Short Communication

Relationship between Low-level Chemiluminescence and Germinability of Soybean Seeds

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The aging of plant seeds is accompanied by a loss of vigor and germinability.^{1,2)} Under poor storage conditions, seeds deteriorate and germinability is generally reduced. The aging of seeds is accelerated by high temperatures and high relative humidities during storage.^{3,4)} Detection of low-level chemiluminescence is thought to provide new information on the biochemical and physical changes in seeds.^{5–7)} Chemiluminescence from biological tissues reflects the production of free radicals accompanied by lipid peroxidation.⁷⁾ The lipid peroxidation reaction involved in deterioration or aging of seeds is considered to be a factor in the chemiluminescence of plant seeds.^{8–11)}

In this study, we attempted to estimate the germinability of soybean seeds by measuring the low-level chemiluminescence spontaneously emitted from the seeds.

The soybean seeds used in the study (*Glycine max* var. Tanrei) were harvested in Miyagi Prefecture, Japan, in 1988 and stored at room temperature. One hundred dry soybean seeds were stored in containers in which relative humidity (r.h.) was controlled by using saturated salt solutions.¹²⁾ Saturated salt solutions such as potassium chloride (84%), sodium bromide (57%), and magnesium chloride

(32%) were used for the humidity adjustment. Low-level chemiluminescence was measured with a Chemiluminescence Analyzer OX-7 (Tohoku Electronic Ind., Sendai, Japan) with a Hamamatsu R-878 photomultiplier tube having a spectral response range of 300–650 nm. Ten soybean seeds (about 2.5 g) stored for two, four, or six days were placed in a glass petri dish (30 mm in diameter) with 5 ml of distilled water and the chemiluminescence was measured. Also, dry soybean seeds were powdered with a coffee milk (Philips, Japan), 2.5 g of the powdered dry seed was placed in a glass petri dish, and the chemiluminescence was measured. Chemiluminescence was measured at 25°C under air in the dark and the intensity was expressed as counts/sec after subtracting background counts (about 110 cps). The experiments were repeated three times for each sample and the deviation was within 10% of the mean value. A chemiluminescence intensity of 500 cps at 550 nm corresponds approximately to 2×10^{-15} W. The germination rate was measured as follows: soybean seeds were soaked with 10% sodium hypochlorite solution for one minute for sterilization, then washed with and soaked in distilled water for two hours at 25°C. Ten imbibed seeds were placed on wet cotton at 25°C under 80% humidity in an auto-humidity incubator for six days and the number of germinated seeds was counted.

The chemiluminescence of soybean seeds stored under 32%, 57%, and 84% relative humidities (r.h.) at 25°C and 50°C for two, four, and six days are shown in Fig. 1. Figure 1(a) shows changes in the chemiluminescence intensity of the water-imbibed whole soybean seeds, and Fig. 1(b) shows those of the powdered dry soybean seeds. The chemiluminescence intensities of both the water-imbibed whole soybean seeds and the powdered dry seed stored at 50°C were stronger than for those stored at 25°C. Generally, chemiluminescence per gram was much stronger in the powdered dry soybean seed than in the water-

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Fig. 1. Chemiluminescence of Water-imbibed Whole Soybean Seeds (a) and Powdered Dry Soybean Seeds (b).

Conditions of 6-day storage: ○, 32% r.h., 50°C; △, 57% r.h., 50°C; □, 84% r.h., 50°C; ●, 32% r.h., 25°C; ▲, 57% r.h., 25°C; ■, 84% r.h., 25°C.

imbibed whole soybean seeds. The hydrogen peroxide-peroxidase or lipoxygenase system were suspected to participate in the chemiluminescence of powdered seeds,^{8,10,11)} and more detailed studies were needed for further mechanistic characterizations of seed chemiluminescence.¹³⁾

Figure 2 shows the correlation between the germination rate and the chemiluminescence intensities for the powdered dry soybean seeds. A negative and high (-0.840) correlation coefficient was observed. The germination rate for seed stored at 25°C was higher than for seed stored at 50°C. For the water-imbibed whole seeds, a negative (-0.629) correlation coefficient between the chemiluminescence intensities and the germinabilities was also observed (data not shown). Soybean seeds with lower germination rates emit intense chemiluminescence. Although the chemiluminescence of soybean seeds with no germinability showed some variations, the deviation was in the range of the correlation as shown in Fig. 2 according to our experience.

In this study, we have shown that it appears possible to estimate the germinability of plant

Fig. 2. Correlation between Germination Rate and Chemiluminescence of Powdered Dry Soybean Seeds.

seeds by measuring chemiluminescence. It generally takes several days to measure the germinability of plant seeds by the usual methods. By measuring the chemiluminescence, it seems that germinability of seed can be estimated more easily and in a shorter period of time.

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