**184. Effect of Plant Extracts and Gibberellin A_3 on
 α -Amylase Production in Embryoless Rice
Endosperm in Relation to Growth-
Promoting Activity**

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It has been found that α -amylase production in barley¹⁾⁻⁴⁾ and rice⁵⁾ endosperms deprived from embryo was increased by the application of gibberellins. It is quite probable that the effect of gibberellins on growth may be closely related to their activity in enzyme production. In the present paper, the effect of growth-promoting plant extracts on the production of α -amylase is preliminarily reported.

Fig. 1 shows the effect of gibberellin A_3 on enzyme production and on growth promotion. The curve for growth promotion is the same as that reported previously,⁶⁾ and that for enzyme production was obtained by the following method. Five endosperms of rice freed from embryos were sterilized with sodium hypochlorite solution and placed in one ml of sterilized test solution and incubated for 4 days at 30°. The endosperms were ground in a glass mortar, extracted with 5 ml of 0.5% calcium chloride solution and filtered. The filtrate was examined for its effect on starch digestion, i.e., one ml of the filtrate was added to 4 ml of 0.25% starch solution of pH 4.8 and incubated for 30 minutes at 40°. After the digestion 0.5 ml of the reaction mixture was pipetted off to a test tube containing 5 ml of 0.5 M acetic acid and 5 ml of 3×10^{-5} N iodine. The coloured solution was measured at 700 m μ by a spectrophotometer. The production of α -amylase was expressed by the formula, $\frac{C-E}{C} \times 100$ (%), where C and E are the optical densities of the solution at the start and at the end of the incubation, respectively.

α -Amylase production was found even with 10^{-5} mg/l gibberellin A_3 and it increased with increasing concentration up to 3×10^{-2} mg/l. Fig. 1 shows that gibberellin A_3 can be detected from the enzyme production with a sensitivity 100 times as high as that shown by growth promotion. No effect on the production of α -amylase was brought about by the application of sucrose, various amino acids, organic acids, vitamins or minerals, and also indoleacetic acid and kinetin.

Fig. 1. Effect of gibberellin A₃ of different concentrations on the production of α -amylase in rice embryoless endosperm and on the elongation of the 2nd leaf sheath of rice seedling.

Extracts from many plant materials are known to promote the elongation of the leaf sheath of rice. It is interesting to investigate whether or not these extracts are also active in enzyme production. Ethanol extracts of 10 g fresh pods of *Lupinus luteus* and 4 cotyledons of 4-day-old seedlings of *Pharbitis nil* were chromatographed on filter paper of Toyo No 51 with a solvent of *iso*-propanol, 7 N ammonium hydroxide and water (8:1:1 v/v/v). The sheets were cut into 15 pieces, each piece was eluted with 2 ml of distilled water. The eluate was tested for its growth-promoting activity on the second leaf sheath of rice seedling.⁶⁾ On the other hand, each eluate was diluted to 1/20 concentration and tested for α -amylase production. The results are shown in Fig. 2. In *Lupinus* the activity for α -amylase production was found at Rfs 0-0.06, 0.13-0.26, and 0.40-0.80, the last one being very active. The activity for leaf sheath elongation was noticed at Rf 0.06-0.46 with a maximum at Rf 0.20-0.26, which corresponds to the second active zone for α -amylase production. Growth promotion of the leaf sheath was not found at Rf 0.40-0.80 where the main active zone for α -amylase production was observed. In *Pharbitis*, activity for α -amylase

Fig. 2. α -Amylase production (top) and leaf sheath elongation (bottom) in eluates from the chromatograms of the extracts of *Lupinus luteus* and *Pharbitis nil*.

production was found at Rf 0.40-0.87, with a maximum at Rf 0.66-0.73. On the other hand, the active zone for leaf sheath growth was noticed at Rfs 0-0.20 and 0.34-0.46, which were entirely different from the active zone for α -amylase production.

Gibberellin A₃ was effective for both growth and enzyme production. However, some substances or extracts from plant materials were effective only for enzyme production but not for the elongation of rice sheath, or *vice versa*. It may be concluded that the substances which promote α -amylase production in an embryoless endosperm are not necessarily effective for growth promotion.

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