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BIOCHEMICAL CHANGES DURING EMBRYOGENY IN *HORDEUM DISTICHUM*

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Key Word Index—*Hordeum distichum*; Gramineae; barley; embryo; nucleic acids; sucrose synthetase; invertase.

Abstract—Changes in fr. and dry wt, soluble reducing sugars, protein, total carbohydrate, DNA, RNA, sucrose synthetase activity and invertase activity were recorded for the developing embryo of *Hordeum distichum* var Julia over the period 18–60 days after anthesis. Fresh wt increased until 45 days whereupon rapid dehydration commenced. Reducing sugar concentration remained low throughout development but total carbohydrate and protein accumulated rapidly over the initial period to reach maximum values at around 50 days. DNA concentration remained relatively constant throughout the middle and later stages of development, but RNA, on the other hand, increased rapidly to reach a maximum value at maturity. Sucrose synthetase (assayed in the direction of sucrose cleavage) was considerably more active with UDP than ADP and reached a maximum value around 35 days after anthesis. When assayed in the direction of sucrose synthesis the peak of activity was slightly later in development and doubled in value. Invertase activity was appreciable and was still present at maturity.

INTRODUCTION

The morphological changes accompanying the initial stages of differentiation during embryogenesis in higher plants have been well described [1]. Little is known, however, of the associated biochemical changes. Jennings and Morton [2] studied the changes in grain composition with maturation. Nitrogen content of the embryos increased steadily from 18 days after anthesis. At maturity the embryo, which constituted *ca* 3% of total grain weight, contained about 7% of total grain N_2. The same workers [3] have shown that total phosphorus showed a similar steady increase and that at maturity 14% of total grain phosphorus was present in the embryo.

Walbot *et al.* [4] in a recent study have described the developmental interaction between the suspensor and the organogenetic part of the embryo in terms of RNA metabolism. They showed that synthetic activity of the suspensor was highest early in development and then declined, whereas synthetic activity of the organogenetic part increased throughout development. The fine structure in cells of mature pea and wheat embryos has been examined by Setterfield *et al.* [5]. The nuclei of all cells were similar, showing nuclear membranes, chromosomes and prominent nucleoli. Other structures present included amyloplasts, extensively developed endoplasmic reticulum, mitochondria and large numbers of ribonucleoprotein bodies—presumably ribosomes. The mature wheat scutellum has also recently been examined [6]. In this case nuclear chromatin was strongly aggregated and the endoplasmic reticulum, while present, was not abundant. Intact mitochondria and plastids were also visible. The fine structure of the developing embryo was not investigated.

Thus although RNA metabolism has been investigated in embryogenesis, the overall mechanism controlling embryo development has not. The present work attempts to characterize the broad features of embryogenesis by describing some of the accompanying biochemical changes. The results will be correlated where possible, with those already reported [7] for the developing seed and its associated tissues.

RESULTS

Overall biochemical changes in the embryo during development

The changes in fr. and dry wt of the embryo are shown in Fig. 1. Fresh wt, after an initial lag phase, increased rapidly from 25 days after anthesis,

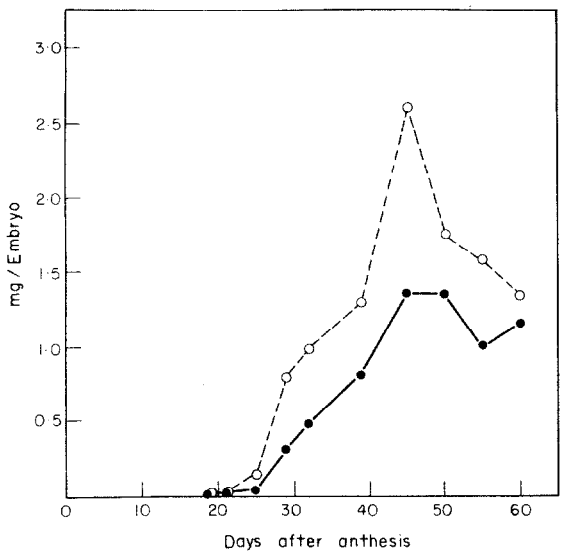

Fig. 1. Changes in DNA and RNA content of developing embryos. ●——● DNA; ○- - - -○ RNA.

