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Mutation Frequency of *Tradescantia* (BNL Clone 4430) Stamen Hairs Exposed to Low Dose of Gamma Ray in the KAERI γ-Field

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저선량율의 감마선 조사에 의한 자주 달개비의 체세포 돌연변이 출현에 관한 연구

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Abstract

For determination of mutation frequency induced by chronic irradiation of low dose gamma rays, *Tradescantia* clone 4430 was exposed to Co-60 γ rays with different exposure rates from 3.6mR/day to 182R/day in or out of the Gamma Field at Kumkok Experiment Farm of KAERI.

Somatic mutations based on pink mutant events of the stamen hair cells were clearly observed by the treatment. The pink mutant events were increased proportionally with increasing exposure rates of gamma ray except for relatively high dose rates of 105 R/day and 182 R/day, indicating saturation effect of mutation. The somatic pink mutations could be fairly detectable even in the low dose rate of 3.6mR/day. Therefore, this stamen hair system of *Tradescantia* clone 4430 seemed to be an reasonable test system for detecting mutability of low level irradiation. These results imply that artificial mutation induction in the fruit and ornamental trees could be expected in the γ-field.

요 약

자주달개비(*Tradescantia*) BNL clone 4430을 본 연구소 감마농장(γ-field)에 거리별로 배치하고 γ-선을 3.6mR/day~182R/day의 선량율로 완조사(緩照射)시켜, 이 식물의 수술털(雄蕊毛)에 나타나는 분홍색 체세포돌연변이(體細胞突然變異)를 대상으로 돌연변이의 출현율을 조사하는 한편 화기(花器)에 미치는 형태변화를 조사하였다.

분홍색 체세포돌연변이율은 22.2R/day의 선량율에서 수술털 1,000개당 85.81±6.45개로 가장 높았고, 이보다 높은 선량율에서는 오히려 변이율이 감소하여 포화선량효과(飽和線量効果)를 초래하였다. 또한 3.6mR/day의 낮은 선량율에서도 이 체세포돌연변이율이 자연돌연변이율보다 증가하였

음을 볼 수 있었으며 비교적 낮은 선량을 조사에서는 꽃, 수술, 수술털등의 형태적 변화가 없이 돌연 변이율만의 증가를 볼수가 있어 감마농장내에서 과수(果樹)나 화목(花木)등 영년식물에 저선량의 완조사를 통해 체세포돌연변이의 유기(誘起)가 가능할것으로 보이며, 한편 이 식물의 특성을 이용하여 저위 방사선에 의한 유전적변화를 탐지하는데에도 적절한 수단이 될 것 같다.

Ⅰ. Introduction

Various techniques for raising efficiency of mutation by radiation treatment are developed depending upon characteristics of the plant. In herbaceous plants, seeds are usually irradiated acutely with relatively high doses for induction of mutation, whereas chronic irradiation to vegetative organs is useful for the perennial vegetatively propagated plants.

The most efficient use of ionizing radiation in the mutation induction in crop plants needs to have various radiological informations, particularly, on the genetic effects by acute and chronic irradiations. A considerable information on biological responses of various living organisms to relatively high dose rates has been given by consecutive radiological researches[1-3], while only a few report was made on the genetic effects at low level exposures[4,5].

Certain species of higher plants were employed in the studies of low level of radiation[6-9], because large size of population can be treated easily and the mechanisms of mutation and chromosome aberrations induced by ionizing radiation are easily detected. *Tradescantia* is one of the higher plants used for radiobiological studies. It has a lot of stamen hairs consisting of a series of single meristematic cells. The stamen hair cells are so sensitive to radiation as to be used effectively for determining somatic mutation rate at low level exposures[8,9].

Present study was carried out to determine a mutability by chronic irradiation of low doses of gamma ray and to find out appropriate chronic irradiation doses for obtaining maximum mutants from the plants grown in the gamma field.

Ⅱ. Materials and Methods

A clone 4430 of *Tradescantia* obtained from BNL, a diploid interspecific hybrid (*T. hirsutiflora X T. Subcaulis*), was used for the present study. Two and four pots containing about 30 inflorescences per pot at flowering stage were placed on nine positions respectively with different distances from the gamma source in and out of the Gamma Field of KAERI. Exposure dose rates at these positions were 182, 105, 22.2 R/day and 1,119, 642, 318, 303, 10.9, 3.6 mR/day, respectively. Check plants were placed as sufficiently apart as possible from the source.

