PLANTS AS WARNING SIGNAL FOR EXPOSURE TO LOW DOSE RADIATION

[1]Rusli Ibrahim, [2]Rosinah Hussin, [2]Nur Suraijah Mohd & [1]Norhafiz Talib
[1]Malaysian Nuclear Agency, Agrotechnology & Biosciences Division, Bangi 43000, Kajang, Selangor
[2]University of Malaya, Faculty of Genetics, Kuala Lumpur

Abstract

The stamen-hair system of Tradescantia for flower colour has proven to be one of the most suitable materials to study the frequency of mutations induced by low doses of various ionizing radiations and chemical mutagens. The system has also been used successfully for detecting mutagenic synergisms among chemical mutagens and ionizing radiations as well as for studying the variations of spontaneous mutation frequency. In this study of radiobiology, the main objective is to observe somatic mutation (occurrence of pink cells from blue cells) induced on stamen hairs of five Tradescantia sp. available in Malaysia after exposure to low doses of chronic gamma irradiation using Gamma Green House. Pink cells appeared only on Tradescantia Pallida Purpurea stamen hairs after 13 days of exposure to irradiation with different doses of gamma rays. The highest number of stamens with pink cells was recorded from flowers irradiated with the highest dose of 6.37Gy with 0.07Gy/h of dose rate. The lowest number of stamens with pink cells was recorded with an average of 0.57, irradiated with the lowest dose of 0.91Gy with 0.01Gy/h of dose rate. There were no pink cells observed on Tradescantia Spathaceae Discolor after exposure to different doses of gamma rays. Similar negative results were observed for the control experiments. The principal cells in this assay are the mitotic stamen hair cells developing in the young flower buds. After exposure to radiation, the heterozygous dominant blue character of the stamen hair cell is prevented, resulting in the appearance of the recessive pink color. Furthermore, no pink cell appears on all species of Tradescantia spathaceae after irradiated with different doses of gamma rays. The sensitivity of the Tradescantia has been used widely and has demonstrated the relation between radiation dose and frequency of mutation observed at low doses which can contribute to the effects of low doses and their consequences for human health. This system carries the advantage of observing meaningful data in a short period of time, being able to meditate effects on human health and to prevent possible accidents, when adopted as periodical monitoring. Tradescantia Pallida Purpurea can be regarded as a biosensor plant or a biological warning signal for exposure to low dose radiation which exhibits a noticeable quantity of cell alteration in a short time following exposure to radiation. Hence, the effects caused on the environment might be anticipated, and by extension on the human being, as a result of its occupation exposition level. The use of this method can be recommended for radiation monitoring, therefore into the environment acclimatization, and may be used, in addition, in the prevention of radiological accidents.

Key Words: Biosensor, low-dose radiation, mutation, stamen hairs, *Tradescantia*

INTRODUCTION

A mutation is defined as a change in DNA sequence, which leads to a heritable change in gene function. Agents (mutagens) that change the sequence of DNA are considered as "toxic" to the gene and are then referred as "genotoxic" (Ribeiro *et al*., 2003). In order to assess and prevent the presence of genotoxic agents in the environment, it is necessary to use sensitive indicators to detect the action of these compounds. There are plants that are considered ideal for the study of mutagenesis, both in laboratory and in situ monitoring, thus acting as bioindicators. Among the tests with the bioindicators, the micronucleus test with *Tradescantia sp*. (Trad-MCN) is considered the most sensitive to genotoxic agents (Ennever et al. 1998; Ribeiro et al. 2003; Saldiva et al. 2002). The genus *Tradescantia* has been used experimentally since the first studies related to gene activity with the action of compounds and chemical agents. The choice is due to a series of favorable genetic characteristics, among which stands out the fact that the cells of almost all parts of the plant provides excellent material for cytogenetic studies (Ma 1979; Grant 1998).

