A New and Efficient Micro Assay Method for Measuring the Gibberellic acid-induced α-Amylase Activity in Barley Seeds

Santokh Singh (Department of Botany, Faculty of Science, University of British Columbia (UBC), Vancouver, BC, V6T 1Z4, Canada)

ABSTRACT

A new micro assay experimental protocol for measuring α-amylase activity in plant tissues was developed. The α-amylase micro assay utilizes a 96-well microplate using only micro liter volumes of the various reagents. The α-amylase activity was determined by measuring the blue color developed by the reaction of starch with the iodine-potassium iodide reagent using a microplate reader. The effect of plant hormones, gibberellic acid (GA_3), and abscisic acid (ABA) on the induction of α-amylase activity in barley (*Hordeum vulgare*) half-seeds (embryo-containing or embryo-less) was investigated using the micro assay method. The α-amylase activity in the embryo-containing half-seeds was much greater than in the embryo-less half-seeds of barley. GA_3 caused the induction of α-amylase activity in embryo-less half seeds, whereas ABA inhibited the GA_3-induced induction of α-amylase. This new method has been introduced to undergraduate research students for their independent research projects in upper-level Plant Physiology and Plant Development research-enriched courses. The new micro assay method for the measurement of α-amylase activity in plant tissues is more convenient, rapid, uses lower quantities of reagents and produces less chemical waste than the traditional α-amylase assays.

INTRODUCTION

Cereal seeds have two parts: the embryo and the endosperm. The embryo consists of an embryonic axis that grows into the seedling and an absorptive region called scutellum, which is involved in both enzyme secretion and absorption of hydrolyzed metabolites and nutrients. The endosperm contains storage food reserves especially starch and is surrounded by the aleurone layer, which is involved in synthesis and/or activation of hydrolytic enzymes, especially α-amylase during germination. The induction of α-amylase in barley seed is under the control of plant hormones, gibberellic acid (GA_3) and abscisic acid (ABA). GA_3 is released from the embryo and act on the aleurone layer of the seed. The aleurone subsequently produces α-amylase, which digests the starch of the endosperm. ABA is known to inhibit the GA_3-induced α-amylase induction in barley seed during germination.

A new micro assay experimental protocol for measuring α-amylase activity was developed in our lab. This method is more convenient, rapid, uses lower quantities of reagents and produces less chemical waste. We used this analytical method to analyze the α-amylase activity in barley (*Hordeum vulgare*) half-seeds treated with plant hormones, gibberellic acid and abscisic acid.

MATERIALS AND METHODS

PART 1: Enzyme Preparation:

- Select about 85 uniform large seeds of barley (var. Himalaya) (Fig. 1).
- Locate the embryo using a dissecting microscope or hand lens, then cut the seeds in half.
- Keep the embryo-containing and embryo-less half seeds separated.
- From this point onward, work in the sterile cabinet. Surface-sterilize each batch of half seeds in separate tubes containing 20-30 ml of 50% commercial bleach. Carefully decant the bleach in an empty flask. Wash the seeds 5 times with sterilized distilled water.
- Add 2.0 mL of sterile 2 mM sodium acetate buffer (pH 4.8), 0.2 mL of sterile 0.2 M $CaCl_2$ solution and 0.2 mL of chloramphenicol (1 mg/mL) solution. Then add 7 barley half seeds (embryo or embryo-less), and hormone or hormone inhibitor solutions of specific concentrations, and incubate at 30^0C for 48 hours.

PART 2: α-Amylase Assay:

- The supernatant in each of the treatment tubes represents the α-amylase extract. The α-amylase micro assay and the starch standard assay in triplicate in conducted in a 96-well Falcon micro titre plate.
- The α-amylase activity is assayed by means of a colorimetric analysis of the blue colour obtained with starch and iodine reagent.
- Measure the absorbance of the starch standards and the α-amylase enzyme samples in micro titre plate at 620 nm using a micro titre plate reader (Fig. 2). Use the starch standard curve to calculate the α-amylase activity.

Figure 1: (a) Barley seed **(b)** Longitudinal section of barley seed

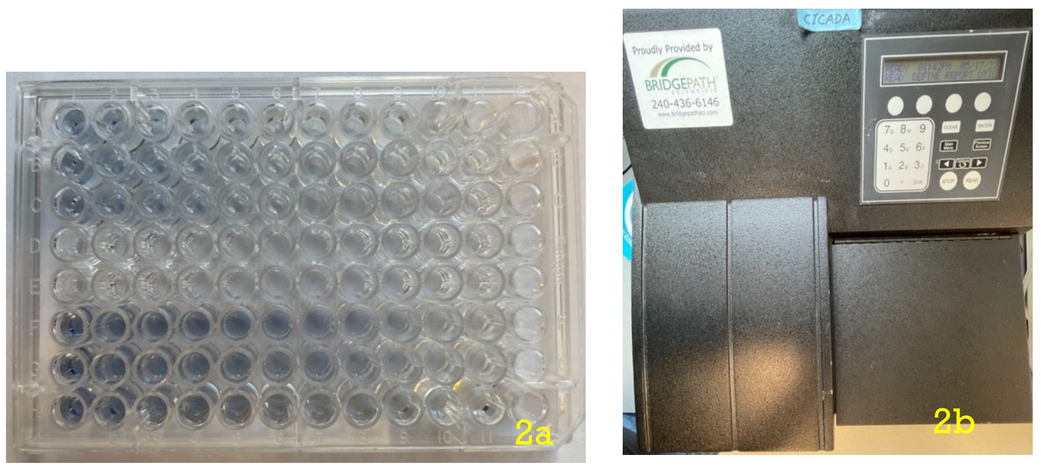

Figure 2: (a) A 96-wells microtitre plate was used for α-amylase micro assay **(b)** The absorbance of starch solution was measured by the plate reader.

