THE EFFECTS OF X-RAY IRRADIATION ON THE GERMINATION AND GROWTH OF SEEDLING IN GinKgo biloba L.

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Introduction

Since 1960, various radiations have been extensively used in the fundamental research on various forest trees and the effect of radiation on the germination, growth and genetic aspects have been carried out by means of acute or chronic irradiation on forest seeds and branches. (12, 15, 22, 23, 24, 33)

For Gymnosperm, Baldwon (1936)[3] reported that a slight retardation of germination in X-ray irradiated Pinus strobus and Pinus silvestris seeds was found when the samples were exposed to approximately 100 KR for 4 minutes at a distance of 8 inches from the X-ray tube at 2mA.

According to Sato et al. (1951)[27], germination was retarded when Pinus densiflora was irradiated with fast neutrons. This was again confirmed by Simak et al. (1953)[30] who observed that X-ray dose of 100 R produced a detrimental effect on the germination of pinus silvestris.

May J. T. et al. (1953)[20] found that in Pinus echinata gamma rays of 5KR produced slight damage to germinability.

Custafsson et al. (1958)[45] found that germination rates of various embryo classes of Pinus silvestris seeds were altered when the seeds were irradiated with X-ray or gamma ray.

Ohha et al. (1961)[22] found that Pinus densiflora seeds with different water content, or of different weight, and Pinus silvestris seeds collected from different provinces or individuals in a population, these all showed different radiosensitivities.

Brandenburg M. K. et al (1962)[5] reported that when a Pinus monophylla tree was acutely irradiated froma position near the base for eight hours with a Multitro Cobalt-60 unit, shoot apices exposed to 1300R-3000R showed almost immediate growth inhibition and were killed at dosages from 500 - 200 R within 4 months.

Sparrow A. H. et al. (1953)[33] found that when seedlings of Pinus strobus were exposed to Co^{60} gamma radiation at dosages from 50 to 1000R for 16.5 hrs during a period of dormancy or a period of active growth, the dormant plants were considerably more resistant than the actively growing plants.

The experiment of Flancois Mergen et al. (1963)[10] demonstrated that Pinus rigida receiving 82R/day of Cs^{137} were unable to resume cell division and differentiation during the spring of 1962, while micro spore mother cells were formed on trees receiving 75R/day.

Fujimoto et al. (1966)[12] studied the effects of gamma(Co^{60}) irradiation on the pollen germination of Pinus densiflora and Pinus thunbergii. They found that gamma irradiation at dosage of 75KR had on significant effects on the germinability of pollen while in accordance with the amount of the irradiation dose the growth in length of pollen tube generally appeared to be more sensitive to the gamma irradiation than the percentage of pollen germination.

Studies were made by Murai et al. (1966)(21), on the effects of chronic gamma irradiation on the growth of Larix leptolepsis in a gamma field with cytological observation Annual height increment was inhibited at about 2R/day, and 50% reduction dose rates were estimated for the height increment of 2.5-4.5R/day and for the breast hight diameter, the leaf length, and leaf number per leaf fascicle 7-9R/day, respectively. In newly sprouted leaves, abnormal cell division represented by the percentages of the cells with chromosomal bridge for all dividing cells had also a plus correlation with the dose rate.

Capella J.A. et al. (1966)(8) concluded that when the sensitivities to acute Co^{60} r-radiation of five species of Gymnosperms were studied, these were a very radiosensitive group of plants; the 50 percent lethal doses (LD_{50}) for the species studied ranged from 500 to 760R and the correlation between radiosensitivity and nuclear (or interphase chromosome) volume for these species agreed quite well with values obtained from other acutely irradiated woody species.

Yim, K.B. (1964)(37) Predicted radiosensitivities to acute Co^{60} r-radiation of some kinds of pine seeds.

Kim, C.S. (1964)(18) studied the effect of therma neutron irradiation on the metabolism of pinaceae seeds. The species were Pinus strobus, Pinus rigida Mill and Biota orientalis. Thermal-neutron irradiation of some pinaceae seeds with 0.98×10^{12} to 12.20×10^{12} N/th. flux per cm^2 greatly reduces seedling development and some specific enzymatic activities.

