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Fluctuation of ATP Content in Soybean and Mungbean Seeds with Germinating Time

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콩(*Glycine max* Merrill) 및 녹두(*Phaseolus radiatus* L.) 種子의 發芽日別 ATP 含量變化

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ABSTRACT

ATP contents of soybean(*Glycine max* Merrill) and mungbean (*Phaseolus radiatus* L.) seeds being germinated with Hoagland solution at 30°C for 6 days were determined. In pregerminated seed, ATP contents in soybean and mungbean were 11.4 and 63.0 μg/g fresh seed respectively. During germination, the highest ATP content of soybean seeds was 550% of initial content on 2nd day and that of mungbean was 480% on 1st day after germination. ATP content in cotyledon of soybean and mungbean were increased up to 4th and 1st day after germination respectively, thereafter both were decreased, but those in the root, including the hypocotyl, of both seeds were continuously increased with germination progress.

INTRODUCTION

Seed germination requires the synthesis of enzymes and organelles for catabolic degradation of reserve food in the storage tissue and for anarobic building up of cellular constituents in the seedling (Ching, 1972). Because the synthesis of protein and nucleic acids is the major event in embryonic tissue, ATP is in great demand during seed germination (Ching, 1972; Marcus, 1969). One of the earliest detactable metabolic processes in germinating seed is the rapid and dramatic increase of ATP content (Ching, 1975; Moreland *et al.*, 1974; Obendory and Marcus, 1974; Walbot, 1972). ATP was found to increase very rapidly upon imbibition of lettuce seeds under optimum temperature and oxygen conditions (Pradet *et al.*, 1968).

Anderson (1977a) reported that during the first three hours in the germination of soybean axes the net increase in adenine nucleotide was partly due to their synthesis from adenine and adenosine. He also reported that the ATP content in soybean axes incubated for three hours in 1mM solution of adenine and adenosine was increased by 100% and 75%, respectively, over axes incubated in water (Anderson, 1977b). These conversions suggest that adenine phosphoribosyl transferase and adenosine kinase act to increase the adenine nucleotide content of germinating seeds. both of these enzymes belong to the salvage pathway which converts purine and pyrimidine bases and nucleosides to nucleotides (Hartman, 1970).

Sobczyk and Kacperska-Palacz (1978) reported the marked increase of ATP content in the leaves of winter rape plants during the first stage of fruit hardening. he also pointed out that the increased ATP content in cold treated leaves were due

to light and dark processes.

The purpose of present study was to determine the fluctuation of ATP contents in root and cotyledon in germinating soybean and mungbean seeds.

MATERIALS AND METHODS

Plant Materials Soybean(*Glycine max* Merrill) and mungbean (*Phaseolus radiatus* L.) seeds were germinated in darkness at 30°C in plastic dishpans containing vermiculate saturated with Hoagland solution. Germinating seeds and seedlings were collected daily and seperated into cotyledon and root inclusive of hypocotyl. Each organ was washed with distilled water and weighed after blotted with paper towel. Before weighing, seed coats of cotyledons were removed. ATP content was measured with 3 g of each sample at intervals of 24hr from the time of seed soaking designated as 0-germination day.

ATP Content Measurement Each sample (3 g) was boiled with 30 *ml* of tris buffer of pH 7.4 for three minutes to kill the cells rapidly and inactivate the ATPase contained in the cells themselves. The boiled solution containing sample was replenished for evaporated moisty to 30 *ml* by adding some precooled tris buffer and then transferred into ice-jacket homogenizer vessel. After homogenizing the solution at 4500 rpm, 0°C, for 10 min., the homogenate was filtered through two layers of gauge, ATP content of filtrate was determined by using a SAI Technology Co. 2000 ATP Photometer which measures the fluorescence of 560~580 nm wavelength, emitted from the photometer cuvette by hydrolysis of ATP component of reaction mixture. The reaction mixture was prepared by adding 0.5 *ml* of enzyme solution to the photometer cuvette containing 0.5 *ml* sample filtrate. After mixing the contents for three seconds, the photometer cuvette was settled in the chamber of ATP-Photometer as rapid as possible to measure the fluorescence. The counting was started within 10 seconds after the enzyme was added to the photometer cuvette. ATP content of filtrate was computed from the peak reading of ATP-Photometer by referring it to the standard curve graphed with the chemical ATP. All operations after boiling were carried out at 0°C.

