種子貯蔵にともなうオオムギのDNA ポリメラーゼ活性の低下

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SHORT COMMUNICATION

DECREASED ACTIVITY OF DNA POLYMERASE IN SEEDS OF BARLEY DURING STORAGE[1)]

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It has long been well-known that plants produced from old seeds are more likely to produce chromosome abnormalities in root tips of the seeds, pollen abortion in plants developed from the seeds and phenotypic mutations in the progenies than plants from new seeds (see, Roberts 1972). There is no doubt now, therefore, that genetic damage is actually produced during aging.

Furthermore, it has been recognized that increased doses of ionizing radiation applied to aged seeds can accelerate loss of viability in them (Nilan and Gunthardt 1956, Sax and Sax 1962, 1964). Recently, a positive correlation between the efficiency of DNA repair and the radiation sensitivity was demonstrated in diverse organisms (see, Hanawalt and Setlow 1975). The role that DNA polymerase plays in the DNA repair was also noted.

In this communication, we studied on the DNA polymerase activity in relation to the aging process of seeds. A decrease in this enzyme activity was observed in the nucleus of old seed.

The experiment was carried out in July, 1975 by using the seeds of *Hordeum vulgare* cultivar Fuji 2-Jyo. They were grown and harvested in 1970, 1973 and 1974, respectively, and stored at room temperature at 5 to 6 percent moisture content sealed in air. The seeds were soaked for 48 h in distilled water at 25°C in darkness. They were continued to grow for further 7 days in the controlled condition illuminating every other twelve hours at 25°C, and then the height of their seedlings was measured. The results were analyzed with the probit method, as shown in Fig. 1. The lower growth rates were found in seedlings derived from aged seeds. It could be suggested that the increased plant-to-plant variation which occurs as a result of seed deterioration during storage is due to the nuclear damage.

Assay of DNA polymerase activity was performed as previously described (Yama-

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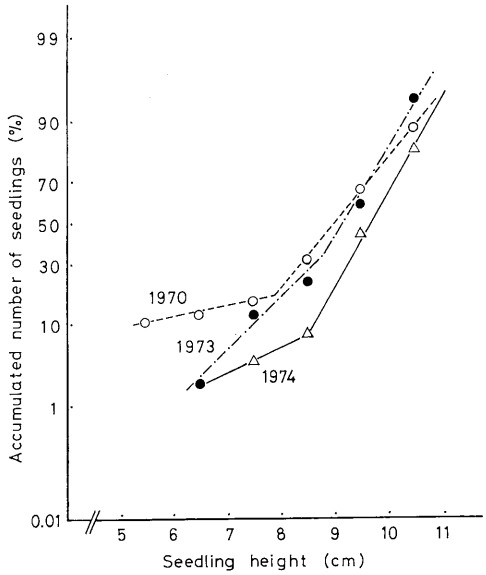

Fig. 1. Seedling height of aged seed.

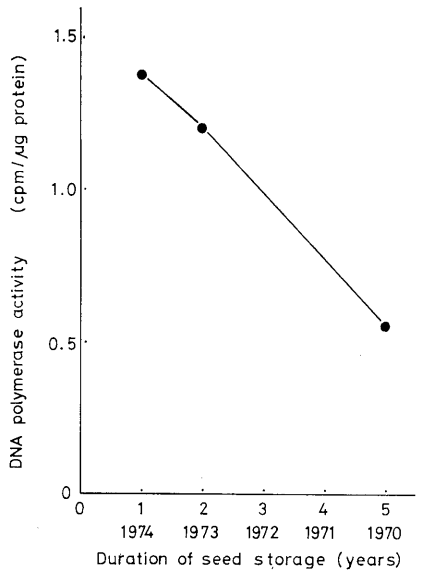

Fig. 2. DNA polymerase activity of aged seed.

guchi *et al.* 1976). All procedures were conducted in the cold. Embryos were prepared from dry seeds stored for 1, 2 or 5 years. They were homogenized in solution of 0.45 M sucrose containing 5 mM $MgCl_2$ and 0.2 mM $CaCl_2$. The extracted nuclei were suspended in 0.04 M Tris-HCl buffer (pH 9.0) containing 1 mM 2-mercaptoethanol, sonicated for 30 min, and pelleted by centrifugation for 10 min at 10,000×*g*. The supernatant was assayed for DNA polymerase activity with activated DNA by treating with pancreatic

DNase, according to the method of Aposhian and Kornberg (1962). Reactions were carried out at 37°C for 30 min with 20 μl of supernatant in a solution of 40 mM Tris-HCl buffer (pH 9.0), 1 mM 2-mercaptoethanol, 8 mM NaF, 0.1 mg/ml activated DNA, 0.08 mM each of dATP, dGTP, dCTP and TTP containing 30 μCi [^{3}H]-TTP (30 Ci/mM). DNA polymerase activity was measured by the polymerization of radioactive dNTP into acid-insoluble form per μg protein with scintilation counting. Protein was determined by the method of Lowry *et al.* (1951).

As shown in Fig. 2, the results suggested an age-associated decline of activity of DNA polymerase in the nuclei of dry seeds. Particularly, the DNA polymerase activity was decreased markedly in the nuclear extract of 5-years-old seeds. Barton and Yang (1975) noticed that the low molecular weight DNA polymerase activity was decreased markedly in the nuclear extract of aged mouse spleens. Recent studies by Linn *et al.* (1976) have shown that DNA polymerase activity isolated from late and early passage cells of the diploid human fibroblast line dropped with increasing passage.

The fact that aged seeds increase the sensitivity to ionizing radiation (Nilan and Gunthardt 1956; Sax and Sax 1962, 1964) might be related with an aged-associated decline of DNA polymerase activity.

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