Physiological Effects of Gibberellic Acid. IV. On Barley Grain With Normal, X-Irradiated, & Excised Embryos [1]

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Introduction

A theory has been advanced suggesting that an endogenous gibberellin acts as an endosperm mobilizing hormone (EMH) (7). In this proposal the embryo is pictured as the origin of an endogenous gibberellin which is released into the endosperm during the early stages of germination. Its presence in the endosperm is indicated by the activation of hydrolytic enzymes which break down starch, storage proteins, and cell walls (5, 6, & 7). Gibberellic acid (GA_3) is the only agent, other than the embryo itself, which has been shown capable of setting these mobilization processes in action.

The theory, however, is largely based on indirect measurements of the effects of GA_3 on barley endosperm; measurements made on the ambient solutions from petri dishes containing the isolated endosperm. It was desirable, therefore, to determine whether similar changes could be observed within the endosperm itself if treated with several GA_3 concentrations and varying germination conditions, in the presence or absence of normal and X-irradiated embryos.

Embryos were X-irradiated to impair their effectiveness as the source of the endogenous EMH. The responses of seeds so damaged were compared with the responses of normal and embryo-less grain. The diminished rate of the mobilization processes occurring in the endosperm of embryo-irradiated grain was compensated for by the application of GA_3. The response syndrome of irradiated seeds treated with GA_3 was also compared with those of normal and embryo-less seeds.

Material & Methods

► Seed. Grain of the 1960 harvest of *Hordeum distichum* auth. var. Tripleawned Lemma (huskless) was used in both experiments. The seeds were separated into two groups: those with normal intact embryos and those which had suffered embryo damage during harvesting, or by insects while stored. Any remnants of the scutellum still present on embryo-less grain were removed by hand before imbibition.

► Irradiation. Results of seed irradiation have been rather variable and sometimes difficult to interpret (1). For this reason widely varying conditions of dose and seed moisture content were chosen. It was hoped that one or both of the experiments would demonstrate impairment of embryo function.

Normal grain with intact embryos was fixed in rows two to four deep with transparent tape. The embryos were exposed, and the endosperm were shielded by 5 mm of lead. Care was taken in placing the lead shield, and, aided by the irradiation-induced browning of the unshielded areas of the petri dish, the grain was culled following irradiation. For the first set of conditions, dry grain at about 9 % moisture content received 45,000 r. Imbibition began 48 hours after irradiation. In the second experiment, seeds were soaked for 3 hours before irradiation, raising their moisture content to about 25 %; the total dose was increased to 75,000 r. The radiation source was a 200 kv, 15 ma unit with a dose rate of 570 r/minute (0.4 mm Cu HVL).

► Germination Conditions. The conditions first investigated simulated those normally employed by many malsters (9). Normal, irradiated, or embryo-less grain (10 g) was soaked in 30 ml of water or GA_3 (2 ppm) at 10 C in 2.5 cm × 15 cm test tubes. The solutions were changed once at the end of 24 hours. After 48 hours, the solutions were decanted and the seeds were allowed to germinate in the test tubes at the same temperature.

The second experiment involved the soaking of 600 normal and 300 embryo-less grains in water at 25 C for 3 hours. The grain was drained, blotted, and exposed to air for 4 hours. During this time, 300 of the normal grain were irradiated. Each group of 300 seeds (normal, irradiated, and embryo-less) was subdivided into six lots and returned to 30 ml of water or GA_3 solutions (varying concentrations) at 25 C for a further 17 hours. The grain were then drained, blotted, and placed on two pieces of filter paper moistened with 3 ml of water, in 5 cm petri dishes. The dishes were incubated at 25 C.

► Analysis.

I. *Grinding.* At the times indicated five seeds

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from each test tube in the first experiment were removed. Embryos were dissected out of the normal and irradiated seeds, and discarded. The endosperm were ground in 10 ml of 0.025 M citrate buffer (pH 5.0) with mortar and pestle at room temperature, for 3 minutes. The solution remained standing a further 2 minutes, after which portions were removed for the assays.

Grain from the second experiment were ground, five at a time following embryectomy from normal and irradiated seeds, in 10 ml of water, for 3 minutes. After standing for a further 2 minutes, portions of the solution were assayed.

