th day of germination.

The rate of synthesis of α-amylase in Klages was more comparable to that of Conquest than to that of Betzes, although Klages is a two-row barley (Fig. 9). Even in the absence of GA, enzyme synthesis progressed rapidly between 2 and 5 days of germination. The rate of synthesis decreased, however, between 5 and 6 days. The total activity at this time was similar to that in GA-treated Betzes but significantly less than that in Conquest.

The addition of GA made only a small difference to the rate of α-amylase synthesis during the first 3 days of germination. However, there was a large difference during the 4th day and, even after 6 days, synthesis was proceeding very rapidly in the presence of GA. Klages was similar to Betzes in that the presence of GA made a large difference in the total α-amylase activity attained after 6 days of germination.

The α-amylase 2 profiles were similar to those of the total activity

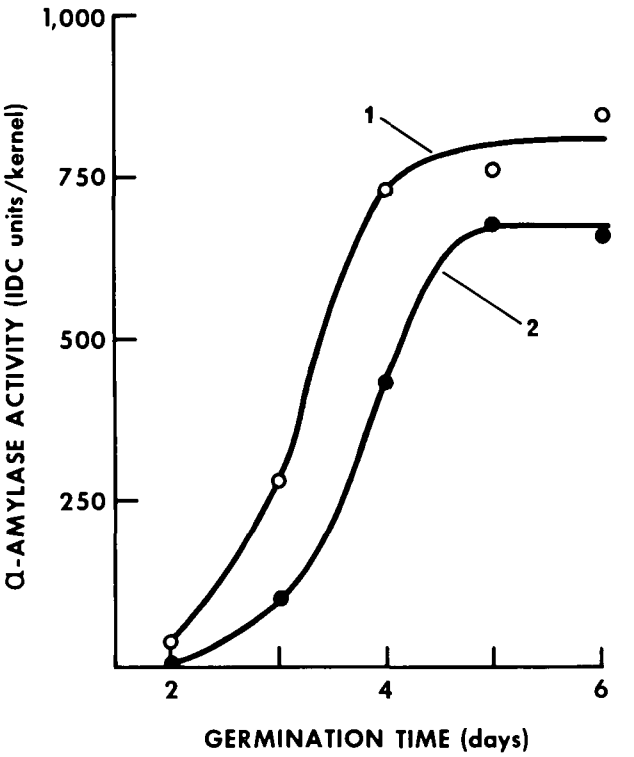

Fig. 6. Formation of α-amylase 1 in Conquest barley during germination. 1) α-amylase 1 in presence of GA; 2) α-amylase 1.

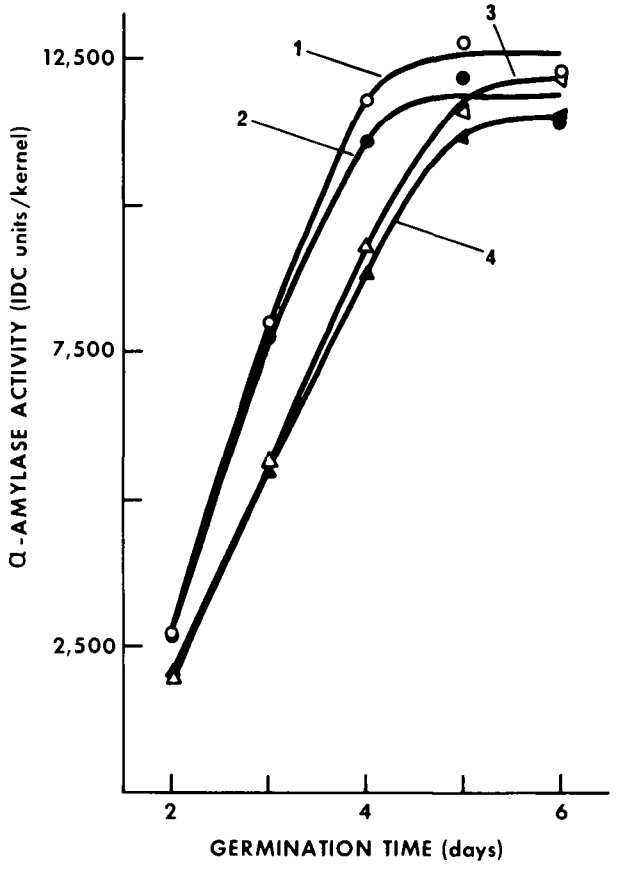

Fig. 5. Formation of total α-amylase and α-amylase 2 in Conquest barley during germination 1) total α-amylase in presence of GA; 2) α-amylase 2 in presence of GA; 3) total α-amylase; 4) α-amylase 2.

