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# Effects of Heat-Shock Treatment and Genotype on Radiosensitivity of Maize Seeds

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In order to clarify the inner and outer factors responsible for radiosensitivity of seed and to aquire mutations more effectively, two experiments were conducted using maize. (1) Seeds of an inbred line were irradiated with $\gamma$ rays at an extremely low temperature (−70°C) and then dipped in hot water (60°C, 30 sec.). Through such heat-shock treatment the radiosensitivity of maize seeds was remarkably reduced: $LD_{50}$ and $RD_{50}$ for growth rose up to as high as about three times and about twice, respectively. (2) Seeds of seven strains including four inbred lines, two single-cross hybrids and one double-cross hybrid were exposed to $\gamma$ rays with ordinary procedure. Hybrids, regardless of single cross and double cross, were clearly proved to surpass their parental strains in radiation tolerance both in survival rate and in culm length. These descents of radiosensitivity were considered mainly due to the increased heterozygosity itself.

## INTRODUCTION

At the present time that the practical way for controlling spectrum of induced mutations is still far from being established, effective obtaining of available mutations depends only upon increasing the frequency of induced mutations. A positive direction with respect to this problem may be to raise the dosage of mutagen as far as possible by means of reducing the biological injuries due to mutagenic treatment.

It has been shown by many investigators that the biological injuries are to be affected by various environmental conditions before, during and after treatment, so far as sparsely ionizing radiations are applied. This suggests that to modify the radiosensitivity of organism, accordingly to make plant seed more tolerant to radiation is attainable by regulating some environmental condition associated with irradiation. One of the successful methods devised with regard to this respect seems to be "heat-shock treatment", where seeds are briefly dipped in hot water to be shocked with heat after having received irradiation at an extremely low temperature. The striking effects of this method on increasing the radioresistance of seeds were found out in barley by Konzak *et al.*[1] and recognized also in rice by the authors.[2]

It appears, however, still difficult at present[3] to elucidate in detail the physical and chemical mechanisms upon which those effects depend. A biological approach, that is, analyzing the difference of radiosensitivity among different genotypes in broad sense, accordingly, may be desired as another important step for solving this problem. The differences of radiosensitivity have been widely shown among genera, species, and cultivars,

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and have been attributed to the difference of nuclear or chromosomal volume,[4~7] action of gene or genes,[8~16] and so on. Among them, however, the effect of genetic heterozygosity as shown by Notani,[11] Sarić,[12] and Stoilov *et al.*[13] was considered worthy to be taken up for the present study, because the vigorous growth expected from heterozygosity must be available for the analysis of radiation-induced injuries.

Based on these considerations, seeds of four inbred lines, two single-cross hybrids and one double-cross hybrid of maize were used, and the effects of heat-shock treatment with one inbred line and the difference of radiosensitivity among all the seven strains were discussed.

## MATERIALS AND METHODS

Maize seeds from Tokachi Agricultural Experiment Station (Hokkaido) were used for the present study. They involved seven strains: two inbred lines of flint corn (N19, CM7) and their single-cross hybrid (N19×CM7), two inbred lines of dent corn (W41A, W79A) and their single cross hybrid (W41A×W79A), and the double-cross hybrid between above two single-crosses (N19×CM7)×(W41A×W79A). This double-cross hybrid named "Heigenwase" prevails now in Hokkaido district as a leading variety. Acute gamma irradiation was administered to the seeds of these strains in May of 1974, using the cobalt-60 gamma-ray irradiation facility for biological studies at Kyoto University.[17] This study is composed of two experiments.

*Experiment with heat-shock treatment* One of the inbred lines, W79A, was used as material. Seven exposures (0, 10, 20, 30, 40, 50, and 60 kR) were provided and each of them involved 50 seeds, which were enveloped in a 1.5×5 cm cloth bag. These bags were stratified in a beaker surrounded with cold ethanol containing some pieces of dry ice, and thus refrigerated to about −70°C, then irradiated with $\gamma$ rays. Immediately after irradiation, each bag was rapidly taken out and quickly soaked in hot water of 60°C for 30 seconds, followed by sowing of treated seeds. Another series of exposure (0～40 kR) was separately prepared as the control for heat-shock treatment, where irradiation was conducted at room temperature. This procedure for the control is hereinafter referred to as "ordinary treatment" as against "heat-shock treatment".

*Experiment with genotypic effect* All the seven strains were used, and 0, 10, 20, 30, and 40 kR of $\gamma$ rays were given to the seeds at room temperature. Each exposure comprised 100 seeds. Treated seeds were sown immediately after irradiation.

