Calorimetry as an Analytical Tool for Germination
Tests of Plant Seeds

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Heat evolution associated with the germination of radish seeds was quantitatively studied in a
calorimeter. After incubation at 30°C for 20 to 50 h, the total heat evolved was correlated with the
length of buds and roots, and with the increase in weight of the germinating seeds. By regression
analysis, the heat produced per unit of bud and root growth was found to be $a_1 = 0.5723 \text{ J/mm of}
bud$ and $a_2 = 0.7093 \text{ J/mm of root}$, respectively, with a correlation coefficient of 0.9577.

From these results we conclude that the calorimetric determination of the heat generated during
germination is a valid reflection of the germination activity of radish seeds. Our calorimetric method
is applicable to the quantitative investigation of the viable activity of plant seeds.

INTRODUCTION

The germinative viability of plant seeds is usually
based on the percentage of seeds that germinate and a gross estimation of seedling size.
However, the information obtained is mostly qualitative. There exists no practical technique
to characterize germination quantitatively. It would be very useful if some physical factor
reflecting the germinative potential of seeds could be measured. One possibility is the
monitoring of heat generated by the biochemical processes involved; such effects are usually
proportional to the metabolic activity of living organisms (1, 5).

In this study, we measured heat evolution
during the germination of radish seeds and
discuss its possible contribution to determining the
germinative viability of plant seeds.

EXPERIMENTAL

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 ar-
ranged in an aluminum heatsink measuring 350
$\times$ 700 $\times$ 120 mm. The basic structure and
mode of operation were essentially the same as
that of an earlier design containing six calorimetric units (3, 4).

Radish seeds (Raphanus sativus (L.) cv. Na-
tsuminowase #3), aged 1 year, were used. The
seeds were rinsed in 70% ethanol for 5 s and
sterilized by immersion for 12 min in the filtrate
from an 8% suspension of calcium hypochlo-
rite. After thorough washing with sterile
water, the seeds were sown singly on in twos or
in threes on the surface of 5 ml of agar medium
in a glass culture vessel with a gas-tight
silicon-rubber stopper. The culture vessels were
then placed in the calorimetric units and the
seeds were incubated in the dark at 30°C for
20 to 100 h. The heat effects arising from ger-
mination were continuously recorded as a func-
tion of time and stored in a computer diskette
for further analysis.

The structure of the calorimetric unit with
the culture vessel is schematically shown in Fig.
1. The amount of oxygen present in the air gap
in the culture vessel was sufficient for the respi-

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RATION OF THE GERMINATING SEEDS DURING INCUBATION.

RESULTS AND DISCUSSION

Figure 2 (a) shows typical examples of calorimetric recordings of the heat produced by germination, i.e., a germination thermogram. The reading of an observed germination thermogram $g(t)$ (the heat effect at each step of incubation) can be corrected for heat conduction by the following equation to obtain the total amount of heat evolved, $f(t)$, during the incubation time $t$.

$$f(t) = g(t) + K \int g(t) dt$$  \hspace{1cm} (1)

where $K$ is the heat conduction constant of the calorimetric unit, including the culture vessel (2, 4, 6). The second term of the equation corresponds to the amount of heat dissipating through the calorimeter wall to the surroundings. The heat evolution processes associated with germination were obtained from the observed $g(t)$ curves by use of the above equation and are shown in Fig. 2(b).

The $f(t)$ curve is the time course of heat evolution when the calorimetric units are placed in a hypothetical adiabatic condition. The $f(t)$ curves thus obtained have a typical upward curvature characteristic of the heat evolved during the differentiation and growth of biological cells. Our data indicates that $f(t)$ reflects the germination process of radish seeds.

Fig. 3 shows germinated seeds taken from the calorimetric units after incubation for 48.5 h. As expected, the estimated germination, as measured by eye, was almost parallel with the heat evolution given in Fig. 2(b). In other words, the greater the amount of heat evolved, the greater the degree of germination.

The quantitative relationship between the amount of heat evolved and the lengths of both roots and buds was investigated. The results are shown in Figs. 4(a) and 4(b) in the form of plots of $f(t)$ vs. the lengths of buds ($B$) and roots ($R$), respectively. The two independent physical quantities, $f(t)$ and $B/R$, were correlated. The solid lines in Fig. 4 were obtained by regression analysis based on a second-order function. The correlation coefficient, $r$, of each mathematical fitting are also given in the figure. The results clearly demonstrate that the amount of heat evolved during germination is an index of the biological activity of the seeds.