Enzyme Solution For the measurement of ATP content, luciferin-luciferase was from a crude extract of 50 mg of firefly lanterns premixed with buffer and desiccated. The enzyme solution was prepared for use by adding 15 *ml* of tris buffer solution to the firefly extract, storing in refrigerator to reduce the background, and filtering prior to use. All enzymes and chemicals used were the products of Sigma Chemical Co.

RESULTS AND DISCUSSION

The ATP concentrations in pregerminated seeds of soybean and mungbean were 11.4 and 63.0 μg/g fresh seed respectively(Table 1). The highest ATP concentration of whole seedling including both root and cotyledon was 550% of the pregerminated soybean seeds on 2nd day and 480% of the pregerminated mungbean seeds on 1st day respectively. Thereafter, ATP concentration of both seedlings decreased with the progress of germination, whereas the total ATP content was increased(Table 1).

Ching and Ching(1972) reported that on average of 540 picomoles of total adenosine phosphates was found in the embryo of mature seeds of ponderosa pine (*Pinus ponderosa* Law.) and 1140 picomoles in the gamatophyte. They also reported that, after stratification, total adenosine phosphates increased

Table 1. ATP concentration of the whole seedling including both root and cotyledon

Germination day	Plant height(cm)		ATP(μg/g)	
	S. bean	M. bean	S. bean	M. bean
0	—	—	11.4	63.0
1	0.7	0.2	50.0	303.2
2	2.0	2.0	62.6	253.7
3	4.5	4.1	59.1	202.6
4	11.5	7.4	55.4	153.1
5	12.0	10.5	50.9	119.3
6	15.1	14.3	45.9	95.6
Mean	7.6	6.4	47.9	170.1
Significance	P<1%	P<1%	P<1%	P<1%

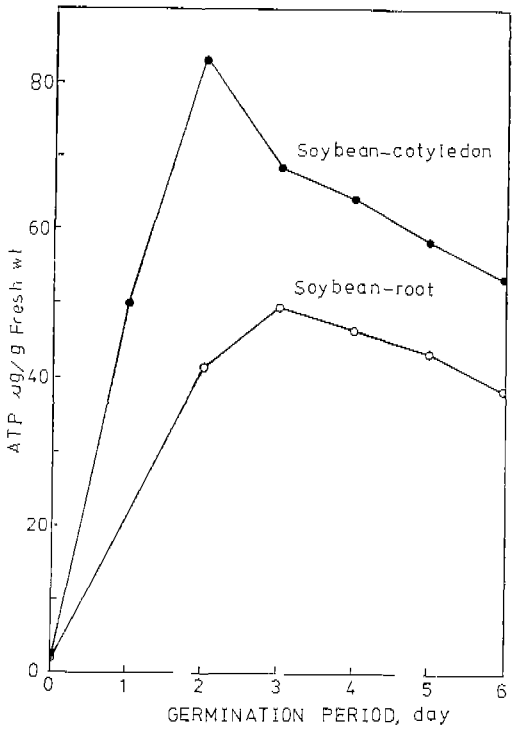

Fig. 1. ATP concentration in cotyledon(significant at 1% level) and root including hypocotyl (significant at 1% level) of soybean with progressing germination.

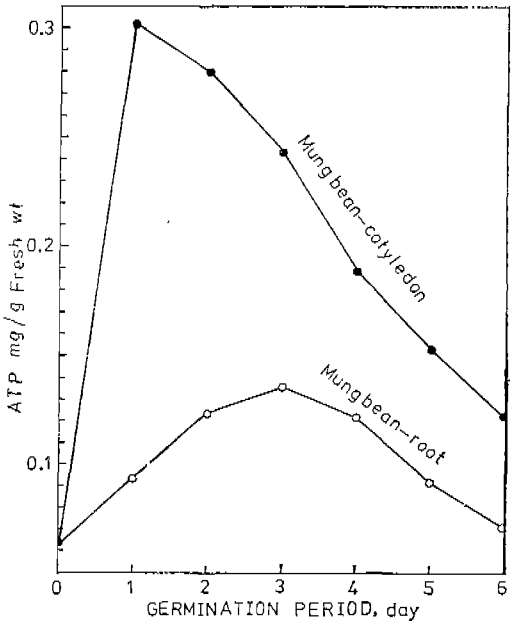

Fig. 2. ATP concentration in in cotyledon and root including hypocotyl of mungbean with progressing germination. Significant at 5% level among periods.

