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稻熱病菌의 放射線感受性에 關한 硏究

權 臣 漢*·吳 正 行*·김 호 원*

Radiation Effects on *Pyricularia oryzae* Cav. Causing Rice Blast Disease Organism

Shin Han Kwon*, Jeung Haing Oh*, Ho Won Kim*

Abstract

In order to establish a dose limits for subsequent induced mutation research in *Pyricularia oryzae*, X-ray sensitivity of the conidia and the vegetative hyphae of the fungus, race N-1, was investigated. Conidia of the fungus irradiated with X-rays reduced significantly in spore germination inversely with radiation doses. A severe suppression of conidial germination in about 80% was found at the dose of 120kR, and the rests of the conidia produce very short and lysed germ tubes. A stimulated effect was observed in the elongation of hyphae from the conidia of 10 kR irradiation at initial stage of the growth. The radiosensitivity of hyphae was extremely higher than that of conidia with the increase of radiation doses. It was also recognized that the frequency of X-ray induced mutation in pathogenicity was directly proportional to radiation doses.

Introduction

The various advantages in the use of microorganisms for induced mutation studies had led to some investigations on radiosensitivity. Even though not all the researches were concerned with phytopathogenic species, dose-survivals based on spore germination rate had been studied for many species of the fungi [1 3 7,9 11 17], in which germination was on indicator of eventual colony formation on agar substrates. Schwinghammer[11] reported that quatitative inoculation of host plant and determination of infection counts were considered to be the most useful method of evaluating urediospore survival. In ionizing radiation treatment, inhibtion of spore gemination occurred only at dose levels far greater than those required for inhibition of host infection in rusts. In *Bacillus subtilis*, the bacterial spores were extremely resistant to ionizing radiations as compared with vegetative cells of the same strain.[15]

Among the fungi that parasitize higher plants, the blast fungus remained unexplored in irradiation researches[6]. Therefore, the dose-survival experiments were attempted primarily to establish dose limits for a subsquent induced mutation research in *Pyricularia oryzae*.

Materials and Methods

Radiosensitivity of conidia of *Pyricularia oryzae* Cav. race N-1, was determined in two different conditions. The intact conidia cultured on potato dextrose agar (PDA) and sporulated on oat meal

* Applied Genetics Lab., Korea Atomic Energy Research Institute, Seoul, Korea.
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agar(OMA) were irradiated at dose of 10, 40, 80 and 120 kR of X-rays on a turn table during exposure.

The irradiated conidia were tranferred on the 1% water agar plate by single spore isolation technique under microscope for determination of survival. In the other procedure, the single spores deposited on the thin layer of 1% agar plate from the same culture as above was irradiated with each dose of X-rays mentioned above. Until the irradiation were completed, all conidia were kept at 5-7°C to avoid their germination which made possible effects on radiosensensitivity owing to changes of physiological state of the spores. Radiosensitivity of the conidia was determined by the criteria of germination percentage which were based on the counts of 150 conidia deposited on the agar plate and the length of germ tube.

For determination of radiosensitivity in vegetative hyphae, the conidia were incubated at 27°C for germination prior to X-ray irradiation, and then tubes were irradiated with X-rays. Survival percent of the germ tubes was determined from the number of colony formed on agar plate after 4 days.

Results and Discussion

Irradiation of conidia of *P. oryzae* with X-ray reduced significantly the percentage of conidial germination inversely with radiation dose (table 1). With X-ray treatment, there appears to be no marked differences in sensitivity between conidia on nonnutrient agar plate and intact cultures on OMA, with possible exception of slightly low survival in intact culture. Severe inhibition of germination in approximately 80% (remaining the conidia with very short, lysed germ tubes) of conidia was found at the does of 120kR. The effect at such high dose levels might be largely due to an inactivation of cytoplasmic componcents. particularly enzyme system [14,15].

Tabel 1. Effect of X-Ray irradiation on germination and mortality of *P. oryzae* at 2 different Periods of treatment.

Dose(kR)	Agar plate				Oat meal agar			
	24 hrsa/		72 hrs		24 hrs		72 hrs	
	Germination percent	Mortality	Survival percent	Mortality	Germination percent	Mortality	Survival percent	Mortality
0	82	0	94	0	80	0	86	0
10	64	21.9	85	9.5	44	49.0	76	11.6
40	41	50.0	71	24.5	32	60.0	65	24.5
80	14	82.9	66	29.8	20	75.0	60	30.2
120	5	93.9	18	80.9	6	92.5	14	83.6

a: denotes time period after X-ray irradiation.