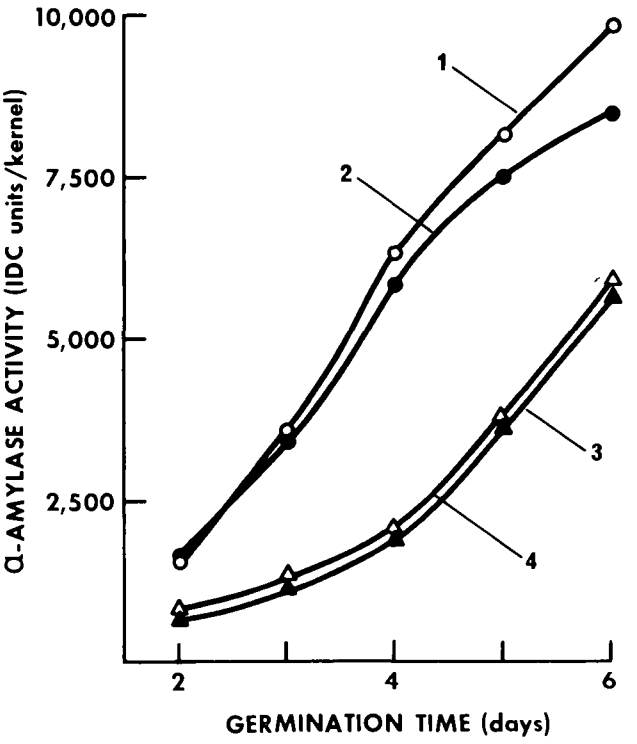

Fig. 7. Formation of total α-amylase and α-amylase 2 in Betzes barley during germination. 1) total α-amylase in presence of GA; 2) α-amylase 2 in presence of GA; 3) α-amylase 2; 4) total α-amylase.

except toward the end of the germination period in the presence of GA. At this stage the α-amylase 2 and total α-amylase curves started to diverge. The reason for this is shown in Fig. 10.

Synthesis of α-amylase 1 was increased fourfold in the presence of GA so that the proportion of this enzyme increased during germination. In the absence of GA, synthesis of α-amylase 1 was slow and the enzyme level reached after 6 days was similar to that of Betzes α-amylase 1.

Klages has the potential to synthesize large amounts of α-amylase. Like Conquest it synthesized the enzyme very rapidly in the initial stages of germination and, like Betzes, it had a marked response to the addition of GA. This combination should yield a Klages malt with the high α-amylase activity usually attributed only to six-row barleys.

These results were obtained with barley samples grown in the same year at the same location. It is possible that the same cultivars grown in different areas and under different climatic conditions would respond differently to the addition of GA. There is evidence to suggest that different lots of the same barley can react differently to GA. However, although environment may affect the α-amylase content of these cultivars there is no evidence to suggest that the α-amylase patterns would be similarly affected.

Direct comparisons between these results and those obtained by malting could be misleading. Germination on filter paper is not identical with the type of germination which occurs during commercial malting practice. The two procedures may give rise to different levels of α-amylase in the germinated samples but, again, the α-amylase patterns should not be affected.

The reasons for some cultivars responding so markedly to GA while others do not are not clear. It is easy to assume that a poor response indicates sufficient GA is already present in the kernel while a large response suggests a deficiency of GA. Detailed analyses of the presence and development of endogenous GA in germinating grain is required to test this assumption. There is evidence that the amount of GA in mature, ungerminated barley is very low (4) and so activation of the aleurone in germinating barley is dependent on synthesis of GA in the embryo followed by movement of the GA to the aleurone layer. Differences in the rates of GA synthesis, movement, or aleurone stimulation in different cultivars would affect not only the rate of germination but also the response to added GA.

Slow germination may be caused also by the presence of abscisic acid (ABA) in barley kernels. Studies have shown that ABA lowers the synthesis of α-amylase in barley (9) and wheat kernels (10). Much more work is required to determine the complex relation between germination inhibitors such as ABA, germination promoters such as GA, and their effect on α-amylase synthesis during malting.

SUMMARY

The malting barley cultivars studied, Conquest, Betzes, and Klages, each synthesized three main groups of α-amylase during germination. Each group was heterogeneous and each cultivar had a different α-amylase pattern. The rate of α-amylase synthesis was different for each cultivar.