It is generally known that Gymnosperms are a very radiosensitive group of higher plants (8, 9, 33, 34, 35). However no report has been found on the effects of radiation on the germination and growth of Ginkgo biloba L. Ginkgo biloba is widely distributed in Korea and is well known to exhibit relatively high dormancy The purpose of this study was to find the effects of radiation on the germination rate, germination damage, early stage growth, and morphological variations in the leaves and branches, of this woody plant.

Materials and Methods

(1) Selection of plants.

The seeds of Ginkgo biloba L. for this study were obtained from Kwang-reung, Yangjoo-kun, korea.

(2) Irradiation procedures.

As a pre-germination treatment to break down dormancy of the seeds, the seeds were stored by means of cold stratification under the ground from December 3, 1967 to March 25, 1968.

After treatment, seeds were selected by means of water. X-ray irradiation was performed on April 3, 1968 with 6 treatments: 100R, 250R, 500R, 1000R, 3000R, 6000R.

X-ray apparatus was operated for irradiation at focus 50cm, filter 0.25mm/al., 250 KVP, and 25mA.

Until sowing. the irradiated seeds were stored at room temperature 15°C±3.

In the greenhouse, they were sown in the germination boxes (70cm×50cm×18cm) filled with soil and sterilized sand in the autoclave.

The experimental design was a randomized complete block arrangement with four replications, with 40 seeds in each treatment. After sowing, the number of germinated seeds were counted at 10 a.m., every day, for 46 days. The seeds were considered to be germinated when the shoots were found on the surface.

Morphological variation and seedling height were observed twice on the 25th and 45th day after germination.

Ten plants in each treatment were selected at random to make leaf index, T/R ratio, fresh weight and dry weight. Leaf index (length/width) was made of the second leaf form surface in each plant.

T/R ratio and fresh weight were calculated first, and then dry weight. The experimental plants were dried at 75°C to 78°C in the drying oven for dry weight measurement.

Results

1) Germination rate.

The early germination of the seeds irradiated at low dose as well as those of the control group can be recognized from the 17th day after sowing, but those at higher doses were delayed in germination with the increasing dose rate.

Maximum germination enei gy was reached on the 42 nd day from sowing. In the early stages (23rd day from sowing) the germinated seeds of control were greater than those of low dose rate, but it is most interesting to note that after that date the low doses of approximately 100R produce stimulatory effects and the germination percentages significantly decreased in accordance with the amount of the dose rate. (Table 1, Fig 1, 2)

Table. 1. Germination percentages of Ginkgo biloba seeds irradiated with X-ray(%)

dose(R) \ date	17	18	19	20	21	22	23	24	25	26	27	28	29	30	33	36	39	42	45	48	51	54
	Days from Sowing																					
Cont	3	9	16	22	26	32	39	43	44	46	48	49	51	53	58	65	71	74	76	77	77	73
100R	0.8	5.8	13	20	22	29	36	45	48	52	56	59	62	65	70	74	77	79	80	82	83	83
250R	—	—	—	0	0.8	6	11	16	22	27	33	37	41	46	54	62	70	73	73	74	76	73
500R	—	—	—	0.8	0.8	5	6	9	12	18	23	27	32	37	43	47	52	58	61	62	63	64
1000R	—	—	—	—	—	—	—	—	—	—	—	—	—	2.5	7.5	9	13	18	18	19	21	23
3000R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1.7	1.7	1.7	1.7	1.7
6000R	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0.8	0.8	0.8	0.8	0.8

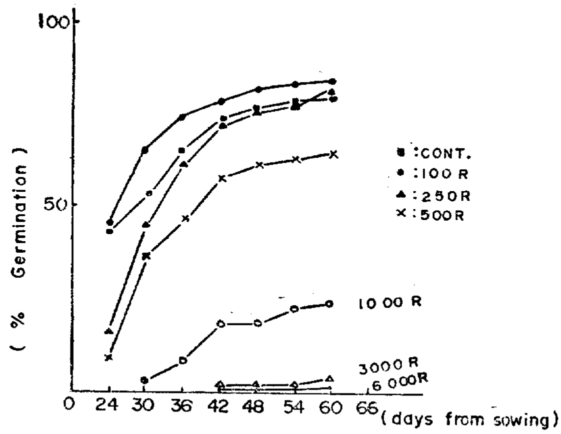


Fig. 1 Germination process of X-ray irradiated Ginkgo biloba L. seeds.