II. *Amylase assay.* 0.1 ml portions of the extracts from the first experiment were added to 5.0 ml of 0.02 % starch and incubated in test tubes at 38 C in a shaking water bath. After 5 minutes 0.2 ml of 0.2 % iodine in 2 % potassium iodide was added; the contents of the tubes were immediately mixed and diluted to 10 ml. The absorption of the resulting solutions was read at 600 mμ on a Unicam SP 350 spectrophotometer. Zero time controls were prepared by reversing the order of addition of the starch and IKI.

No amylase assays were performed on the extracts from the second experiment.

III. *Sugar assay.* Extract from the first experiment (1 ml) was added to 4 ml of water and approximately 1 g IR-120 (H+) resin. After 15 minutes the solution was filtered and 0.5 ml of the filtrate was assayed for reducing sugars with the Somogyi-Nelson test.

The second series was assayed by adding 0.5 ml of extract to 4.5 ml of water and about one gram of resin. The same amount of filtrate was assayed as in the first series.

IV. *Soluble nitrogen assay.* For both experiments 3.0 ml of extract were heated in a boiling water bath for 12 minutes. The resultant solution was centrifuged for 10 minutes and the supernatant was assayed with a micro-Kjeldahl test. When necessary, the supernatant was quick-frozen and stored at −20 C.

Control values were obtained by subjecting dry, untreated endosperm to the same analytical techniques.

Results

Figures 1, 2, and 3 illustrate the endosperm responses of normal, irradiated, and embryo-less seeds treated with water or 2 ppm GA_3 (5.8×10^{-6} M); time was measured from the start of imbibition. There is a fall in the results of the three assays during the first 2 days (soaking period). This is probably a leaching effect (2). In the absence of GA_3, the embryo-less grain showed no change with time in any of the assays. Changes were initiated in the normal and irradiated, untreated grain but these lagged about three days behind the GA_3-treated grain. In all cases, irradiation slightly decreased the rate of response of the intact grain.

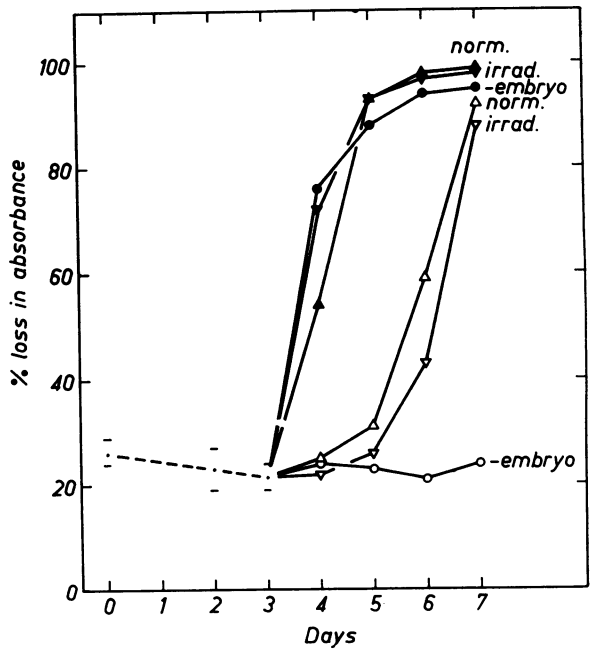

Fig. 1. Amylolytic activity of extracts of endosperm untreated (*open symbols*) and treated with 2 ppm (5.8×10^{-6} M) GA_3 (*closed symbols*).

