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論　文

Application of Calorimetry to Investigate Viability of Crops Seeds

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A high sensitive microcalorimeter which is applicable to the detection of small heat effects due to biological process ocurring in plant cells and tissues was constructed and applied to study germination activity of various crops seeds. The germination tests were performed in the calorimetric units and the heat evolution process associated with the budding and rooting was quantitatively determined. The amount of heat evolved was compared with the extent of germination determined by eyeball observations and found to well reflect the viable activity of seeds.

From the result that there is a good correlation between the heat evolution and the extent of germination, we conclude that the calorimetric method employed here can be an analytical tool in checking viability of useful aged crops seeds and therefore valuable in uniformal selection of seedling materials in aged crops seeds.

INTRODUCTION

Rice seeds have a relatively long period of viability compared with some other crops such as bean or soybean seeds, but after storage for more than about 5 years the percentage of normal germination falls substantially and those seeds which germinate do so at a slower rate and the percentage of abnormal germination increases.

On the hypothesis that loss of capacity for normal germination might be related to changes in plant growth regulator balance within the seed, gibberellic acid (GA_3) was applied to promote the germination in aged rice seeds, and the heat evolution process during the course of the germination was quantitatively determined. During the course of this work a stimulation of the aged rice seeds germination by treatment with GA_3 was observed and the amount of heat evolved was found to well reflect the viability of the seeds.

MATERIALS and METHODS

A multiplex calorimeter based on the conduction principle (designed by K.T.) was used. The apparatus had 25 calorimetric units and, as sensor, semiconducting thermopile plates arranged in an aluminum heatsink measuring 350 × 700 × 120 mm. The basic structure and mode of operation were essentially the same as that of an earlier design containing six calorimetric units [1), 2)]. The heat effects arising from germination were continuously recorded as a function of time and stored in a computer deskette for further analysis.

Rice (*Oryza sativa* L. cv. Koshihikari) seeds aged 7 years were used.

The seeds were rinsed in 70 % ethanol for 5 sec., and sterilized by immersion for 12 min. in the filtrate from an 8 % suspension of calcium hypochlorite. After through washing with sterile water the seeds were sown singly on to the surface of 5 ml of agar medium with various concentration of GA_3 in 25 × 70 ml tubes and were put into the calorimetric unit[1)]. After 4 days incubation in the dark at 30 ℃, the number of viable seeds were counted and abnormal germinaion were investigated by eyeball observation.

The structure of the calorimetric unit with the culture vessel is schematically shown in **Fig. 1**. The amount

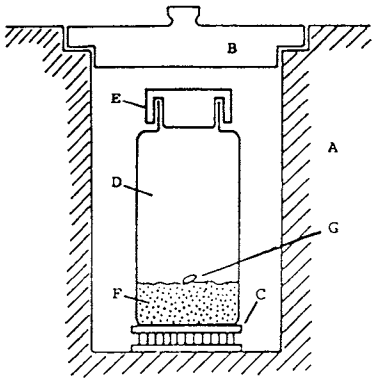

Fig. 1 Diagram of the calorimetric unit. A : aluminum heatsink, B : aluminum lid, C : semiconducting thermopile plate, D : calorimetric vessel (30 ml glass vial) , E : silicon-rubber stopper, F : agar medium, G : rice seed

of oxygen present in the air gap in the culture vessel was sufficient for the respiration of the germinating seeds during incubation.

RESULTS and DISCUSSION

Stimulation of germination by treatment with GA_3 was observed as shown in **Table 1**. The results presented in the Table show that there was a significant increase in the percentage of viable plants in the 7 year old seeds after the treatment with GA_3 compared with the water control. **Fig. 2** shows calorimetric signals observed for rice seed germination and **Fig. 3** is the heat evolution process during the germination which were obtained by computation using the proposed equation.

$$f(t) = g(t) + K \int g(t)\, dt$$

where $g(t)$ is the calorimetric output at incubation time of t and K is the heat conduction constant of the calorimeter. The both Figures reveal the individual activity of the seeds.

Relationship between observed heat evolution and viability of seeds is shown in **Fig. 4**. When the amount of heat evolved was compared with the extent of budding and rooting, it is found to well reflect the viability of the individual seed.

A regression analysis was made on the observed heat evolution and the bud and root lengths on the basis of an equation

$$f(t) = a_0 + a_1 B + a_2 R$$

Table 1 Promotion of germination in aged rice seeds by treatment with GA_3.

