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# Effects of Plant Growth Regulators and Sugars on the α-Amylase Activity in Cotyledons of Germinating *Vigna angularis* Seeds

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Effect of plant growth regulators and end-product on the enzyme activities in cotyledons of *Vigna angularis* during germination was investigated by measuring the changes of α-amylase activities in attached and detached cotyledons applied growth regulators and sugars. The higher levels of α-amylase in detached cotyledons than those in cotyledons attached to the embryonic axis were due to both faster synthesis and slower degradation of the enzyme in the detached cotyledons than in the attached cotyledons. Levels of α-amylase activity were reduced by high concentrations of glucose and sucrose, and it is suggested that this effect was caused mostly by osmotic stress and partly by end-product repression. In detached cotyledons exogenously supplied $GA_3$, IAA, kinetin, or their combinations has a small promotive effect on the developmental patterns of α-amylase activity. ABA and uniconazole both prevented the synthesis of α-amylase. Glucose inhibition of enzyme activity was partly reversed by the application of $GA_3$ and cAMP. $GA_3$ and cAMP seemed to act through a similar mechanism. The addition of inhibitors of protein and RNA synthesis largely prevented the increase of enzyme activity in the presence or absence of exogenous $GA_3$. The pretreatment experiments with canavanine indicated that the earlier the time of addition was, the lower the amylase activity was.

Key words : α-Amylase, Plant hormones, Gibberellin, Canavanine, Seed germination, *Vigna angularis*

## 1. Introduction

The hydrolytic degradation of reserve materials is a conspicuous phenomenon observed following germination of seeds. During the germination of cereals, the stored reserve starch in their endosperm cells is degraded mainly by α-amylase and is mobilized to supply sources of carbon and energy for the growth of the embryo (Bewley, 1994). This enzyme in cereal grains is synthesized primarily in the scutellum and aleurone layer of the grain, and its level is regulated by gibberellin and ABA at the transcriptional level(Garcia-Maya, 1990; Rogers, 1992). In cereals, the mechanism for the development of α-amylase activity in seeds has been studied in detail (Bewley, 1987; Johnes, 1991).

In contrast, in legume seeds, the mechanism for the development of α-amylase activity in reserve tissues is much less clear. Legumes accumulate storage starch in cotyledons that are part of the embryo. Upon germination, the starch is degraded by α-amylase and the resulting sugars are mobilized to support the growth of the embryonic axis. From analogy with the mechanism in cereal grains, it might be expected that the embryonic

axis of leguminous plant seeds regulates the development of amylase in reserve tissues. Available information concerning the regulation of amylolytic activity in cotyledons of germinating seeds of legumes is often contradictory. It has been shown that the increase in α-amylase activity in cotyledons is dependent on the presence of the axis(Morohashi, 1982; Morohashi, 1989). In other studies, on the other hand, it has been reported that α-amylase reached higher levels in detached cotyledons than in attached cotyledones(Koshiba 1983; Taneyama, 1995), or that the development of the enzyme activity in cotyledons is unaffected by excision of the axis(Dale, 1969). The previous paper reported that the presence of the embryonic axis is a prerequisite for the development of α-amylase activity in mung bean cotyledons (Morohashi. 1989). However, α-amylase development could be brought about in cotyledons even in the absence of the axis, if cotyledons imbibed water through the surface not covered by the seed coat.

Results of several other reports dealing with hormonal control of the development of amylase activity in pea and bean cotyledons are not clear and conflicting reports exist. The effect of $GA_3$ upon amylase activity is contradictory, some authors reporting promotion(Mitsunaga, 1993; Taneyama, 1995), while others report no influence or even inhibition (Parys, 1983 : Hirasawa, 1989). It appears that in some experiments GA, or $GA_3$ plus cytokinins, could replace the function of the axis in amylase activity(Locker, 1975), but the opposite results also have been obtained. It is possible that eventual differences in α-amylase activity between varieties arise from differences in $GA_3$ sensitivity. On the other hand, it has been shown that ABA can exert an inhibitory effect upon the expression of α-amylase genes (Jacobsen,, 1995).