Germ tube lengths of irradiated spores are given in table 2. Elongation of germ tubes was also affected by X-ray irradiation, reducing inversely with radiation dose. Lysis and shortening of germ tubes appeared at approximately 40 kR and increased at higher doses, but this was not experimentally demonstrated. It was of interest to note that X-ray dose of 10 kR had a little stimulation effect on hyphal elongation at the initial stage of growth, 24 hours after irradiation. Vasudeva *et al.*[17] reported similar effects that stimulation of the germination and growth of conidia of *Colletrichum falcatum* was observed by irradiation of fast neutrons at a 0.35×10^7n/cm^2/s flux for 10 minutes. When the spores were irradiated with 30-R γ-rays, and 12 kR X-rays, a germination stimulation was induced. The growth of *Ustilago nuda* mycelium was also simulated by irradiation with fast neutrons.

For a comparison of the fungus in conidia spores and the vegetative (hysiologically active) stages, germ tubes were X-rayed a day after germination of the non-irradiated spores (table 3). The radiosensity of hyphae was greater spores irradiated at the same

Table 2. Germ-tube length of X-ray irradiated conidia of *Pyricularia oryzae*.

Dose (kR)	Agar plate		Oat meal agar	
	24 hrs[a]/	48 hrs	24 hrs	48 hrs
0	9.3	85.5	12.5	112.0
10	12.5	87.5	14.4	113.2
40	3.7	21.0	5.2	27.4
80	3.5	12.2	3.6	11.3
120	1.2	4.0	2.0	4.1

[a]/ Germ-tube lengths(μ) were measured under microscope (x 150) in each time period after X-ray irradiation.

doses. The hyphal growth after irradiation was decreased with increase of X-ray dose inversely, as well as survival percent which was calculated by counts of colony formed 4 days after irradiation. There was no stimulation effect in 10kR of irradiation to hyphae while it was observed in spore irradiation with same dose.

It was considered unlikely that the narrow dose-rate range of X-rays would yield significantly different lethality curve (table 4). However, the increase in net growth of hyphae 39 hours after irradiation was considerable as compared with that showed in table 3. This suggests that sensitivity might be affected by degree of initial growth which indicated hyphal length irradiated. The effects by pre-and post-irradiation, temperature, oxygen and moisture conditions as demonstrated for some microorganisms and higher plants[5,11,13] might apply also to some extent for spores of *P. oryzae* as well as their physiologic vigor by cultural conditions under which the spores were grown. For most of the dose-survival experiments, therefors, the spores were stored in a refrigerately after treatment and processed within a day, in a effort to minimize any considerbale these kind of effects.

The regrssion of surval on doses was statistically significant in replications (fig.1). The radiosensitivity in hyphae was makedly higher than that of spores

Table 3. Survival of *Pyricularia oryzae* vegetative hyphae irradiated with X-ray.

Dose(kR)	Hyphal length (μ)					Survival percent after 4 days
	Initial* growth	16 hrs after irrad.	39 hrs after irrad.	Net growth after 16 hrs	Net growth after 39 hrs	
0	5.8	34.5	166.8	28.7	132.3	100
10	4.5	36.6	139.3	27.1	107.7	89.4
40	13.3	39.8	149.0	26.5	109.2	77.7
80	14.3	18.7	28.9	4.4	10.2	11.5
120	15.2	16.0	18.1	0.8	2.1	1.0

* denotes hyphnl length when the hyphae was irradiated by X-rays. A totul of 150 conidia in each dose was germinated on water agar for X-ray irradiation.

Table 4. Survival of *Pyricularia oryzae* when the much progressed hyphae was irradiated with narrow dose range of X-rays.

Dose (kR)	Hyphal length (μ)					Survival percent after 4 days
	Initial* growth	16 hrs after irrad.	39 hrs after irrad.	Net growth after 16 hrs	Net growth after 39 hrs	
40	26.5	70.8	284.4	44.5	213.6	77.3
50	25.7	62.4	244.3	36.7	181.9	77.1
60	23.9	54.6	168.3	30.7	113.7	67.7
70	29.4	59.8	113.7	30.4	53.9	30.0
80	24.9	43.0	46.4	18.1	13.4	22.2

* denotes hyphal length when the hyphae was irradiated by X-rays. 150 condia in each dose was germinated on water ager for X-ray irradiation.

with increase of radiation dose. Terano *et al.* [15] described that bacterial spores were extremely resistant to ionizing radiation as compared with vegetative cells of the same strain. McGrath and Williams[8] and Kaplan[4] suggested that the repairing ability of irradiated *Escherichia coli* cells was one of the important factors determining the rediosensensitivity of the cells, while it was argued by Terano *et al.*[15] that the difference in radiosensitivity between spores and vegetative cells could not be explained by the difference in this DNA rejoining ability. The radio resistance of the spores was thought rather to be dependent on other factors, e.g., the physical state of the DNA involved.

