

A Rapid Germinability Test Using Sugars Exuded from Tea Seeds

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The author and his co-workers have been conducting a study on the long-term storage of nursery plants (grown from cuttings), roots, shoots, pollens, and seeds of tea (*Camellia sinensis* (L.) O. Kuntze). For this study, a rapid method for testing the viability of stored materials is needed. In this connection, it was examined whether the seed exudate method proposed by Takayanagi¹⁾ for testing germinability of rape seeds (*Brassica napus* L.) can be applied to tea seeds or not. The result is presented in this paper.

This method is to estimate indirectly the germinability by measuring the quantity of sugars exuded from seeds, based on the fact that seeds without viability exude a large quantity of soluble substances when soaked in water.

The result showed a very close relation between amount of exuded sugars and germinability of tea seeds, so that the method is applicable to tea seeds.

Procedure of seed exudate method for tea seeds

To establish a procedure of the method, amount of exuded sugar from tea seeds with or without shells was examined firstly. If the seeds with shells exude a measurable amount of sugars, the procedure will become simple because there is no need to remove shells. Open-pollinated seeds of Yabukita, a clonal tea variety, were used after they were kept in the room for 3 months and their germinability was lost. The experiment was carried out in April 1976, using 20 seeds per plot with 3 replications. After sterilized with 0.1% Uspulum

for 30 min., the seeds were soaked in sterile water of 200 ml for 24 hrs at 25°C. After the soaking water was filtered by filter paper, amount of sugars in the filtrate was measured by the Anthron method³⁾. Absorbance was measured by a Beckman DB type (Toshiba) photoelectric colorimeter.

The result showed that only a trace of sugars was exuded from the seeds with shells, while more than 200 µg/ml of sugars, which precludes the measurement without a dilution, was exuded from unshelled seeds. It shows that the removing of shells facilitates the sugar exudation.

Secondly, the degree of dilution required for the measurement (by photoelectric colorimeter) of sugars exuded from 20 unshelled seeds into 200 ml of water in 24 hrs at 25°C was examined. The filtrate obtained from unshelled seeds according to the same procedure as above was diluted to give 10, 20, and 30 times volumes. The result showed that 10 times dilution was not sufficient enough, whereas 30 times dilution was excessive, and the 20 times dilution was appropriate for the measurement.

Based on these results, the procedure for tea seeds was prepared as shown in Fig. 1.

Furthermore, it was made clear that the sugar exudate from tea seeds without germinability was about 100 µg/ml according to the above procedure, and that more than 3 replications are necessary for this germinability test.

Relation between sugar exudate quantity and germination rate

It was reported^{4,5,6)} that tea seeds lost rapidly their germinability when they were kept in the room, but if they were stored at a sealed con-

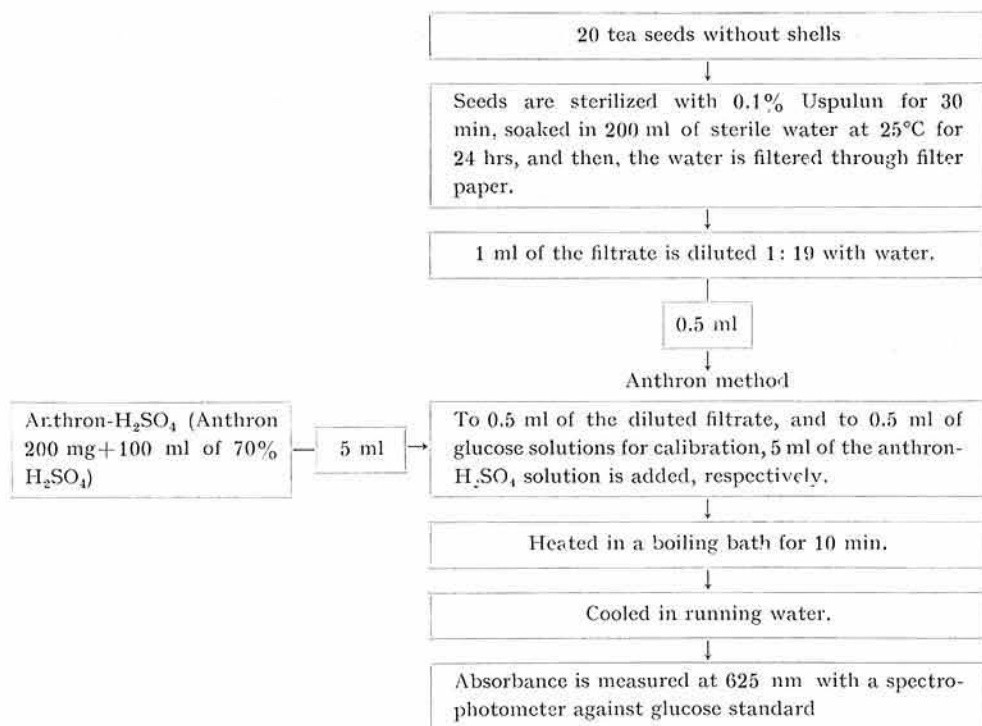


Fig. 1. Procedure of testing germinability of tea seeds by using the seed exudate method.

