Studies on the Recovery from gamma-induced Damage in germinating Rice Seeds

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Dry and pre-soaked seeds of rice were separately treated for 5, 10 and 20 hours with different inhibitors, known to inhibit oxidative phosphorylation and synthesis of protein and DNA and to be a chelating agent, immediately after irradiation with 0~30 kR of ^{60}Co gamma-rays at 10^4 R/hr. And the potentiating effect by these agents of damage, expressed as seedling height at the 10th day after irradiation, was surveyed.

In dry seeds, the effect of caffeine and chelating agent reached the maximum within the first 5 hours of post-treatment, while that of DNP and agent inhibiting protein synthesis was time-dependent up to 20 hours. Furthermore, inhibitor potentiation was considerably lower in pre-soaked seeds than in dry seeds and its degree became to be less pronounced in the progress of germination with all the post-treatments.

Consequently, it is concluded that physiological reversion, which occurs in relation to the metabolic status of irradiated seeds, plays a significant role in recovery process in an early stage of germination.

Introduction

For the practical application of mutagenesis in plant breeding, it is important to survey the treatment procedure that can induce desirable mutations at maximal rate along with low undesirable effects like lethality and sterility. From this point of view, factors affecting the responses of plants to mutagens have been extensively investigated. In the course of these investigations, recently, it is demonstrated that radiation damage is strongly potentiated by the treatment with metabolic inhibitor before, during and after irradiation (AHNSTRÖM 1971 ; KESAVAN *et al.* 1973 ; SWIETLIŃSKA *et al.* 1974), and is interpreted that this potentiation is mostly caused by the inhibition of DNA repair replication (KIHLMAN *et al.* 1973, 1974). However, there are some reports that absent or very weak repair replication is found in irradiated *Vicia faba* (PAINTER *et al.* 1973), *Haplopappus* (TROSKO *et al.* 1968) and *Chlamydomonas* (SWINTON *et al.* 1973), supporting the view that repair process involves neither DNA synthesis nor DNA dark repair, but requires both energy from oxidative phosphorylation and protein synthesis (WOLFF *et al.* 1955, 1956, 1969, 1972, 1973). In any case, cellular processes through which induced lesions have to pass, play significant roles in the final realization of mutations. Therefore, further analyses on the nature of recovery would lead us to obtain the information on the convenient treatment procedure increasing the efficiency and the effectiveness of mutation production.

This report describes experiments designed to analyze the nature of recovery enhanced by supplying glucose in an early stage of germination (INOUE *et al.* 1975), in reference to the metabolic phenomena associated with germination, using post-treatment with metabolic

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Table 1. List of metabolic inhibitors and their concentrations used

Inhibitor	Concentration range tested (M)	Concentration inhibiting growth of control (M)	Concentration potentiating radiation damage (M)
I. DNP	$1.1 \times 10^{-4} \sim 1.3 \times 10^{-3}$	10^{-3}	9.3×10^{-4}
II. actinomycin D	$2.7 \sim 5.0 \times 10^{-5}$	—	5.0×10^{-5}
chloramphenicol	$6.2 \times 10^{-4} \sim 6.2 \times 10^{-3}$	6.2×10^{-3}	4.0×10^{-3}
cyclohexymide	$1.0 \times 10^{-5} \sim 1.4 \times 10^{-3}$	10^{-3}	4.7×10^{-5}
III. 5-fluorodeoxyuridine	$1.0 \times 10^{-4} \sim 1.6 \times 10^{-3}$	10^{-3}	(5.7×10^{-4})
5-bromodeoxyuridine	$1.0 \times 10^{-4} \sim 1.0 \times 10^{-3}$	10^{-3}	(4.6×10^{-4})
caffeine	$5.0 \times 10^{-4} \sim 1.0 \times 10^{-2}$	—	1.0×10^{-2}
IV. EDTA	$7.9 \times 10^{-4} \sim 5.9 \times 10^{-3}$	—	5.9×10^{-3}
sodium diethyldithiocarbamate	$1.0 \times 10^{-3} \sim 1.3 \times 10^{-2}$	10^{-2}	9.0×10^{-3}
8-hydroxyquinoline	$1.0 \sim 4.1 \times 10^{-3}$	4.0×10^{-3}	1.4×10^{-3}

inhibitors.