Storage period		Viable plants (%)
1 year	Water (control)	100
7 years	0 (cont.)	53.8
	Ga_3 0.1 ppm	83.3
	1.0 ppm	100

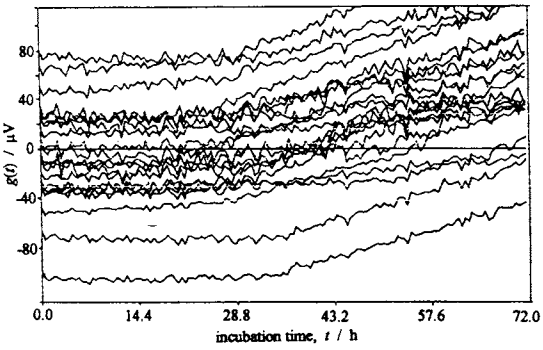

Fig. 2 Calorimetric signal observed for germination of rice seed. (Copy of the computer display)

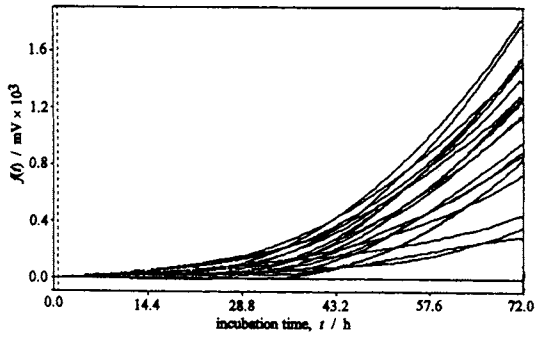

Fig. 3 Heat evolution process during the germination of rice seed. (Copy of the computer display)

where B and R are the length of bud and root, respectively.

The following result was obtained between the heat evolution and the extent of germination. with a correlation coefficient of $r = 0.8285$:

$a_0 = 3.84$ J

$a_1 = 0.1963$ J/mm Bud

$a_2 = 0.1285$ J/mm Root

As far as we are aware, these values are the first figures which may be used to characterize the heat evolution associated with the germination of plant seeds.

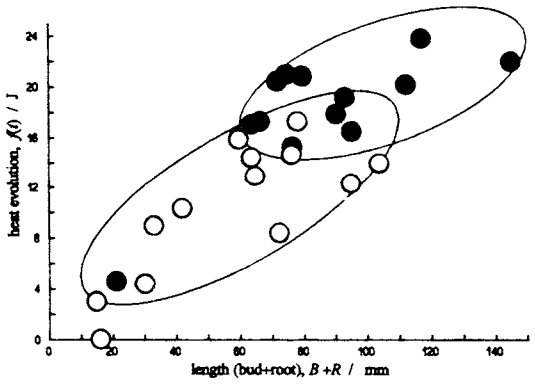

Fig. 4 Relationship between observed heat evolution and viability of each seed.
Bud＋Root : denotes the sum of the both length
Open marks : Water control
Closed one : GA_3 0.1 or 1 ppm

From the above results that there is a good correlation between the heat evolution and the extent of germination, we conclude that the calorimetric method employed here can be an analytical tool in checking viability of useful aged crops seeds, and that at least 12 J (Joule) after 4 days incubation is necessary heat amount to get viable plants from 7 year old rice seeds.

Using this super sensitive calorimeter we are now further checking viability of other crops seeds by treatment with various plant growth substances.

Reference

1) T. Kawabata, Y. Yamano and K. Takahashi : "An attempt to characterize calorimetrically the inhibitory effect of foreign substances on microbial degradation of glucose in soil", *Agric. Biol. Chem.* **47**, 1281-1288 (1983).

2) T. Kimura and K. Takahashi : "Calorimetric studies of soil microbes－Quantitative relation between heat evolution during microbial degradation of glucose and changes in microbial activity in soil", *J. Gen. Microbiol. (London)* **131**, 3083-3089 (1985).

要　旨

作物の生長に伴って発生する極微少熱量を感知することができる高感度熱量計を用いて，作物種子の発芽活性を調べた。材料には，7年間貯蔵したイネ加齢種子（品種：コシヒカリ）を供試したが，発芽時に1粒の種子から発生する極微少熱量を正確に計測することができた。その際，個々の種子の発芽能力と発生熱量との間に高い相関々係のあることが明らかとなった。これは，本方法が発芽の極初期段階において，個々の種子の活性程度をすばやく見極め，活力ある個体を選抜するのに役立ち得ることを示すものである。

国際会議のお知らせ

Announcement of Symposia
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Univ. of British Columbia,
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(Sessions and Chairs)

1. Symposium on the Thermodynamics of Aqueous Electrolytes: Prof. Loren G. Hepler, Univ. of Alberta, CANADA T6G 2G2／Prof. Vladimir Majer, Univ., Blaise Pascal C.N.R.S., FRANCE

2. Symposium on the Themodynamics of Aqueous Non-Electrolytes: Prof. Yoshikata Koga, Univ. of British Columbia, CANADA V6T 1Z1／Prof. Vladimir Majer, Univ. Blaise Pascal C.N.R.S., FRANCE

3. Symposium on Structure Based Thermodynamic Analysis of Protein Folding and Binding: Dr. Javier Gomez, The John Hopkins Univ., U.S.A.／Dr. Vincent J. Hilser, The John Hopkins Univ., U.S.A.

4. Symposium on the Biological Applications of Calorimetry: Prof. Ernesto Freire, The John Hopkins Univ., U.S.A.／Dr. Michael Henzl, Univ. of Missouri-Columbia, U.S.A.

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6. Symposium on the Industrial Application of Calorimetry: Dr. Linda M. Peerey, The Dow Chemical Company, U.S.A.／Dr. David Frurip, The Dow Chemical Company, U.S.A.

7. Symposium on General Topics in Thermodynamics and Calorimetry: Dr. Bill Steele, BDM Oklahoma Inc. NIPER, U.S.A.