The present paper deals with the changes in α-amylase activities in attached and detached cotyledons of *Vigna angularis* seeds during germination and incubation and the effect of both axis removal and the exogenously applied plant growth regulators and sugars on the development of α-amylase activity.

## 2. Materials and Methods

### 2.1. Plant materials

Seeds of *Vigna angularis* were sterilized for 20 min with 1% sodium hypochlorite solution and rinsed thoroughly with sterile distilled water, then allowed to imbibe water for 6 h in the cold. Imbibed seeds were germinated on layers of wet filter paper at 27 ℃ in the dark. Cotyledons were collected from seedlings at defined stages of germination('attached' cotyledons). For experiments with 'detached' cotyledons, dry seeds were cut into halves and their cotyledons were detached from the embryonic axes. These cotyledons were allowed to imbibe water or a test solution for 6 h. They were then surface-sterilized in 1% sodium hypochlorite solution for 5 min, rinsed with sterilized water and incubated on two layers of wet filter paper in Petri dishes containing test solution at 27℃ in the dark. Embryonic axes attached to one side of cotyledons(with the other side removed) were allowed to germinate under the same conditions as above('one-sided' cotyledons). In some experiments cotyledons were detached from seedling on day 2 and day 4 and treated as described above. The cotyledons were harvested at various stages of germination or incubation and stored at -20 ℃ untile use.

### 2.2. Assay of α-amylase activity

Cotyledons(5 pairs each) of *Vigna angularis* at various germination stages were homogenized

with 15 ml of 0.05 M potassium buffer(pH 7.2) in a chilled mortar and pestle. The homogenate was centrifuged at 20,000 xg for 30 min, and the supernatant was assayed for α-amylase according to the method described by Bernfeld (1955) with minor modifications. Amylase activity in the supernatant was measured by incubating 1 ml of 1% soluble starch dissolved in 0.016 M sodium acetate buffer, pH 4.8, that contained 2 μm $CaCl_2$ for 10 min at 30℃ The reaction was stopped by the addition of 1 ml of 3.5- dinitrosalicylic acid solution, and the absorbance of the mixture was measured at 620 nm. One unit of α-amylase activity was defined as 1.0 mg of maltose liberated per h under 1 ml of enzyme solution.

### 2.3. Determination of reducing sugars and protein

The amount of reducing sugars in the extracts was determined according to Somogyi and Nelson(1952) using glucose released as a reference. The protein content was determined by the method of Lowry *et al*, (1951) with bovine serum albumin as the standard. All data represent the means and SE from three replicate experiments.

## 3. Results

### 3.1. Effects of removal of the axis

When seeds of *Vigna angularis* were germinated at 27℃ in the dark, the fresh weight of cotyledons decreased concurrently with the growth of the axis(Fig. 1A). On days 7 to 8, wilted cotyledons tended to fall off the axis. Fresh weights of one-sided cotyledons decreased at a similar rate to attached cotyledons, but the senescence of one-sided cotyledons proceeded less rapidly than that of attached cotyledons, and the cotyledones remained attached to the axis even after 7 days. The fresh weight of detached cotyledons remained almost unchanged over the entire period and remained fresh for 6 days after the start of imbibition.

α-amylase activity in attached cotyledons which was observed in slight amounts in dry seeds, increased from the 2nd to 5th day of