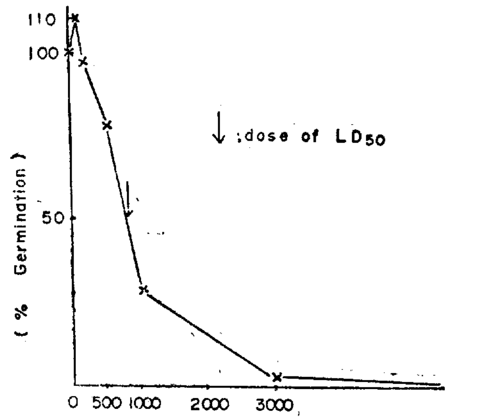


Fig. 2 Germination sensitivity curves of X-ray irradiated Ginkgo biloba L. seeds obtained at the time when the germination energy was reached in control.

Fig. 2.

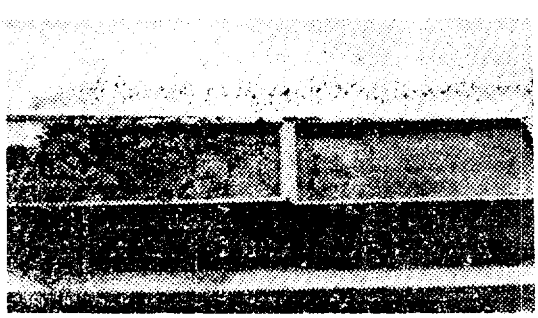


Fig. 3. Seedling damage appeared with increasing dose.

2) Seedling height.

In the early stage (25th day after germination), a slightly increased growth(growth stimulation) was observed at the low dose (100R) over that of the control group. But on the 45th day after germination, the height of the low dose was observed less than the control groups indicating little significance between the low dose(100R) and control.

Seedling height was severely inhibited at the high dose rate. The damage to the seedling increased with the amount of dose rate in each treatment and the growth in each treatment can be of high significance exeept between 500R and 1000R treatment. (Table 2, 3, Fig. 3, 4)

Table 2. Analysis of variance for seedling height.

	D. F	S. S.	M. S.	F.	P.
REP.	3	4109. 116	1369. 705		
TREAT	4	223522. 327	55880. 581	200. 132	P 0. 01
ERROR	12	3350. 617	279. 218		
TOTAL	19	230982. 060			

DUNCAN'S MULTIPLE RANGE TEST

CONT. 100 250 500 1000R

Table 3. Seedling height (Mean values)

	CONT	100	250	500	1000	3000	6000
1	6. 075	6. 335	2. 073	1. 288	0. 625	—	—
2	9. 155	8. 250	2. 853	1. 358	0. 701	—	—

1: 25th day from Grom. (May 24, 1968)

2: 45th day fiom Grom. (Jun 13, 1968)

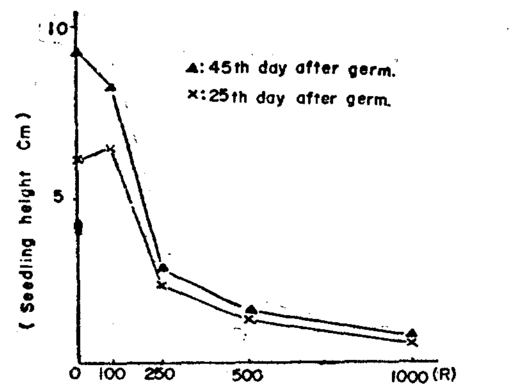


Fig. 4 Seedling height in relation to dose rates. Each point of linear curves represent the mean height of the seedli s 'n each treatment.

3) Leaf index

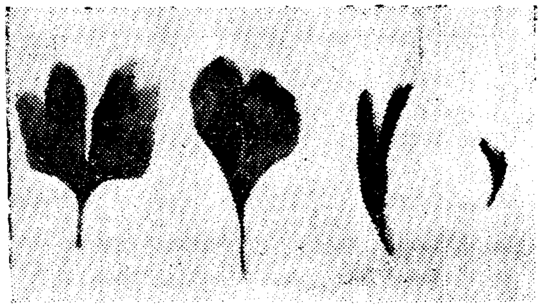
Leaf form of Ginkgo biloba L. observed on the 50th day after germination had significantly with increasing dose rate.

