

# Inhibition of GA<sub>3</sub>-induced Amylase Activity in *Vigna angularis* Cotyledons by Glucose

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## 1. Introduction

Cereals, such as rice, barely and wheat, store reserve starch in their endosperm cells. The starch is degraded mainly by  $\alpha$ -amylase during germination and is mobilized to supply sources of carbon and energy for the growth of the embryo(Bewley and Black, 1994).  $\alpha$ -amylase in these grains is synthesized primarily in the scutellum and aleurone layers of the grain, and its level is regulated by gibberellin and ABA at the transcriptional level(Rogers and Rogers, 1992). By contrast, legume seeds accumulate storage starch in cotyledons that are part of the embryo. Upon germination, the starch is degraded by  $\alpha$ -amylase and the resulting sugars are mobilized to support the growth of the embryonic axis. However, in legume seeds, the mechanism for the development of  $\alpha$ -amylase in reserve tissues is much less clear. The effect of gibberellin on the synthesis of  $\alpha$ -amylase in cotyledons remains ambiguous, since significant levels of expression of the  $\alpha$ -amylase are observed even in *Vigna mungo* cotyledons that have been detached from the embryonic axis(Morohashi et al., 1989; Taneyama et al., 1995). In this study, we examined the changes of amylase activities in cotyledons of germinating seeds of *Vigna angularis* in terms of the possible effects end-products and the effects of both axis removal and exogenously applied plant hormones and growth regulators.

## 2. Materials and Methods

*Plant materials*- Seeds of *Vigna angularis* were sterilized, rinsed, and allowed to germinate on layers of wet filter paper at 26 °C in darkness. Cotyledons were collected from seedlings at defined stages of

germination('attached' cotyledons). For experiments with 'detached' cotyledons, cotyledons were excised from dry seeds and allowed to imbibe water or a test solution for 6 h. They were then sterilized, rinsed and incubated in a Petri dish at 26 °C in darkness.

*Amylase assay*- Amylase activity in the supernatant was measured as described by Bernfeld. One unit of activity was defined as 1 mg of maltose liberated 1 h under 1 ml of enzyme solution.

*Determination of reducing sugars and protein*- The amount of reducing sugars in the extracts was determined by the Somogyi-Nelson. The protein content was determined using the method of Lowry et al.

### 3. Results

Fresh weight of cotyledons in germinating *Vigna angularis* decreased concurrently with the growth of axis, whereas detached cotyledons apparently showed no decrease in fresh weight over the entire period.  $\alpha$ -amylase activity in detached cotyledons started to increase on day 2 ; it increased at a high rate than the activity in attached cotyledons and it seemed to continue to increase even on day 5. The content of reducing sugars in detached cotyledons did not increase during incubation, it remained almost unchanged at the initial level. In contrast, the content in the cotyledons of germinating seeds increased nearly two fold. Protein content in cotyledons from the intact seedling decreased rapidly during germination, but there are slight decrease in the detached cotyledons. We applied plant hormones and growth regulators to detached cotyledons and compared the levels of  $\alpha$ -amylase on day 4 with levels in the controls. The application of GA<sub>3</sub>, kinetin and IAA, or their combinations, showed a little effect on the development of enzyme activity. ABA inhibited the level of  $\alpha$ -amylase activity. Actinomycin D, cycloheximide, canavanine, cordycepin and 5-fluorouracil, inhibitors of protein and RNA synthesis, prevented the increase of enzyme activity, indicating the need for continued protein synthesis in the tissue. Inhibitors with GA<sub>3</sub> showed very little effect on the amylase activity. Exogenously applied glucose, the end-product of amylolytic activities, inhibited the development of amylase activities in cotyledons during incubation. The inhibitory effect of glucose was completely overcome by the addition of exogenous GA<sub>3</sub>. The observation support the view that

glucose interferes with GA<sub>3</sub> biosynthesis.

#### 4. Conclusion

Amylase activity in detached and incubated cotyledons of *Vigna angularis* showed higher levels at the 5th day than in attached cotyledons. In contrast, the contents of the reducing sugars was higher in attached cotyledons than in detached ones. Protein degradation was slowed down by the removal of axis. In detached cotyledons exogenously applied plant hormones, or their combinations, showed a little increase on the level of  $\alpha$ -amylase. GA<sub>3</sub> had a significant effect on the developmental patterns of amylase activity. The increase of enzyme activity in cotyledons was inhibited by high concentrations of glucose, suggesting that this effect was caused mostly by osmotic stress and partly by end-product repression. However, this inhibition was partly reversed by the application of GA<sub>3</sub>. Actinomycin D, cycloheximide, canavanine, cordycepin and 5-FU, inhibitors of protein and RNA synthesis, prevented the synthesis of  $\alpha$ -amylase in the presence or absence of exogenous GA<sub>3</sub>.

#### References

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