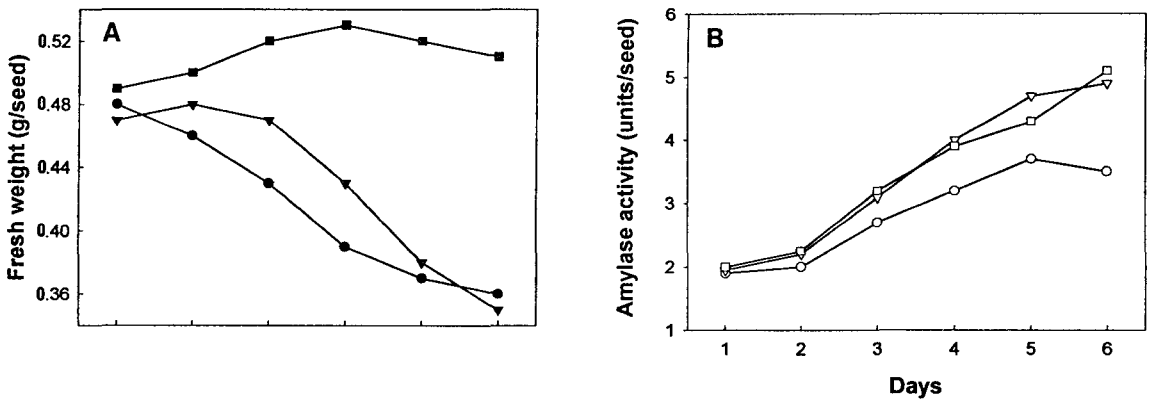


**Fig. 1.** Changes with time in fresh weight(A) and in α-amylase activity(B) in cotyledons during germination and incubation. A. Fresh weight of attached (●), one-sided(▼) and detached(■) cotyledons. B. α-amylase activities in attached(○), one-sided(▽) and attached(□) cotyledons.

germination to reach a maximum and decreased thereafter (Fig. 1B). The activity in detached cotyledons also started to increase at a higher rate than the activity in attached cotyledons and it seemed to continue to increase even on day 6. The activity in one-sided cotyledons increased in a similar manner to that in detached cotyledons but seemed to reach a peak on days 5 to 6.

The content of the reducing sugars in detached cotyledons did not increase during incubation, it remained almost unchanged at the initial level (Fig. 2A). In contrast, the content of the reducing sugars in the cotyledons of germinating seeds increased nearly two fold in this period. These findings indicate that there is an inverse relationship between the concentration of reducing sugars and amylase activity in cotyledons. Protein content in cotyledons from the intact seedling decreased rapidly during germination, but there was a slight decrease in the detached cotyledons during incubation(Fig. 2B). The results indicate that protein degradation was not extensive following axis removal.

When the cotyledons were detached from germinating seeds on day 2 and 4 after imbibition and then incubated, $\alpha$-amylase activity started to increase after excision and reached a higher level than that of attached cotyledons (Fig. 3). Thus, it is likely that in attached cotyledons the increase of amylase activity is due to the accumulation of the enzyme and the decrease occurring after the 5th day of germination is due to the proteolytic degradation of the enzyme, which may already start before the 5th day of germination. On the other hand, in detached cotyledons, amylase was continuously synthesized and accumulated throughout incubation, because of the low level of the proteolytic degradation in this tissue. Such a comparison between attached and detached cotyledons seems to indicate that embryo axis had an undoubtedly positive effect upon $\alpha$-amylase activity in cotyledons during germination. These results raise the possibility that endogenous hormones from the embryonic axis might naturally be involved in the level of cotyledonary $\alpha$-amylase activity. Thus, the influence of plant growth substances on the development of $\alpha$-amylase activity was investigated in the detached cotyledons.