To examine this point more quantitatively, the amount of heat evolved during germination was compared with the increase in the weight of seeds. After the computer recording of the germination thermograms was stopped, the ger-

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**Fig. 1** Diagram of the calorimetric unit. A, aluminum heat sink; B, aluminum lid; C, semiconducting thermopile plate; D, calorimetric vessel (30-ml glass vial); E, silicon-rubber stopper; F, agar medium; G, radish seed.
minated seeds were removed from the calorimetric units and weighed. The increase in weight was determined by subtracting the initial from the final weight of each seed.

In Fig. 5, the amount of heat evolved, \( f(t) \), was plotted against the weight increase, \( \Delta W \). The quantities seem to be correlated. Regression analysis of this plot, on the basis of a second-order function, gave the equation

\[
f(t) = -7.64587 + 0.565437\Delta W - 0.0012283\Delta W^2
\]  

(2)

with a correlation coefficient of \( r = 0.89863 \).

Multiple regression analysis of the heat evolution observed, and the bud and root lengths (\( B \) and \( R \)), was performed on the basis of equation (3):

\[
f(t) = a_0 + a_1B + a_2R
\]

(3)

where \( a_0, a_1, \) and \( a_2 \) are constants. The determined values of \( a_0, a_1, \) and \( a_2 \) are listed in Table 1.

In equation (3), \( a_1, \) and \( a_2 \) are the amounts of heat evolved per unit growth of bud and root, respectively. \( a_0 \) is the amount of heat unrelated to the growth of either buds or roots, and

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**Fig. 2** (a) Germination thermograms observed for the culture of radish seeds, *Raphanus sativus* L., cultured on agar at 30°C and (b) the corresponding \( f(t) \) curves obtained by computation according to the method described in the text. The labels a, b, c etc., given to each tracing correspond to the seedlings shown in Fig. 3.
Fig. 3 Photo of germinated radish seeds (*Raphanus sativus* L.), taken after incubation for 48h on agar at 30°C in the calorimetric units. The corresponding time courses of heat evolution actually observed, are shown in Figs. 2(a) and 2(b), with the same labels.

![Image of radish seeds](image_url)

Fig. 4 Relationship between heat evolution and the lengths of (a) buds and (b) roots. The solid lines are curves fitted by regression analysis on the basis of a second-order function. The correlation coefficients $r$ are given in the figures.

![Graph of heat evolution vs. bud length](graph_url)

**Fig. 4** Relationship between heat evolution and the lengths of (a) buds and (b) roots. The solid lines are curves fitted by regression analysis on the basis of a second-order function. The correlation coefficients $r$ are given in the figures.

![Graph of heat evolution vs. root length](graph_url)
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Fig. 5 Relationship between the heat evolution, \( f(t) \), and the increase in weight \( \Delta W \) during the germination of radish seeds, *Raphanus sativus* L. The solid line is the curve fitted by regression analysis on the basis of a second-order function and the dotted line is its tangent on the vertical axis. The intercept on the vertical axis gives the weight increase because of the physicochemical process of water adsorption before germination, with its generated heat omitted from the calculation of \( f(t) \). The correlation coefficient \( r \) is given in the figure.

Table 1 Regression coefficients for the germination of radish seeds, *Raphanus sativus* L., and rice seeds, *Oryza sativa* L., determined by multiple regression analysis of calorimetric data.

<table>
<thead>
<tr>
<th>Seeds</th>
<th>( a_0 ) (J)</th>
<th>( a_1 ) (J/mm Bud)</th>
<th>( a_2 ) (J/mm Root)</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>radish</td>
<td>-1.098</td>
<td>0.5723</td>
<td>0.7093</td>
<td>0.9577</td>
</tr>
<tr>
<td>rice</td>
<td>3.840</td>
<td>0.1963</td>
<td>0.1285</td>
<td>0.8285</td>
</tr>
</tbody>
</table>

is associated mainly with physicochemical and biochemical processes occurring in the seed itself. To the best of our knowledge, these values are the first figures reported that can be used to characterize heat evolution during the germination of seeds.

The same parameters, monitored in a preliminary study of rice seeds, are also included in Table I for comparison. The values calculated for the radish and rice seeds are quite different. Undoubtedly, all species of plants have characteristic features in their germination process and, accordingly, the calorimetric parameters defined above can be expected to differ widely from species to species. Even with seeds of the same species of plant, the germinative activity likely will vary between samples, depending on the conditions under which the seeds were cultivated, harvested, and stored.

On the basis of these considerations we conclude that the heat generated by plant seeds during germination is a useful determinant for characterization of their physiology and biology. Our calorimetric method can be used to provide valuable quantitative information about germinative viability.

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REFERENCES