Pink mutation frequency and other radiation effects were observed in flower which opened from 7 to 29 days after starting the exposures. The flowers were picked shortly after the flower buds opened in the early morning and stored at 4—6°C on moist filter paper in plastic containers. The stamen hairs were dissected out from the flowers, and the number and the position of the cells mutated in the stamen hair cells were investigated under stereoscopic microscope. The color of flower petals and stamen hair cells is a phenotypically blue and genetically heterozygous.

When a mutation or a deletion of the dominant gene for blue color is induced, the pink color expressed by a recessive gene appears in daughter cells. Therefore, the phenotypic change in pigmentation from blue to pink in mature stamen hair cells was used as a visible marker for scoring mutation.

Ⅱ. Results and Discussions

For determining mutation rate, type of pink mutant event was identified. If a single pink mutant cell was found in a blue stamen hair, the pink cell was termed a single mutant event. Also two or more contiguous pink cells in a hair were considered as a single event. When a hair had two or more pink cells separated by one blue cell or more contiguous blue cells, it was scored as two or more pink mutant events[3]. By this classifying, the case of one pink mutant event in a stamen hair occurred mostly with frequeny of 94%, and two or three pink mutant events in a stamen hair were rarely observed, while the case of more than four events was not found in the surveying period (Table 1).

Table 1. Frequency of Occurrence of Pink Mutation Events on a Stamen Hair in *Tradescantia* Clone 4430

	No. of total stamens examined	No. of mutant events/stamen hair			
		One	Two	Three	Four
No. of stamen hairs	420	394	24	2	0

Somatic mutations as a pink mutant event by chronic radiation treatment were clearly observed as shown in Table 2. The pink mutation frequency which was evaluated with the number of pink mutant events per 1000 stamen hairs observed in every other day from 7 to 19 days after starting the irradiation was increased with the increment of radiation doses except for the two high doses of 105R/day and 182R/day. The highest frequency of the pink mutation in this treatment was 85.81 ±6.45 pink mutant events per 1000 hairs at the exposure dose rate of 22.2R/day. On the other hand, it was possible to detect significantly increased somatic pink mutation with the exposure of 3.6mR/day which was slightly higher than background. Mericle and Mericle[4] reported that the somatic mutation rate increased in the stamen hairs of *Tradescantia* clone 02 by two week exposures of 0.25mR/hr.

Table 2. Numbers of Pink Mutation Events per 1000 Hairs of *Tradescantia* Stamens in Exposed Chronically for 19 Days with Different Dose Rates of γ-ray

Dose rate	No. of hairs investigated	No. of mutant events	Mutant events/1000 hairs
182.00R/day	38,136	1,021	26.77±2.34
105.00R/day	29,496	1,450	49.16±5.38
22.2 R/day	51,006	4,377	85.81±6.45
1.12R/day	56,184	254	4.52±0.35
642.0mR/day	56,940	218	3.83±0.31
318.0mR/day	42,942	148	3.45±0.31
303.0mR/day	50,568	113	2.23±0.22
10.9mR/day	22,968	38	1.65±0.31
3.6mR/day	43,818	64	1.46±0.22
Control*	151,476	145	0.96±0.08

* Background level 0.28mR/day (14 hrs).

Since the development of stamen hair was completed 7 days before flowering, no radiation effect was observed in the stamen hairs collected from the flowers which opened within 7 days after exposure started. Therefore, effective exposure dose at the present experiment was defined as total irradiation dose exposed before 7 days from flowering. The total and effective exposure doses in this experiment were

Table 3. Total Doses and the Effective Exposure Doses for Each Dose Rate Treatment

	Exposure rate								
	R/day			mR/day					
	182	105	22.2	1,119	642	318	303	10.9	3.6
Total doses exposed	3,458	1,995	422	21,261	12,198	6,042	5,757	209	68.4
Effective doses	2,184	1,260	267	13,428	7,704	3,818	3,636	132	43.2

