

# This Week's Citation Classic™

CC/NUMBER 45  
NOVEMBER 5, 1984

**Yomo H.** Studies on the amylase activating substance. (Part 4.) On the amylase activating action of gibberellin. *Hakkō Kyōkaishi* 18:600-2, 1960.  
[Takara Shuzo Co. Ltd., Kyoto, Japan]

When embryo-free ungerminated barley endosperms were aseptically incubated in an artificial medium, gibberellin in the medium increased total amounts of  $\alpha$ -amylase in both the medium and endosperms. The necessary concentration of gibberellin is more than  $10^{-3}$   $\mu\text{g/ml}$ , and 0.1  $\mu\text{g/ml}$  of gibberellin gives maximum amounts of  $\alpha$ -amylase produced. This  $\alpha$ -amylase formation is not observed under anaerobic conditions. [The SCI® indicates that this paper has been cited in over 100 publications since 1960.]

Harugoro Yomo  
9-8-513 Asahioka-cho  
Ashiya 659  
Japan

August 3, 1984

"I started to study  $\alpha$ -amylase formation during germination of barley seeds in the laboratory of Takara Shuzo Co. Ltd. in 1955, when beer production was scheduled to start.

"At that time, there were two theories on the origin of  $\alpha$ -amylase formation, the embryo-origin theory<sup>1</sup> and an endosperm-origin theory.<sup>2</sup>

"I thought at that time that the former theory was correct and wanted to determine experimentally if embryos that grow in an artificial medium can secrete  $\alpha$ -amylase into the medium. Microbial contamination that occurred during embryo growth was overcome by dehussing the barley seeds with 50 percent sulfuric acid and sterilizing the

separated embryos with 0.2 percent bromine water. The growing seedling could produce some  $\alpha$ -amylase, but the small amount secreted into the medium was not enough to explain the level of endosperm  $\alpha$ -amylase of green malt.<sup>3,4</sup>

"However, after the embryos had grown in it, the medium proved able to produce  $\alpha$ -amylase in the embryo-free, ungerminated barley endosperms after aseptic incubation at 20°C for five days.

"I searched for natural substances that contain this kind of  $\alpha$ -amylase-producing substance (the active substance). I found it in green malt and started to purify it. During the purification, using paper chromatography to separate the active substance, I noticed that its  $R_f$  value was almost the same as that of gibberellin. I used a gibberellin solution as the medium for embryo-free endosperm culture. I found that 1 ng/ml (i.e., 3.3 nM gibberellin) produced significant amounts of  $\alpha$ -amylase in both the medium and the embryo-free endosperm after five days of incubation at 17°C, and that 0.1  $\mu\text{g/ml}$  gibberellin produced the maximum amounts of  $\alpha$ -amylase at both sites. This  $\alpha$ -amylase formation was not observed under anaerobic conditions. These are the main findings of my article, which has been cited in several reviews.<sup>5,6</sup>

"I was given an award (Hakkou Kyoukai Shou) on this subject from the Fermentation Association, Japan, in October 1961.

"Gibberellin, one of the so-called plant hormones, induces enzyme formation at a location different from its origin in the plant body. This may account for the frequent citation of my article."

1. DeClerck J. *A textbook of brewing*. London: Chapman & Hall, 1957:58. 2 vols.
2. Brown H T & Escombe F. On the depletion of the endosperm of *Hordeum vulgare* during germination. *Proc. Roy. Soc. London* 63:3-25, 1898.
3. Yomo H. Studies on the barley malt. The sterilization of barley seeds and the amylase formation of separated embryos and endosperms. *Hakkō Kyōkaishi* 18:444-8, 1958.
4. Briggs D E. Origin and distribution of a  $\alpha$ -amylase in malt. *J. Inst. Brew.* 70:14-24, 1964.
5. Paleg L G. Physiological effects of gibberellins. *Annu. Rev. Plant Physiol.* 16:291-322, 1965. (Cited 165 times.)
6. Akazawa T & Okamoto K. Production of  $\alpha$ -amylase in starch-rich cereal seeds. *Kagaku to Seibutsu* 20:38-44, 1982.