Leaf index(length/width) increased in accordance with the amount of dose rate.

The correlation was observed in this plant between the leaf index and the dose rate. (Table 4, Fig. 5, 6)

Table 4. Leaf index and top/root ratio of Ginkgo biloba L. seeds (50th day after germination, June 1968)

	Cont.	100	250	500	1000	3000	6000 R
Leaf Index	1. 48	1. 98	3. 10	4. 07	—	—	—
Top/Root ratio	1. 520	1. 503	0. 790	0. 470	—	—	—



Cont 100 250 500R

Fig. 5. Leaf form of plants narrowed significantly with large dose.

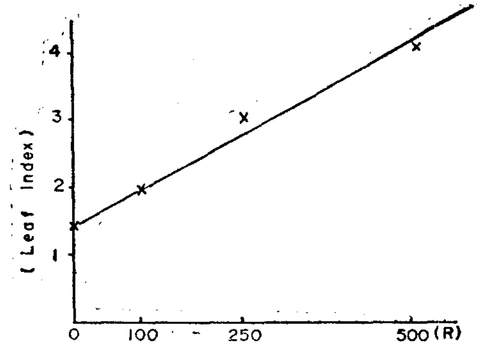


Fig. 6 This figure shows the leaf index(length/width) in relation to dose rates. Each point of linear curves represent the mean leaf Index of the seedlings corresponding to dose rates.

4) T/R Ratio

It was proportionally decreased on top as compared with root at high doses. (Table 4, Fig. 7, 8)

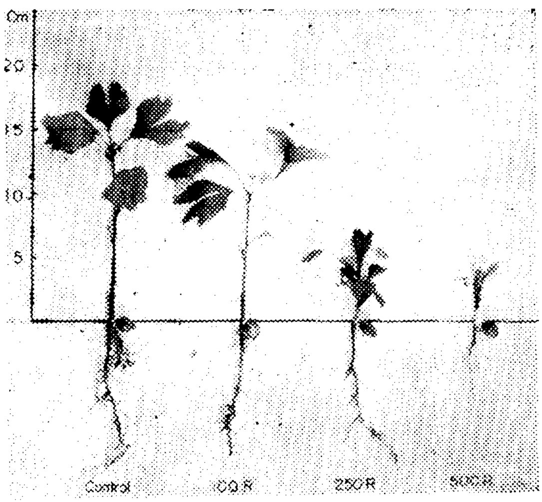


Fig. 8. Rooting was unaffected with increasing dose. but seedling height was damaged.

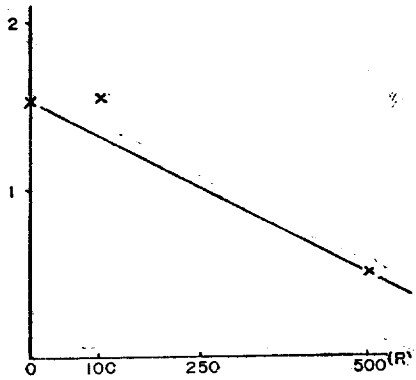


Fig.7 Top/Root ratio in relation to dose rates. Each point of linear curves represent the mean values of the seedling corresponding to dose rates.

5) Fresh and dry weight

With large dose, they were significantly depressed with 250R comparable to 100R. Then, the increasing with 100R showed. (Table 5, 6, Fig. 9)

Table. 5. Analysis of variance for dry matter.

	D.F	S.S.	M.S.	F.	P.
REP.	2	0.0105	0.00525		
TREAT.	3	0.0589	0.01963	4.75	P>5%
ERROR	6	0.0248	0.00413		
TOTAL	11	0.0942			

Duncan's Multiple Range Test

100R Cont. 250R 500R

Table. 6. Fresh weight and dry weight of Ginkgo biloba L.