7-fold and 6-fold in embryo and gametophyte respectively and, during germination, it increased to 80-fold and 20-fold peak on the 9th day in gametophytic tissue and embryo, respectively.

Time course experiment for measuring the ATP content per organ of the germinating plant showed that the highest ATP concentration of cotyledon in soybean was 83.4 μg/g fresh wt. on 2nd day, and that of root was 49.8 μg/fresh wt. on 3rd day (Fig. 1). In the case of mungbean, the highest ATP concentration of cotyledon was 0.303 mg/g fresh wt. on first day and that of root was 0.13 mg/g fresh wt. on 3rd day (Fig. 2).

In the comparision of the two plants, the highest ATP concentration of root was measured on 3rd day for both plants, whereas that of cotyledon was measured earlier by one day in mungbean than in soybean. It was coincided with macroscopic

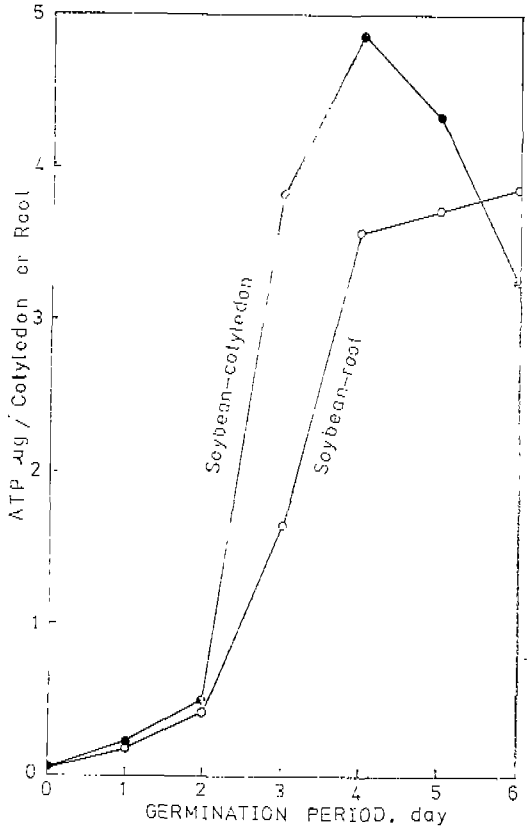

Fig. 3. ATP content of each organ of soybean with progressing germination; root including hypocotyl. Significant at 1% level among periods.

observation in which the germination of mungbean was obviously faster than that of soybean. These data indicated that the content of adenosine phosphates of germinating seeds reflects growth, organogenesis, and morphogenesis, being in accord with previous report (Ching and Ching, 1972). Pilet *et al.* (1978) also reported that the ATP level in the apical end of maize roots including the zone of maximal growth rate was significantly increased.

The highest total ATP content of soybean cotyledon was 4.85μg per cotyledon on 4th day. The ATP content of root increased dramatically till 4th day, although the increasing rate moderated thereafter(Fig. 3). The decreasing ATP content per cotyledon approached to the same level as that of root with about 3.8 mg per cotyledon after 5th day.

In the case of mungbean, the highest ATP content of cotyledon was 7.58 mg per cotyledon on first day. The high increasing rate of ATP content per root was maintained till 5th day, thereafter the increasing rate was moderated (Fig. 4). The ATP content per cotyledon decreased rapidly after first day and approached to the same level as that per root with about 4 mg per cotyledon after 3rd day.

In the comparision of the two plants, the ATP content per cotyledon in mungbean increased so rapidly as to reach to the maximum content earlier than soybean by 3 days, whereas the tendency of fluctuation in ATP content per root was very similar for the two plants.

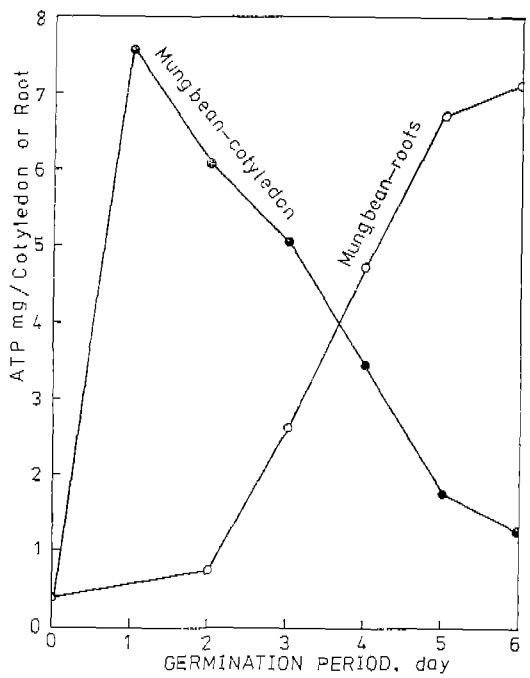

Fig. 4. ATP content of each organ of mungbean with progressing germination; root including hypocotyl. Significant at 5% level among periods.