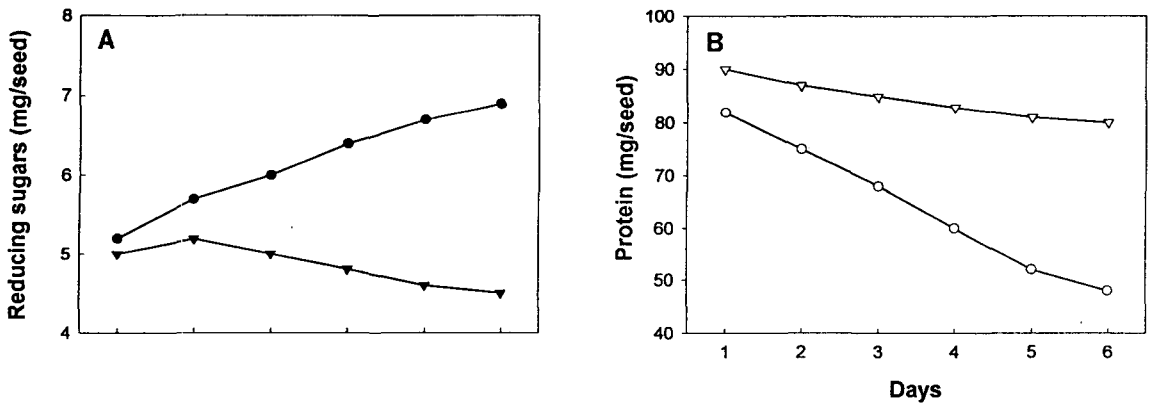


**Fig. 2.** Changes with time in reducing sugars(A) and protein(B) contents during germination and incubation. A. Reducing sugar content in attached(●) and detached(▼) cotyledons. B. Protein content in attached(○) and detached(△) cotyledons.

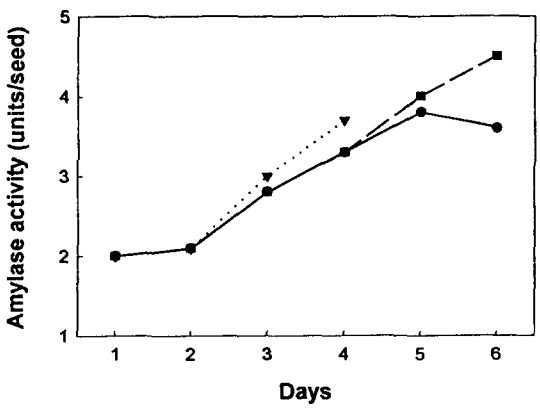


**Fig. 3.** Effect of removing axes on amylase activity after 2(▼) and 4(■) days of germination, compared to attached cotyledons(●).

## 3.2. Effects of plant growth regulators and sugars

Plant hormones and growth regulators were applied to detached cotyledons and compared the levels of α-amylase on day 4 with levels in the controls to which no test compounds had been applied(Table 1). $GA_3$ at $10^{-5}$ M had a slight promotive effect in increasing the level of α-amylase activity in the cotyledons of 4-day-old seedlings. In addition, kinetin and IAA at $10^{-5}$ M showed little significant effect on the increase of α-amylase activity respectively, as compared to the controls. Hirasawa(1989) reported that the embryonic axis had an unequivocally positive effect on the development of α-amylase in pea seedling and that the activity in detached cotyledons was dramatically promoted during incubation with auxins. ABA, an antagonist of the activity of gibberellin, decreased the level by 22% as compared to the control. Uniconazole at $10^{-5}$ M effectively depressed the level of α-amylase in 4-day-old detached cotyledons with 30% inhibition. This triazole-type growth retardant inhibited the biosynthesis of gibberellin and the induced synthesis of α-amylase in a dwarf rice mutant, and the induced synthesis of α-amylase was repressed by uniconazole at 0.1 to 10 μM (Mitsunaga, 1993). The application of combinations of plant hormones at the concentrations used showed little significant effect on the development of enzyme activity. The effect of $GA_3$ on the level of α-amylase was further supported by the supressive effect of sugars and inhibitors of protein and RNA synthesis on the level of the enzyme.

**Table 1.** Effect of plant growth regulators on α-amylase activity in detached cotyledons.

| Growth regulators | Concentration (M) | α-Amylase activity (units/seed) |
|---|---|---|
| None | | 3.72 |
| $GA_3$ | $10^{-5}$ | 4.09 |
| IAA | $10^{-5}$ | 4.13 |
| Kinetin | $10^{-5}$ | 3.95 |
| ABA | $10^{-4}$ | 2.90 |
| Uniconazole | $10^{-5}$ | 2.62 |
| $GA_3$ + IAA | $10^{-5}$ + $10^{-5}$ | 4.27 |
| $GA_3$ + kinetin | $10^{-5}$ + $10^{-5}$ | 4.24 |
| IAA + kinetin | $10^{-5}$ + $10^{-5}$ | 4.18 |

Detached cotyledons were incubated at 27℃ in the dark for 4 days with indicated compounds. The values are the average of three independent experiments.

