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Effects of Natural Extracts on the Radiation-induced Pink Mutations in *Tradescantia* Stamen Hair Cells

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Abstract

The effect of a water-soluble extract from natural materials on radiation-induced mutations was studied by means of TSH assay in *Tradescantia* 4430 stamen hair cells. Inflorescence cuttings, with or without pretreatments of natural extracts for 3 hours, were exposed to 1 Gy of gamma ray. Comparisons were made on the basis of pooled data during the peak interval between the mean pink mutation frequencies of the experimental groups. Pretreatments of FB or FB-I resulted in about two-fold increases of the pink mutation frequencies, compared to those of the control group. Synergism between certain fractions and radiation was a possible cause of the increased DNA damage. FB and FB-I had a radiosensitizing effects on the pink mutations in *Tradescantia* 4430 stamen hair cells ($p<0.001$). On the other hand, the extract PP in a proper concentration significantly reduced the pink mutation frequencies ($p<0.05$). The result means that PP has a protective effect on the radiation-induced cell damage.

Key Words : natural extract, radiomodifier, protective effect, pink mutation, TSH

1. Introduction

Recently many efforts have been made in search for radioprotective or radiosensitizing materials from natural resources such as traditional foods and medicinal plants [1]. Experimental models such as *Tradescantia* stamen hair cell system, mouse ovarian system, and cultured cell line system have been continuously established to screen various candidates of radiomodifier [1]. Among others, the radiation-induced somatic cell mutations in *Tradescantia* has great applicability for studying the effect of some natural candidates. *Tradescantia* 4430 (T-4430) is an interspecific hybrid between *T. hirsutiflora* (blue flower) and *T. subacaulis* (pink flower) heterozygous for the

flower color (blue dominant and pink recessive). The clone was selected because of its known sensitivity to physical and chemical agents and because of its differential sensitivity to some chemical mutagens [2]. It is one of the most radiosensitive plant systems known so far; the dose-response curves have been determined down to doses of 0.25 cGy for X-rays and 0.01 cGy for neutrons [3].

The significant features of *Tradescantia* that make it useful for radiation studies, particularly at low dose region, are its extreme sensitivity to radiation and as well as the relative ease with which the various genetic points in somatic cells can be reliably scored. The flower buds are sufficiently small to permit irradiation which is uniform with respect to both dose and energy. *Tradescantia* stamen hair is essentially of a single-meristematic-cell nature; because it grows by repeated divisions of terminal and subterminal cells. Therefore, the dose response curves for different genetic end points can be compared with data from single cell systems of other organisms. Depending on the dose level, more than 1,500 stamen hairs per one experimental dose point are examined to determine the pink and single pink frequencies.

The assay system based on the stamen hair cells of *Tradescantia* is called TSH assay and has proven to be one of the most suitable materials to study the frequency of mutations induced by low doses of ionizing radiations and chemical mutagens as reviewed earlier [4-7]. The system has also been used successfully for detecting mutagenic synergisms among chemical mutagens and X-rays [8-10]. TSH assay has been applied to a wide variety of radiobiological studies. The mutation frequency of *Tradescantia* 4430 stamen hairs exposed to low dose of gamma ray in a KAERI gamma-field was studied by Kwon et al. [11]. There are many reports which dealt with the biological monitoring of environmental radiations around the nuclear power plants [12,13], and in the air contaminated with radioactive materials after the Chernobyl accident [14]. It is possible to use the TSH assay to compare the relative biological effectiveness of various radiations [15,16] and to estimate the change in the RBE (relative biological effectiveness values) due to boron neutron capture process [17,18]. The present experiment was carried out to test the applicability of TSH assay in association with searching radioprotective or radosensitizing materials.

2. Material and Methods

2.1. Experimental Plants

The experimental plants were provided by vegetative culture from the stock plants of *Tradescantia* 4430 (interspecific hybrid, $2n=12$). Plants were grown under greenhouse conditions where growing and cultivation procedures were maintained as described by Underbrink et al. [4]. An average of 21 cuttings per dose were exposed to γ-radiation and in order to avoid the storage effect [19] the cuttings were irradiated not earlier than 24 hours after cutting from the rooted plants. After irradiation the cuttings were cultivated in the growth chamber at 18~21℃ with the 18-h day and relative humidity 85 %. Flowers in bloom were collected between the 7th day and 20th day after exposure. Then gene and lethal mutations were scored during the interval.