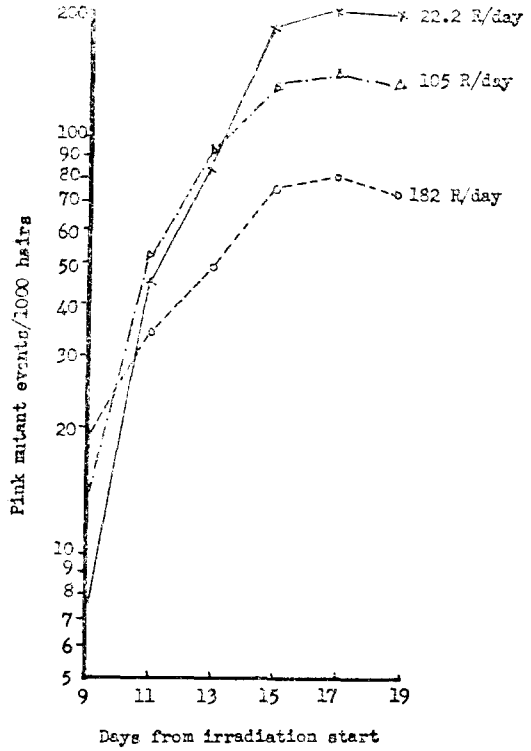

Fig. 1. Pink Mutation Frequency of Stamen Hair in *Tradescantia* Clone 4430 Exposed to γ-ray (22.2R/day-182R/day)

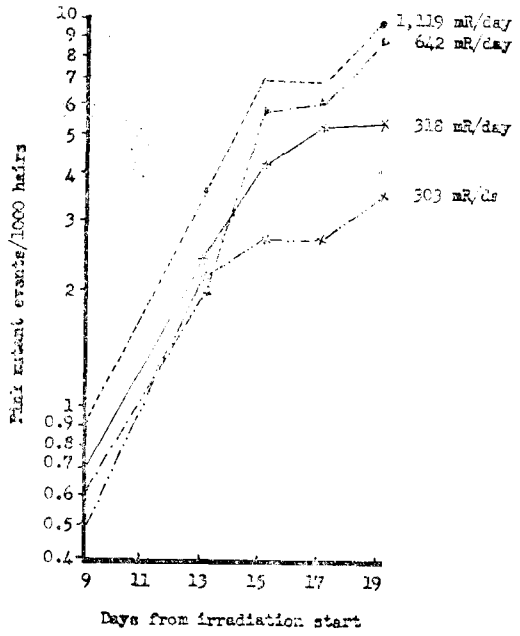

Fig. 2. Pink Mutation Frequency of Stamen Hair in *Tradescantia* Clone 4430 Exposed to γ-ray (303mR/day-1.119mR/day)

shown in Table 3. Figures 1 and 2 showed a trend of increase in mutation frequency with time course after exposure start. The mutation frequency in high dose exposures began to decrease from 17 days after the irradiation start. The mutation frequencies in the relatively high dose rates of 182R/day and 105R/day were higher in the early period of time, while rather low in the late period as compared to that of 22. 2 R/day exposure. This was considered to be due to saturation effect for inducing the pink mutation of stamen hairs of *Tradescantia* clone 4430. On the other hand, the pink mutation events at relatively low doses from 303 mR/day to 1,119mR/day (Fig. 2) increased with the increment of the exposure doses by the time of 19 days after the irradiation started. There was a tendency that the mutation frequency was positively proportional to radiation doses in the relatively low radiation doses.

