First Cell Cycle in the Shoot Apical Cells of Germinating Rice Seeds

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After treating with fixative containing picric acid, the squash preparation by Feulgen staining was made for the shoot apical meristem of germinating rice seeds. The first cell cycle in the cells of the meristem was determined with \(^3\)H-thymidine autoradiography. Radiation mutagenesis at the cellular level was also discussed.

Introduction

It is well known that the cell cycle of higher organisms divides into four stages, i.e., \(G_1\) (pre-DNA synthesis stage), \(S\) (DNA synthesis stage), \(G_2\) (post-DNA synthesis stage) and \(M\) (mitosis stage).

In barley, Savin, et al.\(^1\) reported that the highest mutation frequency was obtained when the treatment of ethyl methanesulfonate or N-nitrosomethyl urea was given at the \(S\) stage. In rice seeds, Yamaguchi\(^2\) suggested that the first \(S\) stage in the primordia cells, from which germinal tissue originates, begins at about 60 hours after soaking at 25°1°C according to the data of the segregation ratio of the mutants appearing in an X₁ panicle-branch progeny and mutation induction with \(^3\)H-thymidine or aminopterin treatment. Furthermore, Yamaguchi\(^3\) found that the highest frequency of chlorophyll mutation was obtained when 58 hours soaked seeds were exposed to \(^{137}\)Cs-\(\gamma\) rays for 200 minutes.

In this experiment, the first cell cycle, especially \(S\) stage, of the shoot apical cells in soaked seeds of rice was determined by the microautoradiography of \(^3\)H-thymidine.

Materials and Methods

Seeds of rice variety Kinmaze were used throughout this experiment. Preceding to the experiment, seeds having specific gravity of more than 1.12 were selected by NaCl solution. Then, moisture content of the seeds was adjusted to about 14% by keeping them with saturated NaClO₃. They were soaked in water at 25±1°C in dark as reported already\(^4\).

Microautoradiography: At various periods from 50 to 63 hours after seed soaking has begun, the seeds were sampled with an interval of one hour between 58 and 63 hours for experiment I and between 50 and 59 hours for experiments II. After the sampling, the embryo was excised from each seed, and then the shoot was obtained by cutting off a tip of the coleoptyle and the root.

In each treatment, 25 shoots were used. They were transferred to a tube containing 3 ml of \(^3\)H-thymidine solution (2\(\mu\)Ci/ml). \(^3\)H-thymidine 6-T(α) was purchased from The Radiochemical Centre, Amersham, England. The specific activity was 25 Ci/mM for experiment I and 20.2 Ci/mM for experiment II. Immediately, the air in the shoots was expelled by a sucker, and they were incubated for 2 hours at room temperature.

At the end of incubation, they were washed three times with distilled water and were fixed for 1 hour in a solution containing the...
following reagents: ethanol 12 ml, chloroform 6 ml, distilled water 4 ml, picric acid 0.2 g, mercury chloride 0.2 g, and 2, 4-dinitrophenol 0.2 g.

After washing with 75% ethanol, they were treated for 1 hour with 75% ethanol solution saturated with iodine so as to remove the mercuric precipitates out of the cell. Without washing in water, then, they were hydrolyzed for 18 minutes in 1 N HCl at 60°C and were stained with the Feulgen reagent for overnight in refrigerator. Under a dissection microscope, the shoot apex including 2nd and 3rd leaves was carefully separated from the other organs of embryo, i.e., coleoptyle and 1st leaf. Such shoot apex was squashed on a slide glass in 45% acetic acid.

After the squash preparations were made, they were frozen on dry ice in order to remove the cover slide. These slides were dehydrated in absolute ethanol and were dipped in the emulsion (Sakura NR-M2). After 3 week’s exposure, the slides were developed in Fuji-Rendol for 4 minutes. These slides were analyzed cytologically to calculate the labeling index (labeled cells per observed cells) of each experiment. In each slide, about 200 apical meristematic cells were scanned. For the counting of the back ground, 58 hours soaked seeds were treated with distilled water instead of 3H-thymidine.

Analysis of mitosis: Using the seeds after soaking from 67 to 77 hours, the M stage of first cell cycle in the apical meristem was determined due to the variation of the mitotic index (dividing cells per observed cells). The squash preparations were made according to the same method mentioned above. The number of the shoot apices sampled for the seed of each soaking time was about 10 or more, and 100 cells in the apical meristem or each shoot apex were observed.