2.2. *Tradescantia* Stamen Hair Assay

TSH assay measures were done under stereomicroscopes (x25). Flowers were taken while in full bloom, normally early in the morning on each day and stored in the refrigerator before scoring. Stamen hairs were carefully removed with

Table 1. Experimental Code for the Group or Material

Code	group or material	remark
CT	Control group	
PP	Pepper paste	
FB	Fermented beans	
FB-I	Fermented beans	Korean style
AP	Artemisia peinceps	

forceps from the flowers and placed onto the slide glass upon which the proper amount of mineral oil was dropped. Scoring was performed to determine the pink, single pink and stunted cells frequency.

Gene mutations, characterized by single or numerous adjacent pink cells in the hair, were counted as one mutational event. The single cell pink event can be considered as an indicator of mutations induced after the S phase of the cell cycle [9]. A ratio between the number of single pink cells and all pink mutation events was considered as a factor indicating cell cycle behavior. The mean values of mutation frequency calculated for the scoring period and expressed as a number of mutations per 100 hairs were used as a measure of the mutation effect caused by the exposure. More than 1,500 stamen hairs were examined in an experimental group during scoring period.

2.3. Preparation and Pretreatment of Natural Extracts

The natural extracts were prepared with fed-batch type extraction for 4 hours at 80℃ in the series extractor (fexIKA 200 Control). Three source materials for the natural extracts were pepper pastes, fermented beans of normal type, and fermented beans of traditional Korean type, which were commercially available. In addition, *Artemia princeps* was collected from the field and used for the extract. Distilled water was used as an extracting solvent. Each experimental group of the inflorescence cuttings was immersed in the diluted extract for 3 hours in an aerating condition. The experimental code for the source material was given in Table 1.

2.4. Irradiation of *Tradescantia* Plants

Irradiation of *Tradescantia* cuttings with gamma ray from ^{60}Co source (source strength about 150 TBq, Panoramic irradiator, Atomic Energy of Canada Ltd.) was done in the air at KAERI. The cuttings were exposed to 1 Gy at which pink mutation frequencies in TSH without pretreatment reached a half maximum value. After irradiation cuttings were transfered into the growth chamber for further cultivation in aerated Hoagland' s solution (6x dilution) [20] with 18-h day at 21℃ (±1℃) and 85 % relative humidity.

3. Results and Discussion

The mean pink mutation frequency of 1 Gy irradiated control group expressed in pink frequency per 100 hairs was calculated from the pooled data during the peak interval was 2.79± 0.61. It was well consistent with those in the previous reports [5,21]. The previous reports demonstrated that the somatic pink mutation frequency in TSH cells induced by ionizing radiation increased linearly with an increasing radiation dose up to 2 Gy. During the whole scoring period, the mutation frequency in TSH cells irradiated with a given dose, i.e. 1 Gy, reached its highest value at a point in time after irradiation and then fell down toward the spontaneous mutation level. The peak interval when the mutation frequencies reside in significantly elevated level can be defined

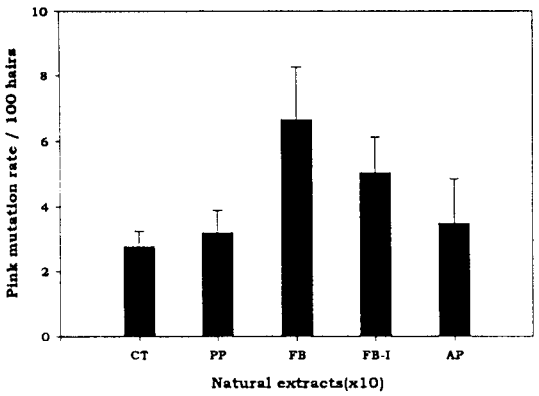

Fig. 1. Pink Mutation Frequencies Induced by Radiation in *Tradescantia* 4430 Pretreated with 10-fold Diluted Natural Extracts

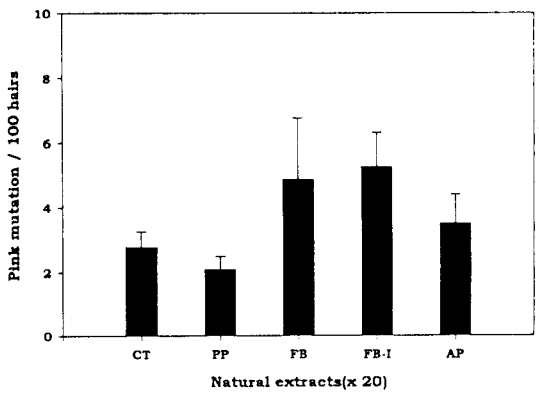

Fig. 2. Pink Mutation Frequencies Induced by Radiation in *Tradescantia* 4430 Pretreated with 20-fold Diluted Natural Extracts

according to the proper criterion. It appeared on day 6 to 10 after irradiation in the control group. On the other hand, the peak interval in the experimental group pretreated with 10-fold diluted extracts (PP, FB and FB-I) appeared during 8 to 16 days after irradiation. There were 2~6 days of frame-shift in the peak interval. Such a shift means that the pretreatment of extracts gave rise to the impact on radiation-induced gene mutations in TSH cells. According to Kim et al. [21], NaCl in proper concentration has a radioprotective effect on radiation-induced DNA demage in TSH cells and a shifting effect on the peak interval, as well. Thus it is possible for NaCl in the extract to play a considerable role in shifting the peak interval since PP, FB and FB-I contain 4~8 % of salt.