The number of stamen hairs in the plants

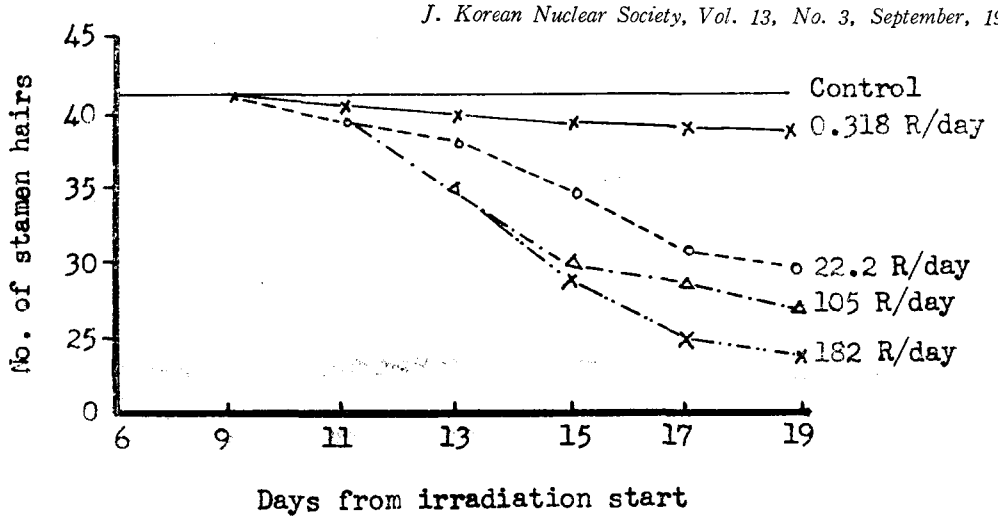

Fig. 3. Changes of Stamen Hair Number Irradiated with Co-60 from 6 to 19 Days from Irradiation

grown under open field averaged 42 per stamen filament, but it varied by position of stamen and growing conditions of the plants. The number of hairs per stamen decreased drastically by irradiation of higher doses whereas no significant decrease was observed in the low dose treatments from 3.6mR/day to 303mR/day (Fig. 3). Average stamen hair was consisted of about 26 cells in mature stamen and basal hair contained more cells. The number of cells decreased gradually acropetally along the stamen filament. In the radiation treatment of 105R/day, the number of cells in teh stamen hair was not affected by the time of 15 days after the irradiation started and thereafter the number was conspicuously decreased (Fig. 4). The development of hair cell is completed about 7 days before anthesis in normal temperature[5,8]. During this surveying period, the temperature at Kumkok Experiment Farm was about 3—7°C which is lower than normal condition (Fig. 5), and the induction of radiation effects seemed to be delayed. The results of this experiment indicated that the stamen hair system of *Tradescantia* clone 4430 was so sensitive as to show a clear response to low level radiation.

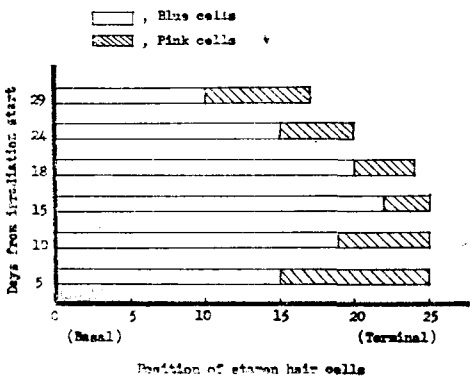

Fig. 4. Changes of Mutation Event Position on the *Tradescantia* Stamen Hairs by Progressing of Exposure

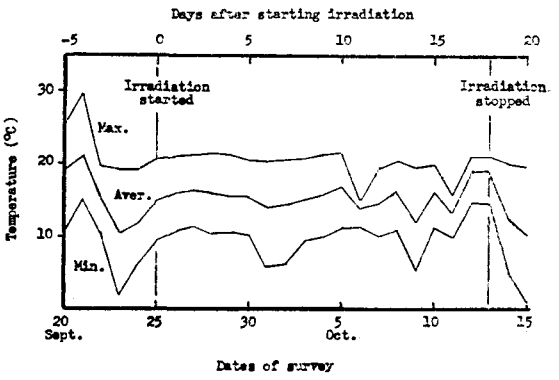

Fig. 5. Temperatures Recorded in Gamma Field during the Experimental Period. Irradiation term Indicated

Ⅳ. Conclusion

Genetic effects by radiation treatment especially chronic irradiation of low doses should be investigated in detail for increasing mutation efficiency. Through the present experiment, mutation frequency based on pink mutation of stamen hair cells more increased in higher dose rates of 182R/day and 105R/day than in a relatively low dose rate of 22.2R/day by the time of 11 days after starting irradiation. But the mutation frequency was higher in dose rate of 22.2R/day from the time of 13 days after starting irradiation. Consequently, the highest mutation frequency was obtained in the relatively lower rather than highe: dose rate. probably this is due to a saturation effect caused by severe physiological disorder of the plant exposed to high dose rate. This effect is usually observed when seeds were treated with acute irradiation of high doses.

In this point of view, the saturation effect by chronic irradiation seems to be considered in gamma field. Furthermore, since a remarkable mutation could be obserⅴed from *Tradescantia* clone 4430 even by the low level exposure of 3.6mR/day, a test system with *Tradescantia* seemed to be available for monitoring low level exposure by further investigation. These results imply that low levels of chronic irradiations in gamma field could be effective in induction of mutations in the higher plants, such as fruit and ornamental trees.

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