To examine the effect of exogenously applied sugars on the level of α-amylase, germinating seeds were allowed to absorb three kinds of sugar: glucose, being the end-product of amylolytic activities; sucrose, being the main transportable form; and mannitol being a non-metabolizable control. Sugars were applied at high concentrations and the influence of these sugars on the growth rate and the level of α-amylase was examined on day 4 after the start of imbibition. Application of glucose and sucrose, but not of mannitol, at 1.0 and 5.0% decreased the loss in fresh weight of cotyledons, as compared with the controls(Table 2). Levels of α-amylase

activity on day 4 decreased gradually depending on the concentration of sugars applied.

Glucose at 1% and 5% inhibited the development of amylase activity in cotyledons by 24% and 39%, respectively(Table 3). The inhibitory effect of glucose was completely overcome by the addition of exogenous $GA_3$. At high concentration of glucose(5%), the severe inhibition of enzyme activity was partially counteracted by $GA_3$. The observation support the view that glucose interfers with $GA_3$ biosynthesis. Interestingly, cAMP was as effective as $GA_3$ in overcoming the inhibitory effect of glucose on $\alpha$-amylase activity in detached cotyledons. Simultaneous addition of $GA_3$ and cAMP with glucose(1%) completely counteracted the glucose-induced inhibition. The results suggest that the inhibition by glucose is not direct but protein and RNA synthesis may be involved.

### 3.3. Effects of inhibitors of protein and RNA synthesis

To study the nature of $\alpha$-amylase formation in the cotyledons, detached cotyledons were incubated in solutions containing inhibitors of protein and RNA synthesis such as actinomycin D, cycloheximide, canavanine, cordycepin and 5-fluorouracil(5-FU) for 4 days. The addition of inhibitors, particularly, canavanine and cordycepin, largely prevented the increase of enzyme activity, indicating the need for continued protein synthesis in the tissue(Table 4). 5-FU, however, was less effective. The presence of 10 $\mu$g/ml actinomycin D less reduced the enzyme activity than cycloheximide. Production of $\alpha$-amylase in rice embryoless seed was less inhibited by actinomycin D than by cycloheximide at similar concentrations(Palmiano, 1972). Inhibitors, when tried together with $GA_3$, the stimulation was sensitive to a large extent with very little effect on the amylase activity.

**Table 2.** Effect of sugars on changes in fresh weight of the detached cotyledons during incubation for 4 days.

| Treatment | Fresh weight (g/seed) | $\alpha$-amylase activity (units/seed) |
|---|---|---|
| Control | 0.42 | 3.84 |
| 1% glucose | 0.47 | 2.73 |
| 1% sucrose | 0.44 | 2.32 |
| 1% mannitol | 0.40 | 2.56 |
| 5% glucose | 0.50 | 2.20 |
| 5% sucose | 0.47 | 2.05 |
| 5% mannitol | 0.40 | 2.17 |

**Table 3.** Glucose-induced inhibition of $\alpha$-amylase activity in detached cotyledons of 4-d-old seedlings.

| Treatment | $\alpha$-Amylase activity (units/seed) | Relative activity ( %[a] ) |
|---|---|---|
| None | 3.75 | 100 |
| $10^{-5}$ M $GA_3$ | 4.02 | 107 |
| $10^{-5}$ M cAMP | 3.95 | 105 |
| $10^{-5}$ M $GA_3$ + $10^{-5}$ M cAMP | 4.13 | 110 |
| 1% glucose | 2.85 | 76 |
| 1% glucose + $10^{-5}$ M $GA_3$ | 3.88 | 103 |
| 1% glucose + $10^{-5}$ M cAMP | 3.69 | 98 |
| 1% glucose + $10^{-4}$ M cAMP | 3.84 | 102 |
| 5% glucose | 2.30 | 61 |
| 5% glucose + $10^{-5}$ M $GA^3$ | 2.81 | 75 |
| 5% glucose + $10^{-5}$ M cAMP | 2.70 | 72 |
| 5% glucose + 10-4 M cAMP | 3.08 | 82 |
| 5% glucose + $5\times10^{-4}$ M cAMP | 3.26 | 87 |

[a]The relative values were expressed by taking the amylase activity for the detached control as 100 %.

