

Jones R L & Varner J E. The bioassay of gibberellins. *Planta* 72:155-61, 1967.
[MSU/AEC Plant Research Laboratory, Michigan State University, East Lansing, MI]

The paper described a bioassay for gibberellins based on the measurement of α -amylase activity released from de-embryonated half-grains of barley. The method was rapid and sensitive to concentrations of gibberellic acid as low as 10^{-10} M. [The SC1[®] indicates that this paper has been cited in over 215 publications.]

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When I joined the Plant Research Laboratory at Michigan State University as a post-doctoral fellow in 1965, my research goal was to define the sites of synthesis of gibberellins. These hormones are present in plants at low concentration, so bioassay methods were used for their determination. Until 1964 bioassays for gibberellins were based on the growth response of shoot tissue, especially that of dwarf cultivars of corn, pea, and rice.¹

In 1960 L. Paleg² in Australia and H. Yomo³ in Japan simultaneously described the response of barley endosperm to added gibberellic acid. They showed that gibberellic acid stimulated α -amylase activity, which caused the breakdown of endosperm starch. The amount of starch breakdown was proportional to the logarithm of the gibberellic acid concentration, and the response formed the basis of a new bioassay.⁴ In this assay, the release of reducing substances from de-embryonated barley grains was measured using the copper-ferricyanide procedure. This assay, as described by Nicholls and Paleg,⁴ was simple and sensitive to as little as 5×10^{-11} M gibberellic acid. Since the bioassay was dependent on the accumulation of reducing groups in solution, it was

subject to interference from impurities in the organic solvents used to isolate gibberellins from plant tissues.

Many of my fellow postdoctorals at the Plant Research Laboratory, including Maarten Chrispeels and John Jacobsen, were engaged in research with Joseph Varner on the effects of gibberellic acid on the synthesis of α -amylase in barley. This group showed that the *de novo* synthesis and release of α -amylase occurred from the aleurone of de-embryonated grain and was regulated by gibberellic acid.⁵

These observations led me to examine the production of α -amylase by de-embryonated grain as a basis for a bioassay for gibberellin. My experiments showed that the synthesis of α -amylase in half-grains of barley was proportional to the logarithm of the gibberellic acid concentration and could be detected at hormone concentrations as low as 1×10^{-10} M. This assay was not sensitive to reducing agents such as those found in organic solvents.

Since this bioassay was a simple and quick method for assaying gibberellins, it was widely used. This no doubt accounts for the high number of citations to this article. The fact that the synthesis of α -amylase by barley aleurone has become a "classic" response to gibberellin and is firmly established as a laboratory exercise in plant biochemistry and plant physiology classes also helped to popularize our work.

Modern methods of plant hormone analysis are replacing bioassays as the methods of choice for determining gibberellin levels. Physical (e.g., gas chromatography—mass spectrometry) and immunochemical methods for estimating gibberellins now offer both superior sensitivity as well as specificity for the wide range of more than 70 known gibberellins. Although the barley half-grain is no longer extensively used as a bioassay, the isolated aleurone layer is widely exploited by us⁶ and others as an experimental system for the study of the molecular basis of gibberellic acid and Ca^{2+} action.

1. Phinney B O & West C A. Gibberellins as native plant growth regulators. *Annu. Rev. Plant Physiol.* 11:411-36, 1960. (Cited 90 times.)
2. Paleg L G. Physiological effects of gibberellic acid. I. On carbohydrate metabolism and amylase activity of barley endosperm. *Plant Physiol.* 35:293-9, 1960. [See also: Paleg L G. Citation Classic. *Current Contents/Agriculture, Biology & Environmental Sciences* 10(13):26, 26 March 1979.]
3. Yomo H. Studies on the amylase activating substance. (Part 5.) Purification of the amylase activating substance in the barley malt and its properties. *Hakkō Kyōkaishi* 18:603-6, 1960. (Cited 35 times.)
4. Nicholls P B & Paleg L G. A barley endosperm bioassay for gibberellins. *Nature* 199:823-4, 1963. (Cited 75 times.)
5. Filner P & Varner J E. A test for *de novo* synthesis of enzymes: density labelling with H_2O^{18} of barley α -amylase induced by gibberellic acid. *Proc. Nat. Acad. Sci. US* 58:1520-6, 1967. (Cited 250 times.)
6. Deikman J & Jones R L. Control of α -amylase mRNA concentration by gibberellic acid and calcium in barley aleurone layers. *Plant Physiol.* 78:192-8, 1985.