	Cont.	100	250	500	1000	3000	6000R
°Fresh Weight	2.64	2.84	2.41	2.09	—	—	—
°°Dry Weifht	0.61	0.72	0.56	0.53	—	—	—

° : 50th day after germ.

°° : 53.d day after germ.

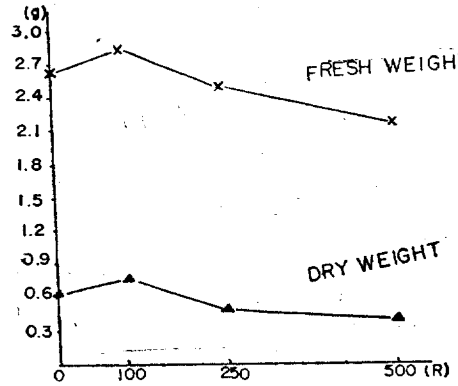


Fig.9 Dry and fresh weight are depressed with treatment.

6) Visible or morphological mutations in plants.

This category of mutants embraced all the changes that appear and remain in the plants after seedling stage.

Among the detected mutants were some affecting plant habit (dwarf type, bushy sprouting from buds.) and leaf traits (tube shape, corpulence, stria, thick green.)

At high doses, sprouting of bushy type in latera buds, thick green and corpulence of leaf were accelerate and tube shape and stria in leaf appeared (Table 7, Fig 10, 11, 12, 13)

Table 7. Leaf variation and axilliary bud aberration of Ginkgo biloba L.

45th day after germination (june 14, 1968)

	Cont.	100	250	500	1000	3000	5000
Leaf variation (tube shape)	0/108 0	32/110 29	10/115 9	1/30 3%	—	—	—
Axilliary bud aberration	0/108 0	7/110 6	52/115 45	24/30 80%	—	—	—

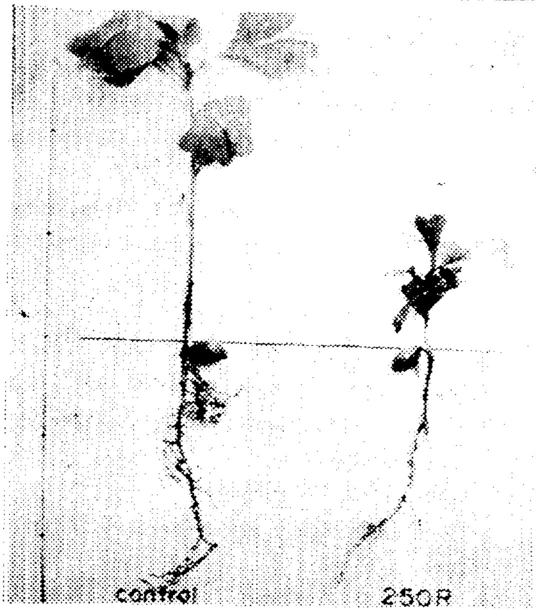
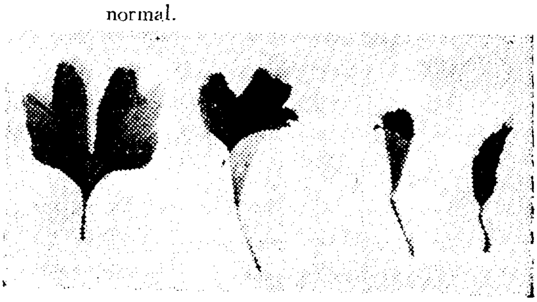


Fig. 10. Photo. taken on seedling stage for sprouting of lateral buds

Cont. 250R

Fig. 11. Sprouting of lateral buds with high dose. There are many sprouts as compared with normal.



Cont 100 250 500

Fig. 12. Morphological leaf variations (tube shape etc.) appered with high doses.