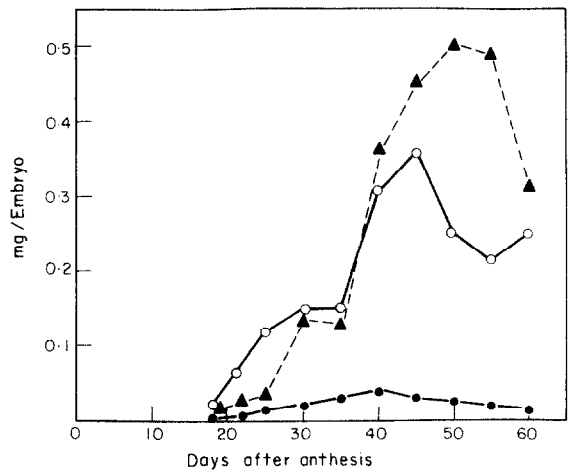

Fig. 2. Changes in reducing sugars, total carbohydrate and protein of developing embryos. ●——● reducing sugars; ○- - -○ total carbohydrate; ▲——▲ protein.

reaching a maximum value around 45 days and declining markedly thereafter. Dry wt also remained low until 25 days, then rose steadily to a maximum value at 39 days and did not fall much below this value with increasing maturity. Thus dehydration commences at 45 days and is almost complete by 60 days.

The variation during embryo development of total carbohydrate, reducing sugars and protein is given in Fig. 2. The level of total carbohydrate is initially low, but appreciably higher than that of reducing sugars at the same age. Rapid accumulation occurs between 18 and 45 days when a maximum figure is attained; levels then decrease slightly. The graph for protein concentration follows a similar pattern rising sharply over the initial period to produce a maximum value at around 50 days. A marked decrease is seen over the final stage of maturation. Levels of reducing sugars remain low throughout, with a gradual increase around 40 days to a broad maximum and a slow decrease thereafter.

After an initial slow increase in concentration, DNA levels remained relatively constant throughout the middle and later stages of development (Fig. 3). RNA concentration, on the other hand, increased rapidly throughout development to reach a maximum value only at maturity.

Changes in the enzymes of carbohydrate metabolism during embryogenesis

The sucrose synthetase (sucrose cleavage) in the embryos was considerably more active with UDP than with ADP (Fig. 4). Activity with UDP could be detected in 21 day embryos and increased very rapidly to a maximum value around 35 days. With ADP, activity was not detectable until 25 days and after reaching a maximum value around 30 days,

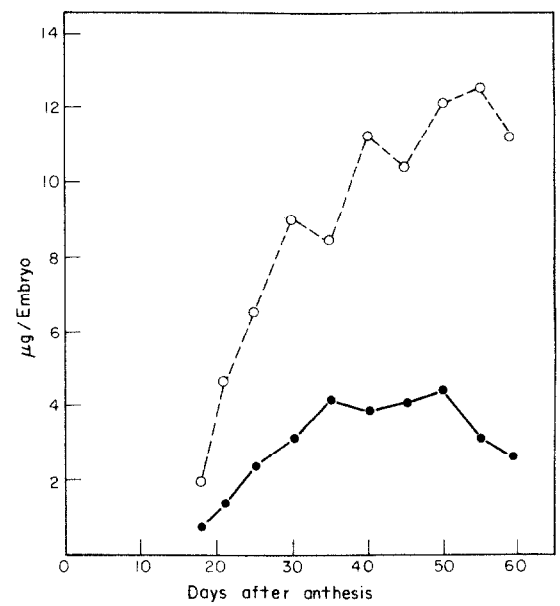

Fig. 3. Changes in fr. wt and dry wt of developing embryos. ○——○ fr. wt; ●——● dry wt.