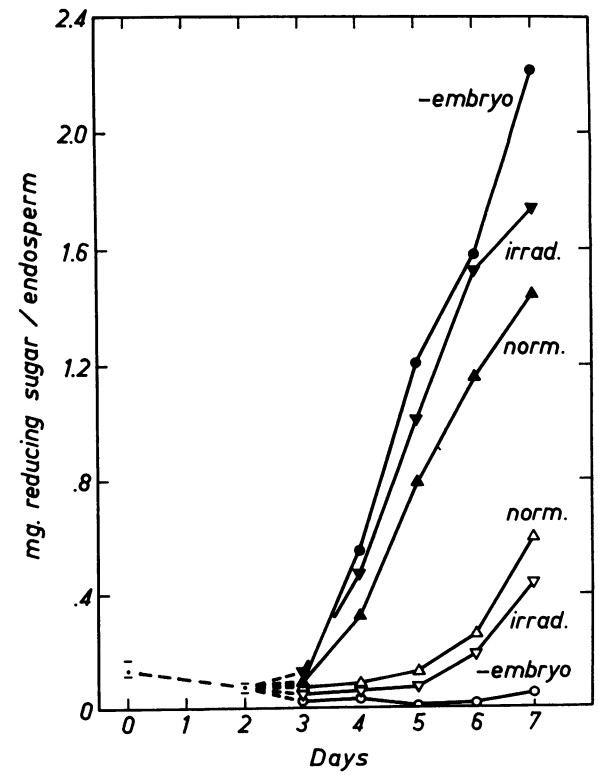

Fig. 2. Reducing sugar content of endosperm untreated (*open symbols*) and treated with 2 ppm (5.8×10^{-6} M) GA_3 (*closed symbols*).

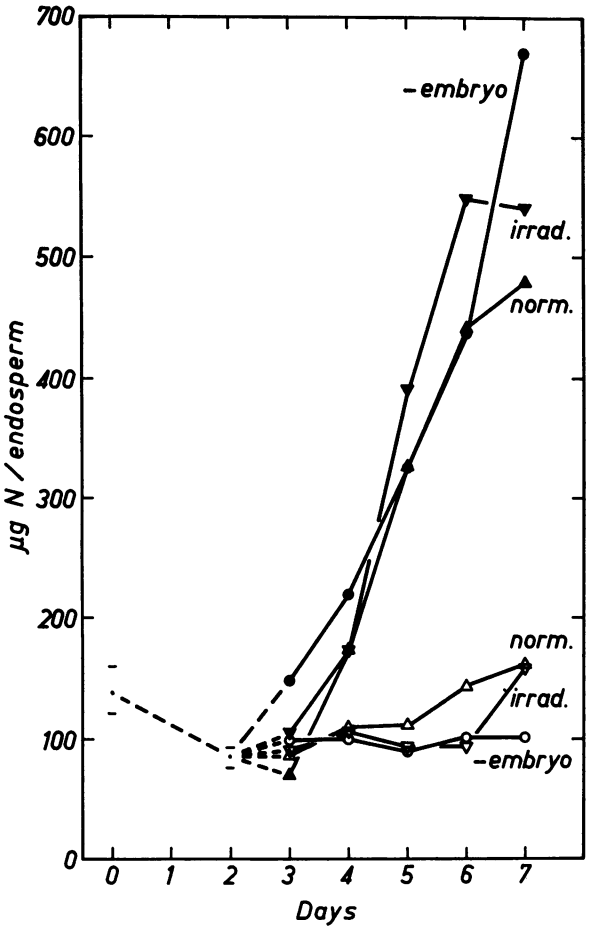

Fig. 3. Soluble nitrogen content of endosperm untreated (*open symbols*) and treated with 2 ppm (5.8×10^{-6} M) GA_3 (*closed symbols*).

In the treated series, the embryo-less grain showed the same responses as the normal and irradiated seeds. However, the magnitude of the sugar and nitrogen responses were greatest in embryo-less grain and least in normal grain. The amylase assay was designed for low levels of activity and consequently little or no effect of treatment was observed after 5 days. It is likely that, had the conditions of the test been modified, the results would have more closely resembled those of the other tests.

To ascertain whether the pattern of response remained similar under widely different conditions, the soaking period, germination containers, and temperature were altered for the second experiment. The effect of varying concentrations of GA_3, from 10 ppm (2.9×10^{-5} M) to 0.001 ppm (2.9×10^{-9} M), on reducing sugar and soluble nitrogen content, may be seen in figure 4. In the absence of either an embryo or exogenous GA_3 under these conditions, there is no evidence of the formation of reducing sugars or the solubilization of nitrogen in the barley endosperm. The presence of an embryo (whether damaged or normal) or GA_3, causes the initiation of both responses. Increasing concentrations of GA_3, up to a maximum of about 2.9×10^{-6} M, increases the slopes of the reaction curves. This relationship holds for the normal, irradiated, and embryo-less seeds, and the latter, again, seem to respond to the greatest extent.

In the absence of an embryo, the endosperm is sensitive to very low concentrations of GA_3; a fairly large response after 3 days is evident at the 2.9×10^{-9} M level. In the presence of an embryo, however, a concentration above 2.9×10^{-8} M appears necessary before an appreciable effect occurs.