Materials and Methods

Dry seeds, with a water content of 13.5%, and pre-soaked seeds of rice (culti. var. Ginbozu) were irradiated with 0~30 kR of ^{60}Co gamma-rays at 10^4 R/hr. Immediately after irradiation, the seeds were immersed in distilled water or in the solution of each metabolic inhibitor, known to inhibit oxidative phosphorylation (I in Table 1), protein synthesis (II) and DNA synthesis (III) and to be a chelating agent (IV), at the concentration shown in right-hand column of Table 1. Each inhibitor, at used concentration, had no effect on the growth of unirradiated seeds throughout the experiments. Following post-treatment for 5, 10 and 20 hours in dry seeds and for 10 hours in pre-soaked seeds, they were washed with running tap-water and planted on filter-paper blotters, and seedling height at the 10th day after irradiation was measured.

Post-treatment and culture of the seeds were carried out in a growth chamber at $25 \pm 1^\circ\text{C}$ with natural day length and light intensity.

The damage potentiating effects of used agents were analyzed in two different ways. Firstly, percent of control in seedling height, separately with or without inhibitor post-treatment, was used. Secondly, potentiating factor, modification index, was calculated by dividing seedling height of irradiated seeds soaked only in distilled water by that of irradiated, inhibitor-treated seeds, for 20 kR-irradiated dry seeds and for 10 kR-irradiated pre-soaked seeds.

Results

Each inhibitor shown in Table 1 enhanced selectively gamma-induced damage. However, 5-fluorodeoxyuridine and 5-bromodeoxyuridine had a very limited effect compared with other inhibitors. Effects of DNP, chloramphenicol, caffeine and EDTA in dry seeds are

Fig. 1. Post-treatment time effect on the seedling growth following gamma-irradiation of dry seeds.

● : irradiated, nonmetabolic inhibitor control.
○, ⊙ : metabolic inhibitor post-treatment for 5, 20 hours.

shown in Fig.1, except for the data in 10 hr post-treatment.

In DNP post-treatment, there is 29 and 41 per cent less growth of 20 kR-irradiated seeds for 5 hr- and 20 hr-treatment respectively, while only 20 per cent in irradiated, non-treated seeds; 20 hr-treatment increases the radiation damage by a factor of 1.36 compared with 1.13 if the seeds are treated for 5 hours. And the degree of damage enhancement with 10 hr-treatment lied between these two values. This trend was more or less the same with all the irradiation doses in DNP and chloramphenicol treatment. Consequently, it is seen that the effect of DNP and chloramphenicol is time-dependent, at least, up to 20 hours studied.

On the other hand, in the case of caffeine and EDTA, it was recognized that the degree of the potentiation by post-treatment for 10 and 20 hours remained almost equivalent to that for 5 hours; for example, in 20 kR-irradiated seeds, each treatment resulted in about 10 per cent more growth reduction as compared to that in non-treated ones. This represents that the full effect of caffeine and EDTA is obtained within the first 5 hours of the treatment-period. Furthermore, other inhibitors, belonging to the group II and IV in Table 1, enhanced the damage to a same level as in chloramphenicol and EDTA, respectively.

The damage potentiating effect of inhibitors was analyzed also in germinating seeds. In view of the evidence of TAKAHASHI (1962), which indicates that, from the pattern of water absorption, the process of germination is divided into three phases, *i. e.* the imbibition stage (phase A), the stage of organization of germination (phase B) and the growth stage (phase C), the seeds pre-soaked for 10, 20 and 40 hours in the present experiments appear to be in middle A-phase, early B-phase and middle B-phase at the time of irradiation, from

Fig. 2. Changes of water content during germination process. The arrow shows "Germination".

Fig. 3. Caffeine post-treatment effect on the seedling growth of gamma-irradiated seeds at different stages of germination.
 ● : irradiated, nonmetabolic inhibitor control.
 ○ : caffeine post-treatment for 10 hours.

Table 2. Differential modification of the seedling growth by metabolic inhibitor as influenced by the stage of germination

Duration of pre-soaking (hr)	Modification index*			
	D N P	chloramphenicol	caffeine	E D T A
0	1.22	1.22	1.19	1.27
10	1.14	1.13	1.09	1.08
20	1.17	1.07	1.08	1.05
40	1.00	1.00	1.00	1.02

* Expressed as the potentiating factor of each metabolic inhibitor, compared to the seedling height of irradiated at 20 kR (0 hr) and 10 kR (10, 20, 40 hrs), nonmetabolic inhibitor control.

the results given in Fig.2. Accordingly, it is conceivable: active water-uptake continues in the seeds pre-soaked for 10 hours; the induction of enzymes requisite for metabolic processes occurring in an early stage of germination takes place, successively their activation follows in the seeds for 20 hours; with increasing enzyme-activity, the release of storage products increases in the seeds for 40 hours.