The effect of preincubation for different times in actinomycin D on the induction of amylase by $GA_3$ is shown in Table 5. This antibiotic, which inhibits DNA-directed RNA synthesis, caused inhibitions of the induction of amylase. This inhibition was fairly marked, although not complete, if the detached cotyledons were preincubated in actinomycin D for 72 h. If, however, the cotyledons were incubated for 48 h then 24 h in actinomycin D before addition of $GA_3$, the

inhibition was less severe.

**Table 4.** Effect of inhibitors of protein and RNA synthesis on α-amylase activity in detached cotyledons of 4-d-old seedlings.

| Treatment | Concentration | α-Amylase activity (units/seed) |
|---|---|---|
| None | | 3.82 |
| $GA_3$ | $10^{-5}$M | 4.12 |
| Actinomycin D | 10μg/ml | 2.26 |
| Cycloheximide | 10μg/ml | 2.07 |
| Canavanine | 50mM | 1.85 |
| Cordycepin | $5\times10^{-4}$M | 1.92 |
| 5-fluorouracil | 1.0mM | 2.76 |
| Actinomycin D + $GA_3$ | 10μg/ml + $10^{-5}$M | 2.71 |
| Cycloheximide + $GA_3$ | 10μg/ml + $10^{-5}$M | 2.52 |
| Canavanine + $GA_3$ | 50mM + $10^{-5}$ M | 2.30 |
| Cordycepin + $GA_3$ | $5\times10^{-4}$M + $10^{-5}$M | 2.34 |
| 5-fluorouracil + $GA_3$ | 1.0mM + $10^{-5}$M | 3.25 |

**Table 5.** Effect of preincubation with actinomycin D on the induction of α-amylase activity in detached cotyledons

| Treatment | α-Amylase activity | |
|---|---|---|
| | units/seed | % inhibition |
| (a) | 2.78 | 30 |
| (b) | 3.24 | 18 |
| (c) | 3.45 | 13 |
| (d) | 3.96 | 0 |
| (e) | 3.75 | |

Inhibition expressed are the inhibition of the activity induced by $GA_3$, i.e. basal levels in the control incubation(e) were subtracted. Detached cotyledons were incubated for a total of 96 h. Treatment was : (a) actinomycin D after 0 h and $GA_3$ after 72 h ; (b) actinomycin D after 24 h and $GA_3$ after 72 h ; (c) actinomycin D after 48 h and $GA_3$ after 72 h ; (d) $GA_3$ after 72 h ; (e) none.

The inhibitory effect of canavanine on $GA_3$ -induced amylase synthesis was less as canavanine was added later after the addition of hormone. No inhibitory effect was observed if canavanine was added 9 h or later after the addition of $GA_3$(Table 6). In fact, an enhancement of α-amylase formation by canavanine added 12 h after $GA_3$ was occasionally observed. Because canavanine had no effect on the degradation of the enzyme, it is suggested that amylase is translated from stable mRNA(Ho and Varner, 1974).

**Table 6.** Effect of canavanine on $GA_3$-induced amylase activity in detached cotyledons.

| Treatment | α-Amylase activity ( units/seed ) | Relative activity ( % ) |
|---|---|---|
| $GA_3$ only | 2.22 | 100 |
| Control(No $GA_3$) | 2.16 | |
| $GA_3$ + canavanine | | |
| Added 0 time | 1.47 | 66 |
| Added 4 h after $GA_3$ | 1.60 | 72 |
| Added 6 h after $GA_3$ | 1.74 | 78 |
| Added 9 h after $GA_3$ | 2.11 | 95 |
| Added 12 h after $GA_3$ | 2.28 | 103 |

Canavanine(50 mM) was added at different times as indicated and cotyledons were further incubated until 30 h after $GA_3$.