Irradiation of the embryo, in this experiment also, slightly slowed the responses in the absence of GA_3 while increasing, to a small degree, the effectiveness of GA_3. In both series, therefore, the effect of irradiation of the embryo was to locate the GA_3 response between that of normal and embryo-less grain. The increase in temperature in the second set of conditions increased the rate of response in the endosperm and resulted in a shortening of the time required to obtain substantial changes in both sugar and nitrogen concentrations. Lastly, it must be noted that the effect of embryo and exogenous GA_3 on the mobilization of endospermal reserves in situ are remarkably similar.

Discussion

These experiments demonstrate anew that control of the initiation of hydrolytic processes in the endosperm resides in the embryo. Following wetting of the grain, an endosperm mobilizing hormone (EMH) is probably produced in the embryo and transported to the endosperm. Yomo suggests that an amylase-activating substance (presumably identical with the EMH) produced in the barley embryo may be a gibberellin (10). His hypothesis rests on similarities between the EMH and GA_3 in barley seedling tests, Rf values and infra-red analyses. The results of the present work also strongly support the view that an endogenous gibberellin is the EMH (7).

It is not yet possible, when considering the way in which the EMH is formed, to choose between synthesis and release; in these experiments either process might be affected by irradiation. Furthermore, it is likely (8) that some readily active gibberellin exists in grain before germination. Lazer et al. have inferred that most of the endogenous gibberellin they identified in normal germinating barley was released from a bound form (3), although the evidence is not strong. They also reported that the maximum amount obtainable from their varieties was about 0.07 ppm (about 2×10^{-7} M). This correlates well with the observation in this work that concentrations of GA_3 above 2.9×10^{-8} M are necessary to produce an effect in the presence of the embryo.

Whether GA_3 can completely, or only partially, replace the effects of the embryo on the endosperm, remains uncertain. The present results suggest that

embryo-less grain respond to GA_3 treatment to a slightly greater extent than do either normal or irradiated grain. Utilization of the measurable components by the embryo may cause such a differentiation. On the other hand, embryo-less grain may take up more GA_3 by virtue of their incomplete pericarp. As a third possibility, the embryo may even secrete a direct or competitive inhibitor of GA_3 action, similar to a suggestion by Naylor and Simpson (4) for oats.

In 1955 Caldecott (1) reported briefly that, with shielding experiments, the growth of the embryo could be impaired by embryo irradiation. It is likely, in studies like these, that scattering results in some irradiation of the endosperm. However, the possibility is very slight that this was the cause of the delayed mobilization in embryo-irradiated grain in these experiments. The figures indicate that such grain can respond to GA_3 treatment at least as well as unirradiated grain. It is more probable that the normal release of the EMH by the embryo can be impaired (but not eliminated) by the dosages employed. By complete removal of the embryo, it was possible to indefinitely postpone the initiation of the mobilization processes. In both of these situations, GA_3 was able