Fig.3 shows the effect of caffeine at different stages of germination. At the dose of 10 kR, caffeine promoted approximately over-ten, ten and several per cent less growth in the seeds pre-soaked for 10, 20 and 40 hours than in non-treated seeds, suggesting that damage potentiating factor became to be less pronounced with the prolongation of pre-soaking time. In order to ascertain this phenomenon, modification index in caffeine at each stage of germination is summarized in Table 2, together with that in DNP, chloramphenicol and EDTA. As mentioned above, modification index in caffeine decreased from 1.19 in dry seeds to 1.09, 1.08 and 1.00 in the seeds pre-soaked for 10, 20 and 40 hours, respectively. Potentiation levels in other inhibitors also followed the same pattern. It therefore appears that damage potentiating effect depends on the stage of irradiated seeds, regardless of inhibitory character of used agents.

Discussion

The results in the present experiments made clear that gamma-induced damage was differentially potentiated by metabolic inhibitor post-treatment. Since each inhibitor, at used concentration, did not affect the growth of nonirradiated seeds, it is conceivable that this potentiation is due to the inhibition of recovery processes by inhibitor. And the results obtained on the ability of used agents to enhance radiation damage are generally in agreement with other reports (AHNSTRÖM *et al.* 1971; KIHLMAN *et al.* 1974; MOUTSCHEN-DAHMEN *et al.* 1969; YAMAMOTO *et al.* 1969). Particularly, the result that modification index becomes to be less pronounced with the advance of germination process is essentially similar to the observations of AHNSTRÖM *et al.* (1971, 1974), which indicate that caffeine is effective early in germination period. The most interesting result of the present experiments is that damage potentiating pattern in the seeds irradiated at the resting stage is different among used agents: the degree of damage enhancement in caffeine and EDTA reaches the full level within the first 5 hours of post-treatment period, while that in DNP and chloramphenicol is time-dependent. From this time- and stage-dependent effect, it may be suggested that recovery process involves metabolic repair system in addition to DNA repair replication, already reported in other plants (KESAVAN *et al.* 1973; SWIETLIŃSKA *et al.* 1974; YAMAGUCHI *et al.* 1974).

In germinating seeds, it was shown that protein and RNA synthesis occurred in early period of germination, where the onset of DNA replication was not recognized (MARCUS *et al.* 1964; CHEN *et al.* 1968, 1970), and also that the inhibition of this early protein synthesis resulted in the interference of subsequent DNA replication (MORY *et al.* 1972). Further evidence that impaired metabolic processes could be counterbalanced by the supply of exogenous substances, like gibberellin and glucose, was reported in gamma-irradiated wheat (HABER *et al.* 1960; ANANTHASWAMY *et al.* 1971) and rice (INOUE *et al.* 1975). Considering the above reports, it can be suggested that physiological reversion from metabolic disturbances plays a significant role in recovery process in an early stage of germination. Then, in view of inhibitory character of DNP, as the agent inhibiting oxidative phosphorylation, and that of chloramphenicol, as the agent inhibiting protein synthesis, the possibility that the time-dependent effect of these agents is caused by reduced physiological reversion must be admitted. Concurrently, modification index reduced at prolonged presoaking times indicates that the metabolic status of irradiated seeds is concerned in such physiological reversion, which takes place effectively in impairment of metabolic phenomena occurring during and immediately after water imbibition.

On the other hand, we have recognized the fact that the full effect of caffeine and EDTA is obtained in 5hr-treatment of the seeds irradiated in the resting stage, suggesting that repair of gamma-induced damage is more or less completed during the critical period up to 5 hours from the beginning of soaking. Subsequently, if repair process at the germinating stage, where gamma-sensitivity increases, occurs in a similar fashion as at the

resting stage, it would be expected to find same level in damage-enhancement between these stages. Nevertheless, it was observed that caffeine and EDTA promoted lesser enhancement of damage in the seeds pre-soaked for longer period, as well as in other inhibitors. It seems possible, therefore, that there is a differential repair capacity related to the metabolic status of irradiated seeds. DAVIES *et al.* (1960) reported that barley seeds kept their radiosensitivity for a long time in the absence of oxygen and at low temperature, where little or no metabolism took place. Furthermore, the evidence that protein and RNA synthesis were more sensitive to caffeine than DNA synthesis was demonstrated by ŽUK *et al.* (1973). Accordingly, the conclusion to be drawn from the above results is that caffeine and EDTA applied before DNA synthesis delay the repair process, finally enhancing the damage by inhibiting repair of damage in the next S-phase (MORY *et al.* 1972; EL-MAHDI *et al.* 1972). It also appears that whether induced damage remains repairable or not during this repair retardation, depends on the metabolic status of irradiated seeds.