## 4. Discussion

α-Amylase plays an important role in starch mobilization in germinated legume seeds. In *Vigna angularis* cotyledons the enzyme activity showed a marked increase about 2 d after the start of imbibition(Fig. 1B). It has been indicated that the development of α-amylase in mung bean is primarily regulated at the transcriptional level (Morohashi, 1989; Koizuka, 1995). Thus, the expression of α-amylase in cotyledons is developmentally controlled during and following seed germination. The previous papers described that α-amylase expression in mung bean cotyledons was dependent on the presence of the axis and that the expression in axis-removed cotyledons was severely retarded.(Yamauchi, 1994). In attached cotyledons, the relative amount of α-amylase per seed increased rapidly to reach a peak on day 2 and then decreased rapidly, whereas the amount in detached cotyledons was

maximal on day 4 and remained high until day 10. In attached cotyledons, storage starch, as well as storage protein was actively mobilized to the growing axis(Minamikawa, 1979), and both the synthesis and the degradation of hydrolytic enzymes might take place in such cotyledons. By contrast, the mobilization of starch and storage protein is arrested in detached cotyledons and thus the cotyledons exhibited delayed senescence (Fig. 2). Under such artificial conditions, α-amylase might tend to accumulate rather than to be degraded in detached cotyledons. Such a comparison between attached and detached cotyledons seems to indicate that the embryonic axis had an undoubtedly positive effect upon α-amylase in cotyledons during germination. These results suggest that the development of enzyme activity and reserve metabolism may be controlled by the mediation of plant hormones produced in axis portions.

The results in Table 1 indicate that $GA_3$ at $10^{-5}$ M was effective in increasing the level of α-amylase activity in cotyledons of 4-day-old seedlings of *Vigna angularis*. The effect of $GA_3$ on the level of α-amylase was further supported by the suppressive effect of ABA and inbibitors of protein and RNA synthesis in the level of the enzyme. However, it should be noted that, in contrast to the essential role of gibberellin in the inducible synthesis of α-amylase in isolated aleurone layers of cereal plants, exogenously applied gibberellin was not a prerequisite for the synthesis of α-amylase in detached cotyledons of the seeds of *Vigna angularis*. Exogenous $GA_3$ was effective only in stimulation of the level of α-amylase in the cotyledons. The difference may reflect different source-sink relationships in the endosperm-embryo system of monocot cereal grains and the cotyledon-axis system of legume seeds. The present results suggest the possibility that gibberellins and, presumably, other plant hormones as well increase the level of α-amylase in detached cotyledons by suppression of the degradation of the enzyme. Of the endogenous auxins, IAA in *Pisum sativum* had a small effect on the development of enzymatic activity in the detached cotyledons during incubation at 1 μM and 10 μM (Hirasawa, 1989). However, synthetic auxins, 2,4-D and 2,4,5-T, dramatically promoted α-amylase activity. Exogenous IAA may be susceptible to decomposition in the metabolically active cotyledons. The kinetin had a small effect on the development of enzymatic activity. Hirasawa(1989) reported that BA promotes about 2-fold increase in α-amylase activity in detached cotyledons, whereas $GA_3$ had little promotive effect on the development of α-amylase activity. By contrast, ABA at a concentration of 10 μM repressed 50 % of the α-amylase activity in pea cotyledons. In intact seedlings an auxin from the embryonic axis may induce α-amylase activity in the cotyledons during germination. $GA_3$ and ABA act in opposite direction in the control of gene expression during seed development and germination(Jacobsen, 1995). ABA is known to reduce the production of α-amylase in barley aleurones(Chandler, 1991). Pagano *et al.*(1997) reported that differences between varieties of *Sorghum* are not limited to differences in the susceptibility to germinate on the mother plant, but they include differences in the activity of α-amylase. These results suggest that the participation of both $GA_3$ and ABA in the regulation of the α-amylase activity in developing *Sorghum* caryopses. In the presence of uniconazole, the synthesis of α-amylase by dwarf rice mutant during germination was depressed(Mitsunaga, 1993). The repression caused by uniconazole could be overcome by a subsequent treatment with $GA_3$. These results indicate that the levels of active $GA_3$ in the normal cultivar treated with the growth retardant