Results

S stage

Fig. 1 shows the silver grains incorporated into the cell nuclei of the apical meristem. The labeling index (L. I.) was calculated according to the method of Chiba⁴.

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L. I. = 1 - \frac{Y_0}{P_0}
\]

where \(Y_0\) shows the proportion of cell nuclei having no silver grains to the total cell nuclei in a given apical meristem and \(P_0\) shows that of the apical meristem in which 3H-thymidine was not administered. \(P_0\) was 0.7370 in experiment I and 0.8625 in experiment II. After calculation of \(L. I.\), the difference of the specific activities of 3H-thymidine between the two experiments was corrected.

As shown in Fig. 2, the labeling indices had a clear peak when 58 hours soaked seed was treated. From this figure, it is obvious that the cells of the apical meristem entering into the first synthesis stage of their chromosomal DNA with partial synchronization. Thus, it could be concluded that the first S stage begins at 57.5 hours and ends at 60 hours after seed soaking.
**M and G2 stage**

Fig. 3 shows the mitotic index and relative frequency of the cells being at each phase of mitosis in the apical meristem of rice seed that was soaked for various periods. There was also a clear peak in the mitotic indices. This indicates that the cells of the apical meristem enter into M stage as a partially synchronizing cell population. Thus, M stage was determined to be from 71.5 to 77 hours after seed soaking. G2 stage being between the end of S stage and the beginning of M stage was determined to be from 60 to 71.5 hours after seed soaking.

These results are summarized as follows:

- **G1** seed soaking..........................57.5 hours ..........................60 hours 2.5 hours
- **G2** ..........................71.5 hours ..........................77 hours 11.5 hours 5.5 hours

**Discussion**

The nuclei of rice somatic cells have been stained poorly by the most common method of acetic alcohol fixation and Feulgen staining. Therefore, we tried to use a fixative containing ethanol, chloroform, distilled water, picric acid, 2,4-dinitrophenol and mercuric chloride. This was a modification of the method used by Östergren and Heneen for studying Agropyron chromosome. Fixation of more than 2 hours induced a destruction of rice cells. A satisfactory result was obtained when the materials were fixed for 1 hour. Since mercuric chloride produced often some precipitates in cell, it was rinsed in iodine for 1 hour. Following these procedures, we were able to observe the good cytological figures from the squash preparation of rice cells.

A preliminary experiment with \(^3^H\)-thymidine of 1 \(\mu\)Ci/ml was unsuccessful because few silver grains were found per cell. Since \(\beta\)-rays from highly radioactive thymidine caused the disturbance of cell division and induced chromosome aberration, \(^3^H\)-thymidine concentrations higher than 2 \(\mu\)Ci/ml had to be avoided. If air was expelled from the excised shoot by a sucker, the incorporation of \(^3^H\)-thymidine was accelerated. Thus, a concentration of \(^3^H\)-thymidine was satisfactory with 2 \(\mu\)Ci/ml.

Cell cycle has been divided to the four stages of G1, S, G2 and M. It had been believed that the embryo of dormant seed is mostly composed of G1 cells. By autoradiographic and microphotometric analyses, Avanzi, et al. demonstrated that in the embryo of dormant wheat seeds every cell constituting the shoot apex or the 3rd leaf stayed exclusively in G1 stage and most of the cells in the 1st or the 2nd leaf were belonging to G1 stage. In the embryo of dormant rice seeds, cells in the apical meristem seem to stay in G1 stage since squash preparations did not allow to find mitosis before 67 hours soaking time. Furthermore, this observation that the cells in the apical meristem belong to G1 stage may be supported by the result shown in Fig. 2. This figure revealed that DNA synthesis in the apical meristem took place with a partial synchronization.

Yamaguchi has estimated that de novo synthesis of DNA in the cells from which germinal tissue originates might take place about 60 hours after the beginning of seed soaking. The finding that the S stage is from 57.5 to 60 hours after seed soaking certifies the above estimation.

In Paramecium, Kimball has presented a hypothesis that the premutational damage produced at G2 stage repairs very efficiently and establishes as mutation at S stage. From the previous data of radiation induced muta-
tions and the present data of chromosomal DNA synthesis, it is apparent that such hypothesis is valid for germinating rice seeds.

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