RESULTS AND CONCLUSIONS

KEY RESULTS:

- The new micro assay experimental protocol was very rapid and efficient method for measuring the α-amylase activity in barley seed.
- GA_3, especially at a concentration of 100 µM increased the α-amylase activity in the embryo-less half seeds of barley (var. Himalaya) (Fig. 3).
- In contrast to the effect GA_3, the ABA treatment showed much lower levels of the α-amylase activity in barley seed.

CONCLUSIONS:

- A new more rapid, efficient and convenient micro assay experimental protocol for measuring the α-amylase activity was developed.
- This method was used to analyze the α-amylase activity in barley (*Hordeum vulgare*) half-seeds treated with plant hormones, gibberellic acid and abscisic acid.
- This method uses much lower quantities of the chemical reagents and produces less chemical waste.

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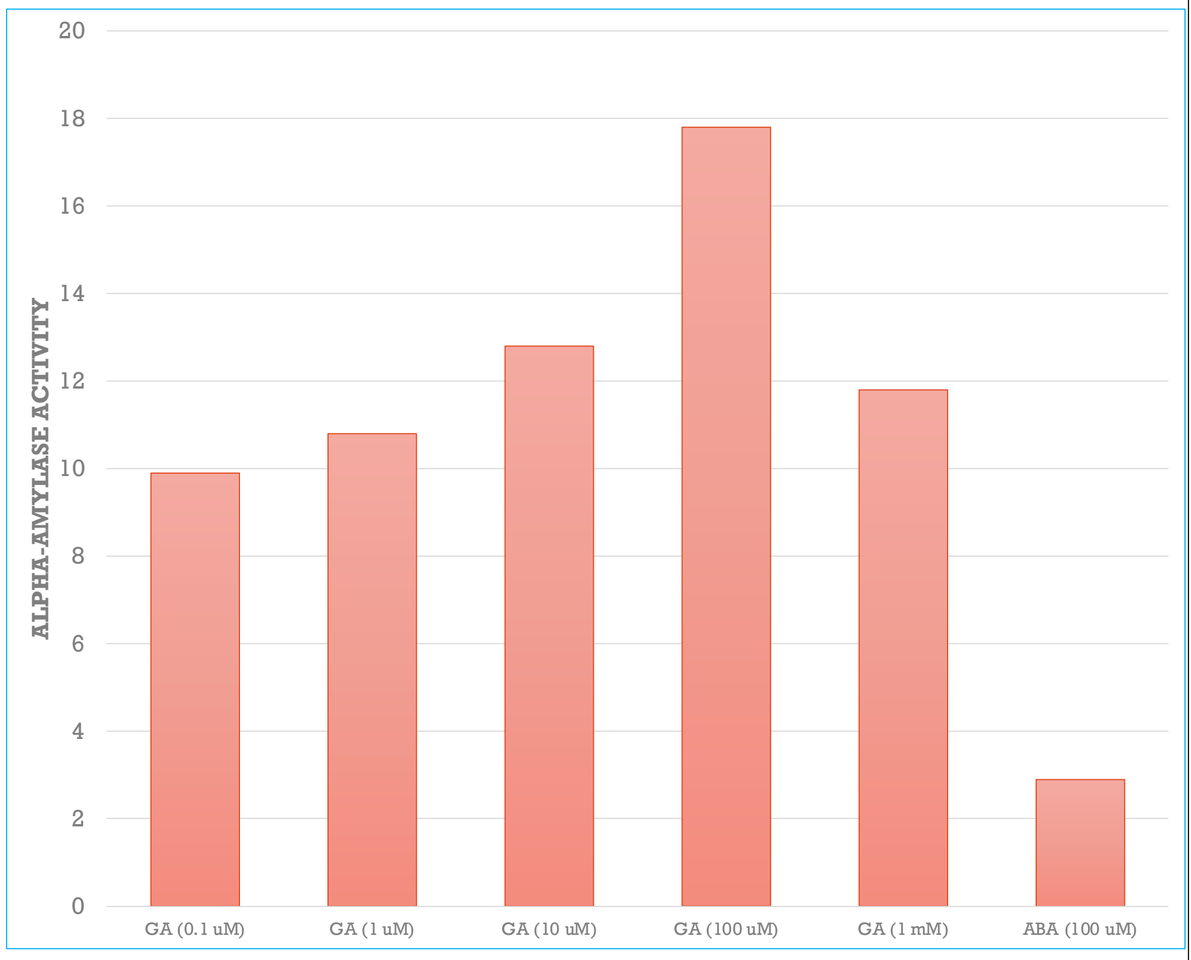

Figure 3: The α-amylase activity in barley embryo-less half seeds treated with plant hormones, gibberellic acid (GA3) and abscisic acid (ABA)