(Note) Glucose solutions for calibration: 0, 25, 50, 75, 100, 125, 150, 175 and 200 $\mu\text{g}/\text{ml}$ of water.

dition at low temperature their germinability was maintained for more than 6 months. Therefore, using the seeds kept in the room without sealing and those stored at a sealed condition at 1°C , amount of sugar exudate and germination rate were examined in November 1977. Open-pollinated seeds of Yabukita, that had been stored at a sealed condition in a low temperature thermostat at 1°C for 20 days after seed collection, were used. On 27 November, the seeds were taken out from the thermostat, and divided into lots of 20 seeds each, weighing 30 g. A half of them were sealed into polyethylene bags and stored at 1°C (low temperature storage plot), while the other were placed into paper bags and kept at room temperature without sealing (room temperature, no seal plot). From both plots, 3 lots each were sampled every week to measure sugar exudate according to the procedure given in Fig. 1. Seeds, which were soaked in water for 24 hrs, were then sown on the germination

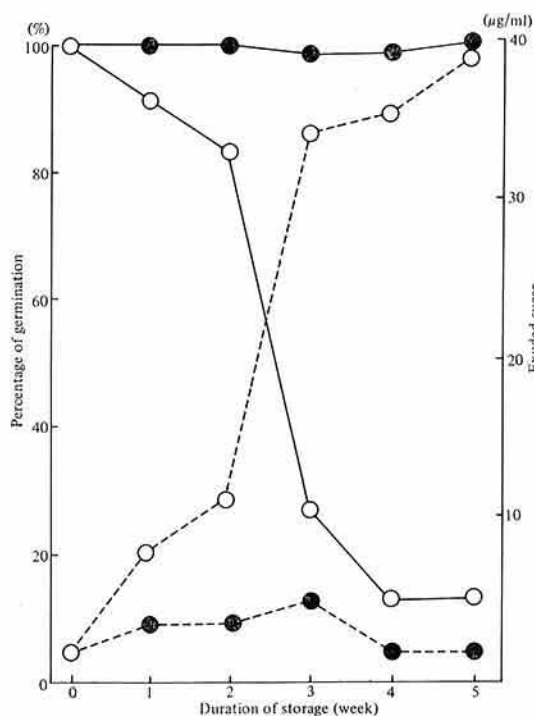
bed, made of 160 g of perlite + 600 ml of water (in closed vessels) to examine germination percentage.

As shown in Fig. 2, the low temperature storage plot maintained almost 100% germination and sugar exudate not exceeding $5 \mu\text{g}/\text{ml}$ in a period of 5 weeks. On the contrary, in the room temperature, no seal plot, germination decreased rapidly, giving only 10% at the 4th and 5th weeks, while the sugar exudate increased linearly up to $40 \mu\text{g}/\text{ml}$ in the 5th week. In the latter plot, the correlation coefficient between germination percentage and sugar exudate was -0.994^{**} , showing a linear relation. The linear regression equation is as follows, and it is significant at 1% level.

$$y = -2.50x + 108.5$$

where, x = sugar exudate in $\mu\text{g}/\text{ml}$, and y = germination percentage.

This result indicates clearly that germination percentage can easily be estimated by knowing



- ...● Exuded sugar, low temperature storage plot
- Germination(%), low temperature storage plot
- ...○ Exuded sugar, room temperature, no seal plot
- Germination (%), room temperature, no seal plot

Fig. 2. Relation between germinability of tea seeds and exuded sugar in two storage conditions

the quantity of sugars exuded from seeds.

A further experiment is needed to know when and how the germination will be decreased in the low temperature storage plot. Cases in which the relation between germination and sugar exudate is not necessarily linear are also

observed²⁾, depending upon the season when seeds were kept without sealing in the room.

In any case, however, it is clear that the relation between germination and sugar exudate is very close, so that the sugar exudate method can be used for estimating the decrease of germinability of tea seeds easily and with a high practicability. Namely, the following estimation can be made by the quantity of sugar exudate obtained from 20 seeds according to the procedure shown in Fig. 1:

Sugars (µg/ml)	Germinability
About 5	Completely maintained
>10	Begins to decrease
>40	Decreased considerably
>100	Completely lost

Thus, the sugar exudate method is considered to be an efficient means to estimate the germinability in the long-term storage of tea seeds.

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