The addition of GA to whole kernels had no effect on the pattern of α-amylase enzymes. However, it did increase the rate of synthesis of both amylase components studied in all cultivars and gave greatly increased levels of total α-amylase in Klages and Betzes

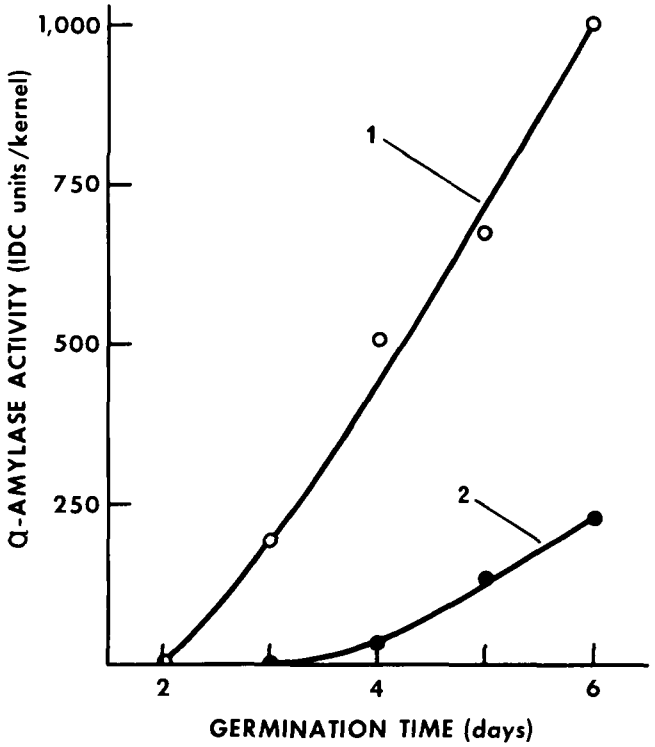

Fig. 8. Formation of α-amylase 1 in Betzes barley during germination. 1) α-amylase 1 in presence of GA; 2) α-amylase 1.

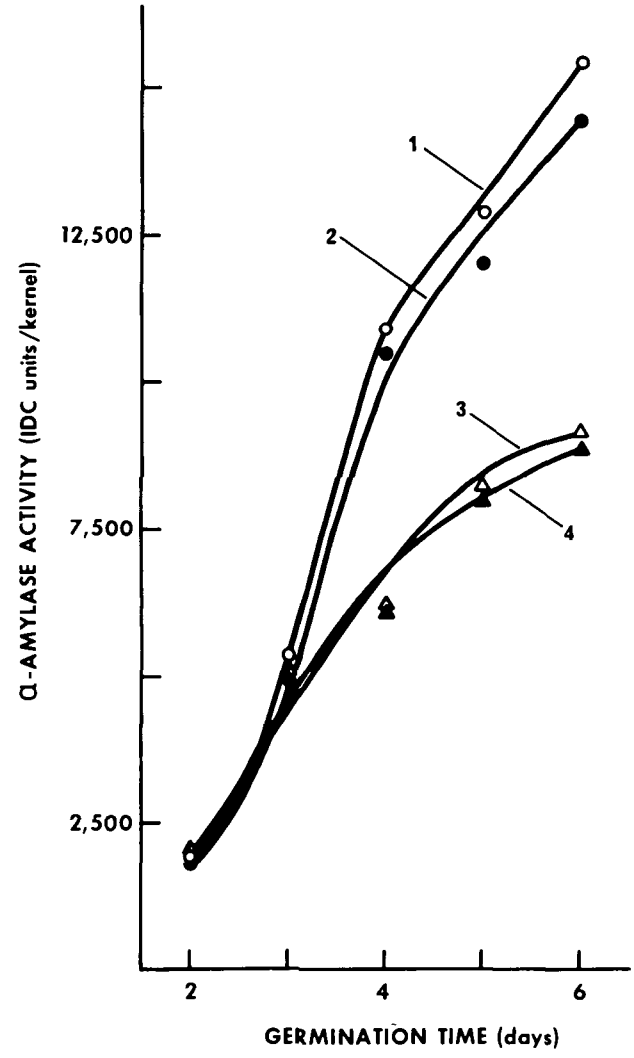

Fig. 9. Formation of total α-amylase and α-amylase 2 in Klages barley during germination. 1) total α-amylase in presence of GA; 2) α-amylase 2 in presence of GA; 3) total α-amylase; 4) α-amylase 2.