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摘 要

本 硏究는 콩(*Glycine max* Merrll) 및 녹두(*Phaseolus radiatus* L.) 種子의 發芽日數 經過에 따라 根 및 子葉의 部位別 ATP 含量變化를 調査함에 그 目的을 두었다. 콩과 녹두의 種子를 30°C 暗所에서 Hoagland solution으로 6日間 發芽시켜 發芽日別 ATP 含量을 ATP-Photometer(model 2000)으로 測定하였다. 그 結果 種子의 發芽日別 g當 ATP 最高含量은 콩의 境遇 62.6 μg/g로서 發芽 2日째, 녹두의 境遇 303.2 μg/g로서 發芽後 1日째였다.

콩 子葉의 ATP含量은 發芽後 4日까지 漸次 增加하고 그後 漸次 減少하였으며 콩 根의 ATP含量은 發芽後 6日까지 계속 增加하였다. 子葉과 根의 ATP含量이 같아지는 時期는 發芽 5日 後이며 약 3.8 μg/organ로서 비슷한 含量에 到達하였다. 녹두 子葉의 ATP含量은 發芽 1日째까지 增加하나 그 以後부터 漸次 減少하였으며, 녹두 根의 ATP含量은 發芽 6日까지 계속 증가하였다. 녹두의 子葉 및 根의 ATP含量이 같아지는 時期는 發芽 3日後이며 그때의 ATP含量은 약 4 mg/organ이었다.

REFERENCES

Anderson, J. D. 1977a. Adenylate metabolism of embryonic axes from deteriorated soybean seeds. *Plant Physiol.* 59: 610—614.

———. 1977b. Responses of adenine nucleotides in germinating soybean embryonic axes to exogeneously

applied adenine and adenosine. *Plant Physiol.* 60 : 689—692.

Ching, T. M. 1972. Metabolism of germinating seeds. *In:* T.T. Kozlowski, ed., Seed Biology, Vol. 2. Academic Press, N. Y. pp.103—218.

_____. 1975. Temperature regulation of germination in crimson clover seeds. *Plant Physiol.* 56 : 768—771.

Ching, T. M. and K. K. Ching. 1972. Content of adenosine phosphates and adenylate energy charge in germinating ponderosa pine seeds. *Plant Physiol.* 50 : 536—540.

Hartman, S. C. 1970. Purine and pyrimidines. *In:* D. M. Greenberg, ed., Metabolic pathways Ⅳ. Academic Press, N.Y. pp.1—68.

Marcus, A. 1969. Seed germination and the capacity for protein synthesis. *Symp. Soc. Exp. Biol.* 23 : 143—160.

Moreland, D. E., G. G. Hussey, C. R. Shriner and E. S. Eaxmer. 1974. Adenosine phosphate in germinating radish (*Raphanus sativus* L.) seeds. *Plant Physiol.* 54 : 560—563.

Obendory, R. L. and A. Marcus. 1974. Rapid increase in adenosine triphosphate during early wheat embryo germination. *Plant Physiol.* 53 : 779—781.

Pilet, P. E., P. Steck and G. Mayer. 1978. ATP in growing roots: Coreaction and light effect. *Plant Sci. Lett.* 12 : 343—348.

Pradet, A., A. Narayanan and J. Vermeersch. 1968. Etude des adenosine-5′-mono, di-et tri-phosphates dans les tissues vegetaux. Ⅱ. Metabolisme energetique au cours des premiers stades de la germination des semences de Laitue. *Bull. Soc. Fr. Physiol. Veg.* 14 : 107—114.

Sobczyk, E. A. and A. Kacperska-Palacz. 1978. Adenosine nucleotide changes during cold acclimation of winter rape plants. *Plant Physiol.* 62 : 875—878.

Walbot, V. 1972. Rate of RNA synthesis and t-RNA end-labeling during early development of *Phaseolus*. *Planta* 108 : 161—171.

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