From this series of experiments, it may be concluded that more efficient recovery from damage in dry seeds results mainly from the physiological reversion in such a way that the primary reversion from metabolic disturbances initially present affects the subsequent repair process.

Literatures cited

- AHNSTRÖM, G. and A. T. NATARAJAN 1971. Repair of gamma-ray and neutron-induced lesions in germinating barley seeds. *Int. J. Radiat. Biol.* **19** : 433~443.
- 1974. Repair processes in germinating seeds : Caffeine enhancement of damage induced by gamma-radiation and alkylating chemicals. *Mutation Res.* **26** : 99~103.
- ANANTHASWAMY, H. N., U. K. VAKIL and A. SREENIVASAN 1971. Biochemical and physiological changes in gamma-irradiated wheat during germination. *Radiation Botany* **11** : 1~12.
- CHEN, D., S. SARID and E. KATCHALSKI 1968. Studies on the nature of messenger RNA in germinating wheat embryos. *Proc. N. A. S.* **60** : 902~909.
- and D. J. OSBORNE 1970. Ribosomal genes and DNA replication in germinating wheat embryos. *Nature* **225** : 336~340.
- DAVIES, D. R. and E. T. WALL 1960. The influence of oxygen and temperature on the radiosensitivity of soaked barley seeds. *Int. J. Radiat. Biol.* **2** : 261~267.
- EL-MAHDI, A. M., J. SHAEFFER and T. MERZ 1972. The effect of BUdR on the cycle of irradiated and unirradiated meristematic cells of *Vicia faba*. *Radiation Botany* **12** : 1~6.
- HABER, A. H. and J. LUIPPOLD 1960. Effects of gibberellin in gamma-irradiated wheat. *Am. J. Bot.* **47** : 140~144.
- INOUE, M., H. HASEGAWA and S. HORI 1975. Physiological and biochemical changes in γ -irradiated rice. *Radiation Botany* **15** : 386~395.
- , ——— and ——— 1975. Effect of EI-treatment in relation to physiological and biochemical traits in rice : Delay in germination and its recovery with provision of glucose. *Radiation Botany* **15** : 397~404.
- KESAVAN, P. C., S. TRASI and A. AHMAD 1973. Modification of barley seed radiosensitivity by post-treatment with caffeine. I. Effect of post-radiation heat shock and nature of hydration. *Int. J. Radiat. Biol.* **24** : 581~587.
- KIHLMAN, B. A., S. STURELID, B. HARTLEY-ASP and K. NILSSON 1973. Caffeine potentiation of the chromosome damage produced in bean root tips and Chinese hamster cells by various chemical and physical agents. *Mutation Res.* **17** : 271~275.
- , ———, ——— and ——— 1974. The enhancement by caffeine of the frequencies of