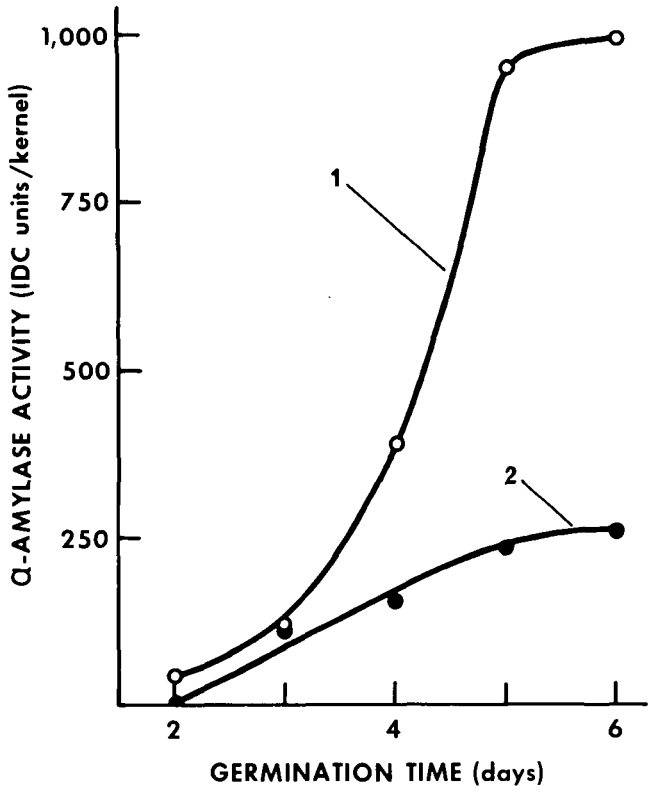

Fig. 10. Formation of α-amylase 1 in Klages barley during germination. 1) α-amylase 1 in presence of GA; 2) α-amylase 1.

after 6 days of germination.

More detailed information is required on the nature, synthesis, and movement of gibberellins in barley and on the action of gibberellins on aleurone layers from a number of barley cultivars. Such results would lead to a clearer understanding of the synthesis of α-amylases during germination.

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Literature Cited

1. BILDERBACK, D. E. *Plant Physiol. 53*: 480 (1974).
2. BRIGGS, D. E. *J. Inst. Brew. 67*: 427 (1961).
3. BRIGGS, D. E. *J. Inst. Brew. 69:* 13 (1963).
4. BROOKES, P. A., and MARTIN, P. A. *J. Inst. Brew. 81*: 357 (1975).
5. FRYDENBERG, O., and NIELSEN, G. *Hereditas 54:* 123 (1965).
6. HAYASHI, T. *J. Agr. Chem. Soc. (Japan) 16*: 531 (1940).
7. HORGAN, R. *Proc. Aust. Sec. Inst. Brew. 11th Convention, 1970,* p. 69.
8. JACOBSEN, J. V., SCANDALIOS, J. G., and VARNER, J. E. *Plant* Physiol. *45*: 367 (1970).
9. KHAN, A. A., and DOWNING, R. D. *Physiol. Pl. 21:* 1301 (1968).
10. KING, R. W. *Planta 132:* 43 (1976).
11. LUERS, H., and RUMMLER, W. *Wschr. Brau. 50*: 297 (1933).
12. MacGREGOR, A. W. *Cereal Chem. 53*: 792 (1976).
13. MacGREGOR, A. W. *J. Inst. Brew. 83*: 100 (1977).
14. MacGREGOR, A. W., LaBERGE, D. E., and MEREDITH, W. O. S. *Cereal Chem. 48*: 255 (1971).
15. MacGREGOR, A. W., THOMPSON, R. G., and MEREDITH, W. O. S. *J. Inst. Brew. 80:* 181 (1974).
16. MITCHELL, E. D. *Phytochemistry 11:* 1673 (1972).
17. MOMOTANI, Y., and KATO, J. *Plant and Cell Physiol. 8*: 439 (1967).
18. PALMER, G. H. *Eur. Brew. Conv., Proc. Congr. 13th, Estoril, 1971,* p. 59.
19. SANDEGREN, E., and BELING, H. *Eur. Brew. Conv., Proc. Congr. 7th, Rome, 1959,* p. 278.
20. TANAKA, Y., and AKAZAWA, T. *Plant Physiol. 46*: 586 (1970).
21. VARNER, J. E. *Plant Physiol. 39:* 413 (1964).
22. Von ONCKELEN, A., and VERBEEK, R. *Planta 88:* 255 (1969).

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