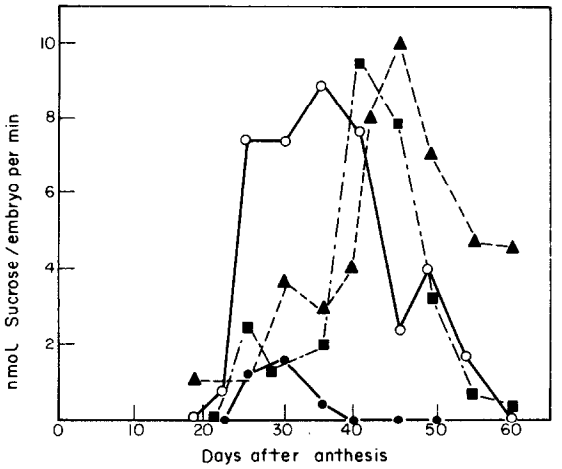

Fig. 4. Changes in UDP and ADP dependent sucrose synthetase activity (sucrose cleavage) and invertase activity of developing embryos. ○——○ UDP dependent sucrose synthetase; ●——● ADP dependent sucrose synthetase; ▲----▲ invertase; ■-·-·-■ sucrose synthetase activity (sucrose synthesis).

fell rapidly to zero at around 39 days. Activity with UDP fell to zero only at 60 days after anthesis. Invertase activity was always measurable in embryos. After an initially slow increase, enzyme activity increased rapidly to reach a maximum value at 45 days. Although activity decreased thereafter appreciable levels were still present at maturity.

Sucrose synthetase (sucrose synthesis) had a similar developmental pattern (Fig. 4) to UDP dependent sucrose synthetase (sucrose cleavage). The peak of activity was slightly later in development and almost doubled in value. The initial rate of increase was, however, much less than that of the cleavage enzyme.

The results for sucrose phosphate synthetase (sucrose synthesis) are not reported since: (a) at every age examined, activity with fructose-6-phosphate was much less than with fructose and (b) hydrolysis of fructose-6-phosphate (as measured by release of inorganic phosphate in the absence of added UDPG) was considerable and made it impossible to conclude that activity was due to fructose-6-phosphate rather than to fructose itself.

DISCUSSION

It is interesting to note that while the embryo initially grew fairly slowly, in terms of increase in fr. and dry wt, carbohydrate, protein, enzyme activity and RNA concentration increased very rapidly over this period. Indeed, a major part of the young embryo must be composed of carbohydrate and, to a lesser extent, of protein. As development proceeded, however, the rate of protein accumulation exceeded that of carbohydrate. Final concentrations were similar.

These results contrast strongly with those described [7] for the developing barley endosperm, in which protein concentration varied little and constituted less than 20% of the mature tissue. Presumably the increase in protein levels is associated with the synthesis of enzyme protein since it parallels the increases in activity of most of the enzymes investigated.

While the reducing sugar levels remain low throughout embryogenesis carbohydrate synthesis and accumulation is rapid. The low levels thus do not reflect their probably high turnover rate. It is likely that, while the results do not show initially high levels of reducing sugar, embryo carbohydrate is synthesized from a precursor pool of reducing sugars [7]. Some of this carbohydrate is likely to be starch [20] and certainly our observations with the light microscope, of sections stained with I_2/KI, suggest that the barley embryo contains many small amyloplasts. The wheat [6] and barley [21] scutella apparently contain no amyloplasts and presumably, therefore, no starch.

The pattern of RNA accumulation follows very closely that described [22] for embryos of *Phaseolus vulgaris* L. except that synthesis tails off only at the very last stages of maturation. Thus protein synthesis, which also continues to maturity, is probably mediated by newly synthesized RNA.

Of particular interest was the observation that the DNA concentration remained relatively constant per embryo throughout the middle and later stages of development. The significance of these results in relation to cell division and differentiation will not be clear until more is known of the DNA concentration per cell at different stages of development.