For an application of subsequent induced spore mutation studies, relative mutation frequency in virulence was determined (fig. 2). Since rice variety Tong-il shows almost immune reaction to original race N-1, mutants were detacted at the basis of infection on the leaf blades of the variety,as indicated by diameter of blameter of blast lesion size. Out of 105 isolates obtained, twelves were classified as mutants in pathogenicity and the mutation frequency was determined with the per-cent mutant to radiation

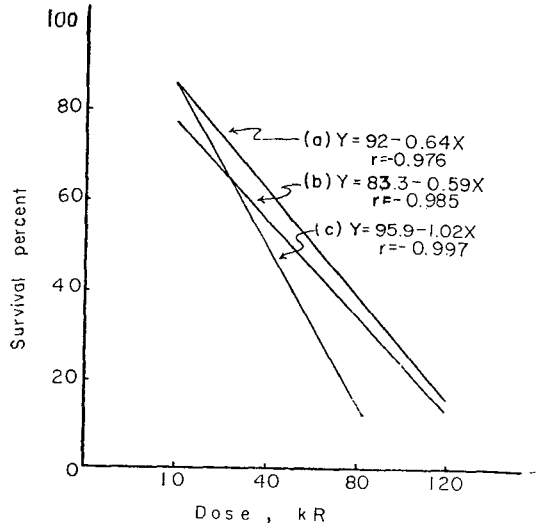

Fig. 1. Radiosensitivity of *Pyricularia oryzae* exposed to X-ray. Survival of spores on water agar plate (a), spores on sporulation media (b), and germinating hyphae on agar plate (c).

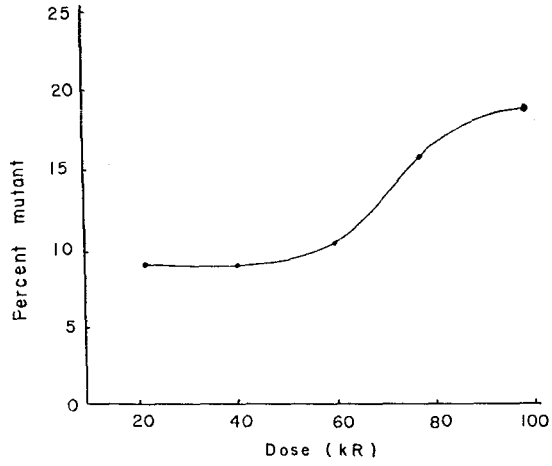

Fig. 2. Mutation frquency for pathogenicity in *Pyricularia oryzae* race N-1 irradiated with X-ray.

dose. It was recognized that the frequency of X-ray induced mutation(avirulence to virulence) was diretly proportional to radiation dose up to high lethality level. This is anology the results in higher plant that higher mutation rates appear at higher dose[5]. In view of the many known cases of mutation effects in higher plants, mutations in ionizing radiations have most frequently been ascribed to point change (intra genic) or to loss of chromosome sections following chromosome abberation, of which the yield is proportional to radiation doses[2]. Neverthlesee, further cytological and genetic studies are needed to clarify the mutation mechanism in plant pathogens.

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摘 要

放射線을 利用한 稻熱病研究의 基礎資料로서 稻熱病菌의 胞子와 發芽菌糸에 各各 X-ray 10, 40, 80, 120 kR를 照射시켜 그들의 放射線感受性 및 生存率을 調査한 結果는 다음과 같다.

1. 稻熱病菌의 胞子發芽率은 X-ray 照射線量의 增加에 反比例하여 減少되었으며 120 kR에서는 거의 生存하지 못하였다.
2. 比較的 低線量인 10 kR 照射에서는 初期發芽管伸長의 촉진 현상을 보였으며 線量의 增加에 따라 菌糸의 死滅 현상이 顯著하였다.
3. X-ray 照射에 依한 發芽菌糸의 生存率 및 伸長은 線量의 增加에 反比例하여 減少하였다.
4. X-ray를 照射받은 發芽菌糸는 胞子에 比하여 높은 放射線 感受性을 보였으며, 高線量일수록 그 差異는 顯著하였다.
5. X-ray 照射에 依한 稻熱病菌의 突然變異率은 照射線量에 正比例하여 增加하였다.