Based on the mean values of the pooled data during the peak interval, the pink mutation frequencies in the groups pretreated with 10-fold diluted FB and FB-I extracts were nearly two times that of the control group (Fig. 1). The pink mutation frequency of TSH cells was 6.66 ± 1.60 in the FB pretreated group, and 5.06 ± 1.08 in the FB-I pretreated group.

When pretreated with 20-fold diluted FB, the pink frequency of TSH cells was 4.89 ± 1.87, which was still higher than that of the control group (Fig. 2). In the case of FB-I, it gave the similar value of pink mutation frequency in both concentrations. Such a large increase in the somatic mutation rate resulted from the synergistic action of the extract and γ-radiation in both FB and FB-I pretreated groups. The combined treatment of the extract and irradiation induced higher mutation rates in TSH than irradiation alone. In other words, FB and FB-I have a radiosentizing effect on TSH pink mutation system ($p<0.001$). However, the activity of FB or FB-I is not concentration-dependent.

On the other hand, the pretreatment of 10-fold diluted PP extract resulted in the pink mutation frequency which was not actually different from that of the control group. However the lower concentration of the PP extract lead to an interesting result. The pretreatmet of 20-fold diluted PP extract reduced the gene mutation frequency in TSH cells by a statistically significant level ($P<0.05$). It is normally expected that the response of biological systems to natural extracts can be dose-dependent or concentration-dependent. The effect of the PP extract was concentration-dependent and protective against

Table 2. Effect of Natural Extracts on Radiation-Induced Pink Mutations in the Experimental Groups

Dilution factor	Experimental Group	Effect	Remark
x20	PP	protection	*
	FB	sensitization	*
	FB-I	sensitization	***
	AP	-	
x10	PP	-	
	FB	sensitization	***
	FB-I	sensitization	***
	AP	-	

* ; $p<0.05$, *** ; $p<0.001$

the damage of genetic materials induced by radiation.

It is also expected that the extract can be toxic to the TSH system. In *Tradescantia*, the number of hairs per stamen and the number of cells per hair are bioindicators to reflect the toxicity of treated materials. The numbers of hairs per stamen and of cells per hair in all of the pretreated experimental group counted during the scoring period showed no recognizable difference from those of the control group. Such a result indicated that the pretreatment of the extract in the experimental concentration did not cause any physiological damage such as incomplete development of flower buds, inhibition of flowering, etc. In addition, a ratio between the number of single pink cells and all pink mutation events was also evaluated in all groups. The ratio, as a factor indicating cell cycle behavior, also assured that abnormal physiology of plants was not invoked by the extracts. The concentration of the extracts available in this study proved non-toxic, and thus the results obtained are thought to be physiologically valid.

Such results proved promising from the biological point of view in that it is possible to effectively screen the radioprotective or radiosensitizing candidates from various kinds of natural materials (Table 2). This study was done on a small scale without any assurance of the possible data fluctuations invoked by the slight difference in the arrangement of inflorescence samples and in absorption efficiency of an individual cutting. Since the effect of the natural extracts was based on the comparisons between the pink mutation frequencies of the experimental groups, the limitation in the sample size could also be a key factor affecting the statistics. Further study on such confounding factors mentioned above will make it possible to measure precisely the increase or decrease in gene mutations of TSH cells.

4. Conclusions

The effects of some natural extracts on the pink mutations induced by ionizing radiation were studied. *Tradescantia* 4430 is an interspecific hybrid heterozygous for flower color and thus is one of the plants sensitive to radiation and mutagenic chemicals. The pink mutations in *Tradescantia* stamen hair cells have a distinct feature that can directly be applied to measure the radiomodifying activity of natural materials. Among natural extracts tested, FB and FB-I showed radiosensitizing effects to increase the pink frequencies in TSH cells by about two-fold compared to that of the control. Those extracts did not act on the TSH pink mutation system in a concentration-dependent manner. On the other hand, the extract PP gave quite different results according to its concentrations applied to the plant cuttings. In the lower concentration, PP showed a protective effect on the radiation-induced damage to reduce significantly pink mutation frequency in TSH cells, while it did not affect the pink mutation frequency at all when applied in the higher concentration.

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