The developmental pattern of the enzymes involved in sucrose cleavage was very similar to that recorded previously for endosperm [7]. Thus the increase in enzyme activity immediately preceded gains in dry wt, a major part of which was due to carbohydrate. Sucrose synthetase (sucrose cleavage) was considerably more active with UDP

than with ADP. It is therefore likely that the UDP enzyme is involved preferentially in the utilization of translocated sucrose. However, invertase was present at 18 days while neither ADP nor UDP dependent sucrose synthetase (sucrose cleavage) activity was detectable. It is possible that in the very early stages some sucrose is cleaved by invertase during entry and diffuses as monosaccharides to the embryo cells. The resynthesis of sucrose from monosaccharides, derived either from invertase or the cleavage enzymes, is then possible utilizing UDPG dependent sucrose synthetase (sucrose cleavage)—an enzyme shown here to be twice as active as the ADP dependent cleavage enzyme at its maximum. A similar mechanism was suggested by Shannon [23] for developing maize endosperm. The overall mechanism of carbohydrate synthesis in the embryo cannot however be established until the sites of action of enzymes such as invertase are determined. Edelman *et al.* [24] have shown that in germinating cereal seeds invertase activity is confined to the root and shoot and very little is found in the scutellum. On the other hand Palmer [21] has shown that in barley after 2 hr germination, invertase activity was measurable in root, shoot and scutellum. Certainly invertase was noteworthy in that its activity did not fall to zero at maturity. It may be that, like β-amylase [25], it is reactivated from a latent form in germination.

Thus, while some of the broad features accompanying embryogenesis in barley have been described, the details remain to be established. In particular the origin of the nutrients supplied to the developing embryo is subject to some speculation. It is generally supposed that the embryo requires the endosperm for development and presumably some, at least, of the carbon and nitrogen required is derived from the immature endosperm. These, and related problems are currently under investigation in this laboratory.

EXPERIMENTAL

Plant material. The two row barley *Hordeum distichum* (L.) Lam. cv. Julia, was used throughout. Conditions of growth and methods used to determine the date of anthesis were as described by Merritt and Walker [8].

Embryo extracts. The outer layers of glumes, paleae and pericarp were first taken off the intact grains. The embryos, together with the scutellum were then removed by hand and analysed immediately. The term "embryo" thus refers to the scutellum together with the organogenetic part. A minimum of 10 embryos from the grain aged between 18 and 60 days after anthesis was used for each analysis.

Fresh and dry weights. Fresh wts were first determined and the embryos then dried to constant wt at 80°.

Soluble reducing sugars. Embryos were homogenized vigorously in 1·0 ml of 0·01 M NaF. The homogenate (which included a further 0·5 ml H_2O used for washing the homogenizer) was then centrifuged at 3000 *g* for 10 min. The supernatant was removed, the pellet re-extracted with a further 0·5 ml H_2O and the suspension centrifuged as before. A suitable volume, i.e. 0·1–1·0 ml depending on size of the embryos, was then assayed directly for soluble reducing sugars by the Somogyi–Nelson procedure [9, 10]. Deproteinization was not necessary.

Total carbohydrate was measured using the anthrone reagent [11].

DNA, RNA and protein were measured in intact embryos by the method described by Rozijn and Tonino [12]. The values for RNA were additionally verified after sodium lauryl sulphate–phenol extraction as described by Stern [13].

Invertase. Embryos were homogenized in 1·0 ml of 0·2 M acetate buffer pH 4·8 and the suspension assayed for invertase at pH 4·8 as described by Tsai *et al.* [14]. The reducing sugars released were measured as described above.

Sucrose synthetase (sucrose cleavage). UDP and ADP dependent sucrose synthetase was assayed in the 3000 *g* for 10 min supernatant as described by Pressey [15]. The incubation time was 10 min. Controls were run in the absence of UDP (ADP) to correct for invertase activity. The fructose released was measured by the Somogyi–Nelson procedure.

Sucrose synthetase (sucrose synthesis). The reaction mixture contained 20 μmol HEPES buffer pH 7·4, 2 μmol fructose, 0·5 μmol UDPG and 0·05 ml enzyme in a total vol. of 0·25 ml. After incubation for 10 min at 37° the sucrose released was measured by the method of Roe [16] as modified by Cardini *et al.* [17]. Controls were run simultaneously in which either UDPG or fructose were added after incubation.

Sucrose phosphate synthetase (Sucrose synthesis). This was assayed as described for sucrose synthesis above except that fructose was replaced by fructose-6-phosphate and Tris–HCl buffer pH 6·4 replaced the HEPES buffer [18]. Inorganic phosphate released by fructose-6-phosphate under these conditions but in the absence of added UDPG was measured by the method of Allen [19].

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