In both the experiments, radiation effects were measured by survival rate and seedling height 15 days after treatment, and culm length after maturing.

## RESULTS AND DISCUSSION

### 1. Effects of heat-shock treatment

As shown in Fig. 1, the survival rate in ordinary treatment started an abrupt decrease at 30 kR and dwindled into zero at 50 kR, and the $LD_{50}$ exposure was estimated about 25 kR. In heat-shock treatment, on the other hand, only a slight decline was observed at the highest exposures, 50 and 60 kR. $LD_{50}$ may be inferred from the curve tendency

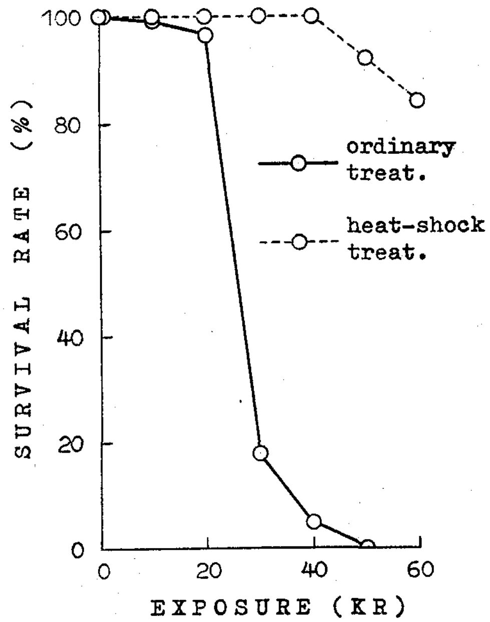


Fig. 1. Effect of heat shock on survival rate 15 days after irradiation.

to be at least more than 70 kR; almost three times as much as that in the ordinary treatment.

As to also the dose responses of seedling height and culm length, a conspicuous difference was observed between the two kinds of treatments, though the decreasing trends

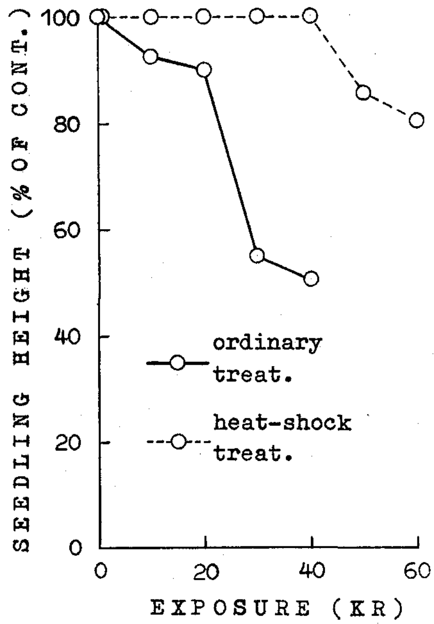


Fig. 2. Effect of heat shock on seedling height 15 days after irradiation.

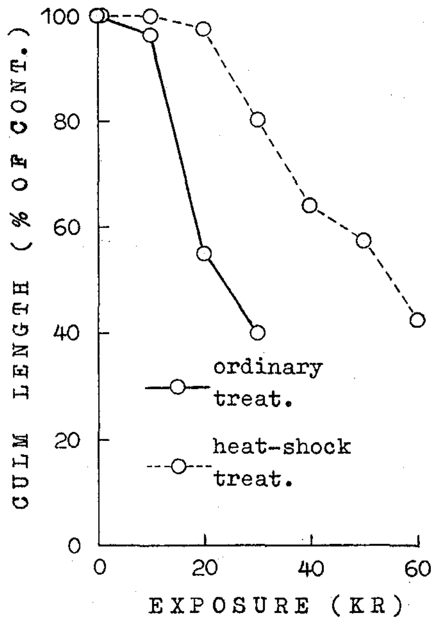


Fig. 3. Effect of heat shock on culm length after maturing.

of seedling height were not entirely consistent with those of culm length. Based on the results shown in Figs. 2 and 3, $RD_{50}$'s for seedling height could be deduced 40 kR in ordinary treatment and more than 70 kR in heat-shock treatment, while those for culm length about 25 kR and 55 kR, respectively. Consequently, it may be concluded that $RD_{50}$ for growth was enhanced about twice by heat shock.

Thus it was clearly proved that heat-shock following irradiation at an exceedingly low temperature gave a pronounced radiation tolerance to seeds of maize. Konzak *et al.*[1] and Nilan *et al.*[18] suggested that a decreased effect of oxygen took part in such a heat-shock-induced reduction of biological injuries. Though the adequacy of this interpretation still remains unexplained as far as the present experiment is concerned, the remarkably increased tolerance against radiation seems to promise a practical utility for mutation breeding.