- chromosomal aberrations induced in plant and animal cells by chemical and physical agents. *Mutation Res.* **26** : 105~122.
- MARCUS, A. and J. FEELEY 1964. Activation of protein synthesis in the imbibition phase of seed germination. *Proc. N. A. S.* **51** : 1075~1079.
- MORY, Y. Y., D. CHEN and S. SARID 1972. Onset of deoxyribonucleic acid synthesis in germinating wheat embryos. *Plant Physiol.* **49** : 20~23.
- MOUTSCHEN-DAHMAN, J. and M. and J. GILLOT-DELHALLE 1969. Modifications with chelating agents of the effects of γ -ray fractionated exposures on chromosome aberrations. *Experientia* **25** : 998~1000.
- PAINTER, R. B. and S. WOLFF 1973. Apparent absence of repair replication in *Vicia faba* after X-irradiation. *Mutation Res.* **19** : 133~137.
- SWIETLIŃSKA, Ż. and J. ŻUK 1974. Effect of caffeine on chromosome damage induced by chemical mutagens and ionizing radiation in *Vicia faba* and *Secale cereale*. *Mutation Res.* **26** : 89~97.
- SWINTON, D. C. and P. C. HANAWALT 1973. Absence of ultraviolet-stimulated repair replication in the nuclear and chloroplast genomes of *Chlamydomonas reinhardtii*. *Biochim. Biophys. Acta* **294** : 385~395.
- TAKAHASHI, N. 1962. Studies on germination of rice seed with special reference to genetical factors. *Bull. Inst. Agr. Res. TOHOKU Univ.* **14** : 1~77.
- TROSKO, J. E. and V. H. MANSOUR 1968. Response of tobacco and *Haplopappus* cells to ultraviolet irradiation after posttreatment with photoreactivating light. *Radiation Res.* **36** : 333~343.
- WOLFF, S. and H. E. LUIPPOLD 1955. Metabolism and chromosome-break rejoining. *Science* **122** : 231~232.
- and ——— 1956. The biochemical aspects of chromosome rejoining. pp. 217~222. In, *Progress in Radiobiology*.
- and D. SCOTT 1969. Repair of radiation-induced damage to chromosome Independence of known DNA dark repair mechanisms. *Exptl. Cell Res.* **55** : 9~16.
- 1972. The repair of X-ray-induced chromosome aberrations in stimulated and unstimulated human lymphocytes. *Mutation Res.* **15** : 435~444.
- and J. E. CLEAVER 1973. Absence of DNA repair replication after chemical mutagen damage in *Vicia faba*. *Mutation Res.* **20** : 71~76.
- YAMAGUCHI, H., T. NAITO and A. TATARA 1974. The inhibiting effect of EDTA on the repair of gamma-ray induced lesions in germinating barley seeds. *Japan. J. Breed.* **24** : 188~193.
- YAMAMOTO, K. and H. YAMAGUCHI 1969. Inhibition by caffeine of the repair of γ -induced chromosome breaks in barley. *Mutation Res.* **8** : 428~430.
- ŻUK, J. and Z. SWIETLIŃSKA 1973. Effect of caffeine on nucleic acids and protein synthesis in *Vicia faba*. *Mutation Res.* **17** : 207~212.

水稻発芽過程におけるガンマ線照射障害の回復

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種子の放射線感受性が酸素封入，窒素封入，貯蔵，温度処理等の照射条件により変化することはよく知られているが，さらにカフェイン等の代謝阻害剤処理によっても障害が増加することが明らかにされつつある。特に後者の場合にはこれら代謝阻害剤が DNA 損傷の修復合成を抑制することにより生じるものであると説明されている。しかし *Vicia faba*, *Haplopappus* あるいは *Chlamydomonas* では障害の回復が起るにも拘らず，そのような修復合成が認められないかあるいは非常に微量であるとの報告もある。著者らは，既に，ガンマ線照射した水稻種子をグルコース溶液中で発芽・生育させることにより照射障害が減少することを報告したが，ここでは，気乾種子および浸漬種子にガンマ線を照射した後種々の代謝阻害剤処理を行なった時の照射障害の変更を調査して，発芽過程における照射障害の回復について検討した。

水稻品種「銀坊主」の気乾種子（水分含量 13.5%）および 10, 20, 40 時間浸漬種子に 0~30 KR の ^{60}Co

ガンマ線 (10^4 R/hr) を照射した後、気乾種子については 5, 10, 20 時間の、浸漬種子については 10 時間の代謝阻害剤処理を行ない、照射後 10 日目の幼苗草丈を調査した。使用した代謝阻害剤は呼吸、蛋白合成および核酸合成を阻害するものならびにキレート剤で (第 1 表)、それぞれ、その処理濃度での非照射種子に対する生育抑制効果は認められなかった。なお種子の浸漬、後処理ならびに育成は $25 \pm 1^\circ\text{C}$ の温度条件下で行なった。

FUdR および BUdR 以外の阻害剤処理により照射障害が増加したが、その程度ならびに様相は阻害剤の種類あるいは浸漬条件により異なることが認められた。

まず、気乾種子照射後の DNP および蛋白合成阻害剤処理では処理時間が長くなるとともに障害の増加が顕著になり、例えば 20 KR 照射区では、無処理区に比べ 5 時間区で約 10%, 20 時間区で約 40% の増加率を示した。しかしながらカフェインおよびキレート剤処理ではそのような処理時間効果は認められず、5, 10, 20 時間区とも 10% 程度の増加にとどまった (第 1 図)。

一方、浸漬種子照射ではいずれの阻害剤処理においても障害の増加率は気乾種子照射の場合よりも低く、かつ照射前浸漬時間の長い種子程その値が減少する傾向にあった (第 3 図, 第 2 表)。

以上の結果から、発芽過程初期における照射障害の回復は吸水期あるいは発芽準備期初期に起る生理的障害の修復によるものであること、生理的障害の修復は DNA 複製後修復とともに発芽過程での照射障害の回復過程において重要な役割を果しているであろうことが推察された。