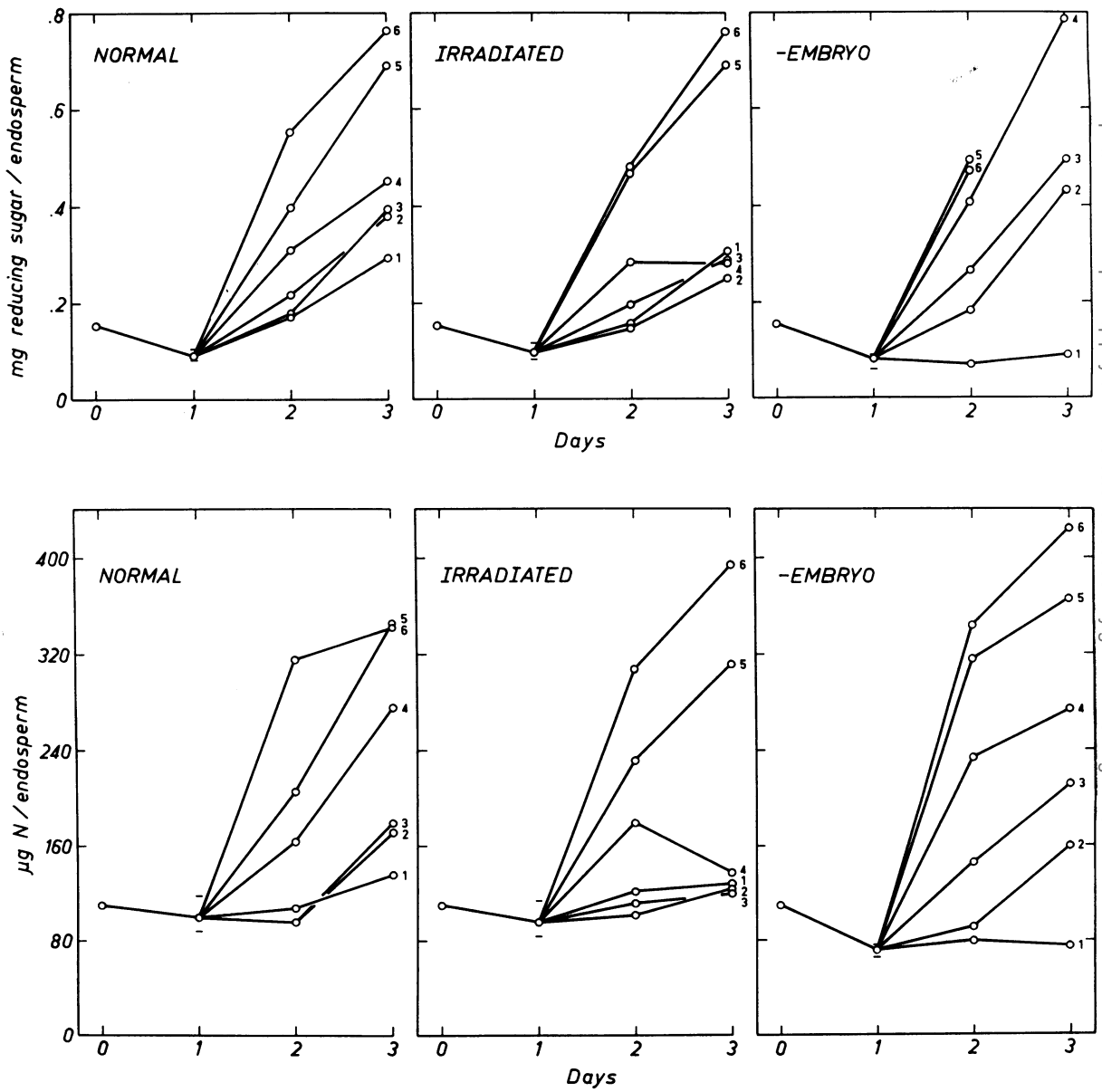

Fig. 4. Reducing sugar content (*upper*) and soluble nitrogen content (*lower*) of endosperm treated with increasing concentrations of GA_3 (curve 1 = control; 2 = 2.9×10^{-9} M; 3 = 2.9×10^{-8} M; 4 = 2.9×10^{-7} M; 5 = 2.9×10^{-6} M; 6 = 2.9×10^{-5} M).

to overcome the artificially imposed barriers to hydrolysis.

The lack of responsiveness of excised endosperm and the irradiation-induced lag in mobilization can be counteracted on a quantitative basis. In other words, a series of response levels can be obtained dependent primarily upon the GA_3 concentration, and secondarily on the prevailing temperature. Figure 4 demonstrates that it is possible to exactly replace the embryo effect by application of the proper concentration of GA_3.

There is a close similarity between these results and those reported earlier (7), where ambient solutions were analyzed. The variety used in this work, Triple-awned Lemma, is different from that used in previous reports (Prior). It does, however, respond in the same way as Prior to GA_3 treatment. The conclusion can be drawn that the responses occurring within the tissues of the excised endosperm are accurately manifested by changes in the ambient solutions as measured in previous work. The results presented here support the hypothesis that GA_3 can replace the action of the barley embryo on the endosperm, and that mobilization of endospermal reserves is probably initiated by an endogenous gibberellin secreted into the endosperm by the embryo.

Summary

Measurements were made of the amylolytic activity, reducing sugar, and soluble nitrogen contents of barley grain with or without normal or X-irradiated embryos. The results indicate that the embryo is responsible for the initiation of the mobilization processes which occur in the endosperm during germination. X-irradiation delays this initiation and complete removal of the embryo indefinitely postpones it. Gibberellic acid can hasten the responses of endosperm in the presence of normal or damaged embryos and can initiate them when the embryo is absent. A concentration above 2.9×10^{-8} M gibberellic acid was needed to produce an effect in the presence of the embryo, suggesting that this level may be close to the amount of endogenously produced gibberellin.

Acknowledgment

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