## 2. Genotypic effects

Gamma irradiation of seeds of the seven strains brought about the result that dose response remarkably differed with different genotypic constitutions of materials as shown in Figs. 4 and 5. As to the rate of survivals 15 days after irradiation (Fig. 4), there was recognized a wide variation among four inbred lines: the most radioresistant line, W41A (dent), seemed to require about one and a half times as high the dosage as the least resistant line, N19 (flint), for falling to the same level of survival rate at higher exposures. As compared with those four inbred lines, however, the two single-cross hybrids showed a distinctly high resistance, while dent cross (W41A×W79A) appeared somewhat more resistant than flint cross (N19×CM7). Superiority of the double cross hybrid to single crosses could not be detected because of the high resistance of single crosses.

Culm lengths of the control were 54.9 (N19), 65.2 (CM7), 67.8 (W41A) and 79.0

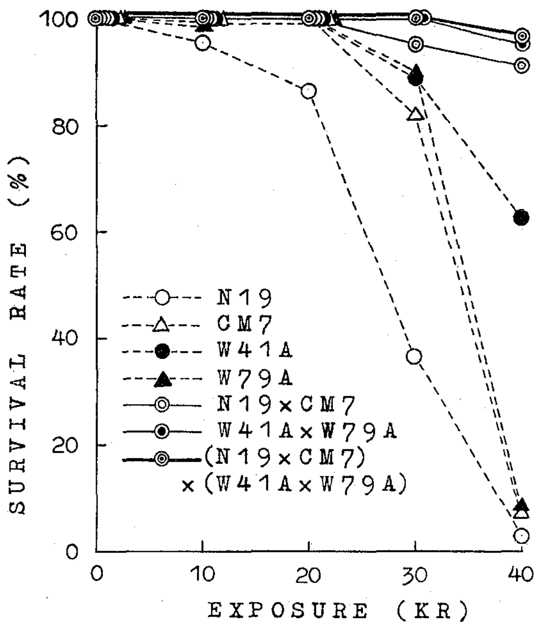


Fig. 4. Dose responses in survival rates 15 days after irradiation.

cm (W79A) for inbred lines, 87.4 (N19×CM7) and 108.6 cm (W41A×W79A) for single crosses, and 100.7 cm for the double cross. Thus both the two single-crosses showed a manifest heterosis, but the double cross was rather less vigorous than the single-crosses.

As seen in Fig. 5, the difference of culm length among four inbred lines was generally

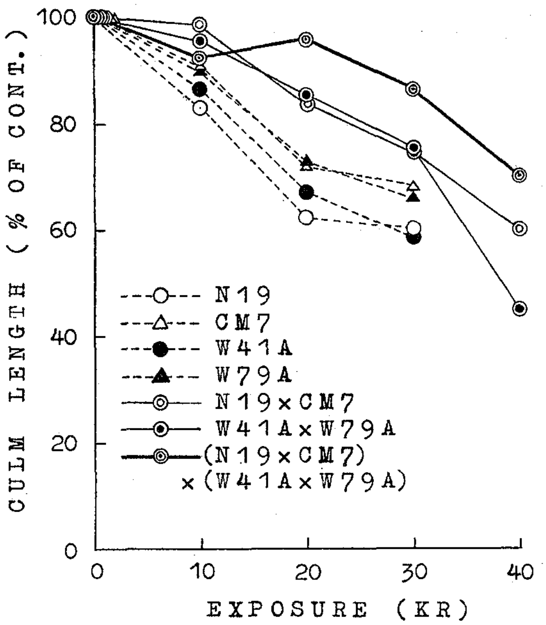


Fig. 5. Dose responses in culm length after maturing.

too small to decide the ranking, though N19 behaved as the most sensitive as observed in survival rate. Two single-cross hybrids were also indistinguishable each other, and distinctly surpassed the inbred lines in radiation tolerance. The double cross hybrid was still more resistant than the single crosses.

Thus it was clearly shown that the hybrids of maize, both in single-cross and in double-cross, exceeded their parental strains in radiation tolerance. This indicates naturally that the radiosensitivity is strongly affected by genetic heterozygosity and/or heterosis. Taking into consideration that the double-cross hybrid did not show any apparent heterosis, however, it may be presumed that the sensitivity depends upon heterozygosity as Sarić[12] suggested rather than heterosis as proposed by Notani[11] and Stoilov *et al.*[13] This appears one of the interesting problems remaining to be solved for learning the biological dependency of radiosensitivity.

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