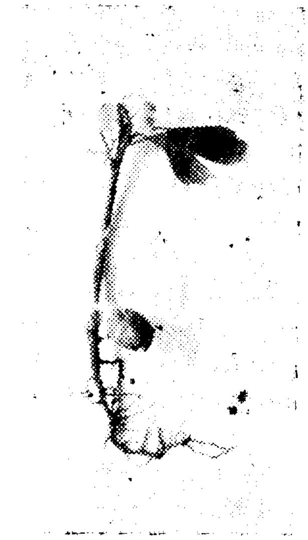


Fig. 13. Leaf mutant (stria)

Discussion

While the germination percentage of treated seeds in cereal crops appears unaffected by the various irradiation doses, there was faster germination within a limited dose but beyond that dose sensitivity in Gymnosperm was very severe. (2, 4, 6, 11, 19)

According to this fact, Gymnosperm was higher than Planted crops in radiation response. The germination LD_{50} reported in Gymnosperm until now, has been indicated with 1.9 KR in P. densiflora, 7.2 KR in P. rigida, 11.3KR in P. banksiana. (37)

The data calculated in this experiment seemed to be 765R in LD_{50}. (17, 37)

If the sensitivity that was high in Gymnosperm could be related to nuclear volume, the sensitivity in Ginkgo biloba L. also high in volume may be due to the same reason(8, 9, 33, 34, 35)

It was most interesting to note that the stimulating effect of low doses of radiation on seeds increased. the evidence of a physiological stimulating effect for the phenomena was frequently observed. (36)

Discussing the effects of small doses in plants, several prevous investigators have drawn attention to permeability of the outer membranes as the site of action of radiation besides the hypothesis of enzyme release after the destruction of inner membranes. (28) In many cases an increased uptake and release of substances was observed as a consequence of irradiation.

Here there was confirmation of the findings by Bacq Z.M. et al, Bruce, Higinboth am, Hug, Pouard, Rothstein etc. (1, 16, 26.)

There was a dose proportional delay in germination, which was the first indication of physiological damage. (7, 19, 29.)

In addition to an increase of plant height in early stage with low dose, the increasing of dry and fresh weight as compared with the non-irradiated controls were also considered with provocative germination vitality and germination capacity. (16, 26, 28, 36.)

The depressed response with large doses of irradiation indicates that there may be a relation between the amount of irradiation and germination delay, and absorption delay in growth. (24)

It was the general opinion that the effect of ascendence for leaf index with treatments may be caused by narrowing leaf width, corpulence of leaf (25).

Moreover, it was easy to find morphological changes with high level radiation. By doing so, these distinguisable genetic variability appeared as thick green, stria, tube shape in leaves and sproutings of bushy type from lateral buds (14). It could be concluded from observations in many pieces of literature that the alternation in metabolism concerned by irradiation could often be compared with the effect of physiological active substances. (29, 33)

Summary

Ginkgo biloba L., which was distributed in Korea, was x-rayed with 100, 250, 500, 1000, 5000, 6000R.

The results of responses about germination, morphological variations in seedling and early stage growth may be summarized as follows:

1. The germination percent was decreased with X-ray increasing dose from 500R except the slight stimulation of 100R.

2. The germination LD $_{50}$ seemed to be 765R.

3. The seedling height was depressed significantly but only slightly in 100R.

4. The leaf index was increased proportionally to high dose but T/R ratio was reduced apparently.

5. The dry or fresh weight decreased from 250R. It was highly significant except 100R.

6. Many morphological variations were indicated in plants.

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摘 要

우리나라 全體에 分布 栽植되고 있는 은행나무 種子에 X—線을 照射하여 發芽率, 發芽障害, 枝葉의 形態的變異및 初期 生育을 調査한데 대해서 몇가지 결과를要約하면 다음과 같다.

1. 對照區에 比해서 低線量區(100R)에서는 發芽가 약간 促進되는 경향이 있으나 500R區에서 부터는 현저히 低下되었으며

2. 發芽의 LD_{50}는 765R으로 推定된다.

3. 苗長에 있어서 低線量區(100R)에서는 減少의 差異가 가미있으나 他處理區의 減少現象에 대해서는 有意性이 認定되었다.

4. 線量增加에 따라 葉面指數는 거의 直線的인 關係로써 增加하며

5. T/R率은 反比例的으로 적어졌다.

6. 生體重과 乾物重에 있어서는 對照區에 比해서 100R區에서는 약간 增加되어도 하나, 250R區에서부터는 減少되어서 有意性이 認定되었다.

7. 線量에 따라서 植物體 各部分에서 多樣한 形態的인 變異를 볼 수 있었다