are sufficient to retard the elongation of shoots, but not to inhibit the induction of α-amylase, suggesting that the levels of active $GA_3$ required for elongation of shoots.

The regulation of enzyme activity was affected by the external addition of sugars. The glucose and sucrose arrested the growth of seedlings after day 4(Table 2). The growth rates and α-amylase level were much influenced by sugars and they decreased gradually depending on the concentration of sugars applied(Taneyama, 1995). The effects of sugars at high concentrations on the activity of α-amylase in cotyledons of *Vigna angularis* might be due mostly to osmotic stress and also to end-product repression of synthesis of the enzyme to a lesser extent. High osmotic concentrations might prevent the synthesis of the enzyme and stimulate the degradation of the enzyme(Bewley, 1994). The expression of a gene for α-amylase in the scutella of isolated rice embryo was shown to be repressed by a variety of sugars, and the level of expression has shown to be inversely related to the concentration of sugars in cultured cells (Karrer, 1992; Huang, 1993). The accumulation of sugars inhibited the synthesis of $GA_3$, which in turn prevent the synthesis of α-amylase in barley(Briggs, 1973). $GA_3$ and cAMP was capable of reversing the inhibitory effect of glucose on α-amylase activity in the cotyledons(Table 3). Both $GA_3$ and cAMP was acted through a similar mechanism and control various processes by synthesizing new mRNAs specific for different hydrolysing enzymes(Kapoor, 1987).

Recent reports suggest that α-amylase induction depends on increase of translatable mRNA for α-amylase and protein synthesis. The increase was due to a direct enhancement of transcription of α-amylase gene by $GA_3$ (Bernal-Lugo, 1981). Koizuka,(1995) reported that the activity of α-amylase in mung bean cotyledons increased markedly in response to wounding and the increase in α-amylase mRNA level in wounded cotyledons was severely inhibited by cordycepin. The stimulation of RNA synthesis was extremely sensitive to the action of inhibitors of RNA synthesis in the presence or absence of exogenous $GA_3$(Table 4). These results corresponded with the inhibition of ribonuclease with respective inhibitors(Kapoor, 1987). The inhibitors data suggest that a part of the enzyme is synthesized *de novo* and amylase is translated from stable mRNA.

The addition of actinomycin D prevented the increase of enzyme activity, but the inhibition was less severe, if the cotyledons were incubated for 48 h then 24 h in actinomycin D before addition of $GA_3$(Table 5). This result suggests that some of the RNA necessary for enzyme production is produced in the first 48 h, i.e. before addition of $GA_3$. It is possible that the incomplete inhibition by actinomycin D is the result of poor penetration or destruction of the antibiotics.

The addition of canavanine after 12 h of incubation after $GA_3$ did not show any inhibitory effect on the enzyme activity(Table 6). However, the earlier the time of canavanine addition was, the lower the enzyme activity was. The results suggest that canavanine is incorporated in proteins, thus leading to the synthesis of functionally active proteins. Inhibition of $GA_3$-induced amylase activity by canavanine in barley half seed seemed to be due to affection of canavanine on transcriptional stage including mRNA metabolism rather than on translation of amylase protein(Park, 1981). Taken together these experiments in legume cotyledons many anabolic activities take place independently of growing axis. Detached cotyledons can and do synthesize many enzymes. Yet the hydrolysis of the macromolecular reserves and the senescence which follows it appears to depend on a growing

axis. Therefore, it is concluded that there must be a feed-back loop that regulates amylolytic activities in the axis and in the cotyledons during germination.

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