The influence of chemical and physical agents (especially radiation) on the frequency of mutations has been extensively studied through analysis of changes observed in *Tradescantia* (Carvalho 2005). Among the features that allow the detection of agents that affect the stability of the genome in *Tradescantia*, some were selected as indicators in bioassays for evaluating genetic toxicity: testing the pollen tube mitosis and cell color change to pink in hair stem (Trad-SH). The evaluation of genotoxicity in Tradescantia can also be made by detecting fragments or segments of DNA induced by genotoxic agents in the air, soil and water (Carvalho 2005), was developed as a cytogenetic test that is based on the micronucleus (Trad-MCN). Thus, the Trad-MCN test is

based on the formation of micronuclei, which are a result of chromosome fragmentation, visualized in the tetrad stage of stem cells grain (Ma 1979; Rodrigues et al. 1997). Micronuclei are counted and the frequencies in which they occur indicate the toxicity of the environment. The micronucleus test in *Tradescantia* (Trad-MCN) has long been used in environmental monitoring, which is due to its effectiveness in detecting chromosomal damage, the simplicity with which it is executed and its low financial cost of its methodology.

Sparrow et al. (1974) and Schairer et al. (1978) reported that the stamen hairs of *Tradescantia* were a very useful system for the detection of induced mutations and for evaluating the effects of ionizing radiations and chemical mutagens including environmental pollutants. There are two types of mutagens used to induce somatic mutation on *Tradescantia* hair stamen; chemicals and internal irradiation. As for the chemical mutagens, there are three types of dose-response relationships between *Tradescantia* stamen hairs and mutagens as reported by Shegemitsu (1987) which are a linear relationship (e.g. Ethyl methanesulfonate treatment), a sharp increase of the mutation frequency at low dose range and leveling off at high dose range (e.g. N-nitroso-N-ethylurea treatment) and a slow increase at low dose range and a sharp increase at higher dose range (e.g. N-nitroso-N-methylurea treatment).

Tradescantia shows a slow increase of mutation frequency at low dose range in N-nitroso-Ndimethylamine and N-nitroso-N-methylurea treatment may due to the enzymatic recovery of the methylated DNA with methyltransferase (Ishizaki et al. 1986). Razin and Riggs (1980) suggested that methylation of cytosine to 5-methylcytosine 213 plays an important role in gene regulation in relation to DNA replication and protein-DNA interaction. In the *Tradescantia* test system, ethylation is much more effective than methylation per unit of dose in the case of a small amount of chemical application. Therefore, the different efficiency of the mutation induction in *Tradescantia sp.* may reflect enzymatic activity such as methyltransferase which was reported by Ishizaki et al. (1986).

On the other hand, most of the investigations carried out on the effects of radiation on mutation induction in Tradescantia stamen hair cells using external irradiation such as gamma rays (Ichikawa 1971; Sparrow et al. 1972; Nauman et al. 1978; Underbrink et al. 1980; Ichikawa et al. 1981). In addition, differences in the efficiency of mutation induction by internal irradiation are dependent on the amount of incorporation and retention of the applied radionuclides and the labeled compounds in plants.

Tradescantia is also known as spiderworts and it is a genus of an estimated 71 species of perennial plants in the family Commelinaceae. They are weakly upright to scrambling plants and able to grow from 30 to 60 cm tall. Besides that, they are commonly found individually or in clumps in wooded areas and fields. The leaves are long, thin and bladelike from 3 to 45 cm long. The flowers color can be white, pink, purple, and the most commonly bright blue. Each flower of *Tradescantia sp.* consist three petals and six yellow anthers. However, there are limited numbers of species which can be found in Malaysia due to narrow adaptability to local climatic conditions.

Tradescantia sp. can perform two type of bioassays; *Tradescantia*-Micronucleus assay (Trad-MCN) and *Tradescantia*-Stamen Hair Mutation (Trad-SHM). These two bioassays are able to determine the clastogenicity and mutagenicity of various pollutants in water and air. However, the most used *Tradescantia sp.* as bio-indicator are *Tradescantia* Clone 4430 which are highly sensitive to chemical mutagents and *Tradescantia* Clone 02 which are highly sensitive to radiation (Shigemitsu 1987). The advantages of using *Tradescantia* sp. as bio-indicator, they are easy to handle and have high accuracy (Shigemitsu, 1987). There are many example of past research using Tradescantia species or experiment carried out by the researchers and scientists all over the world such as radioisotopes, radiation-contaminated substrates (Cebulska-Wasilewska 1992), high level background radiation from monazite sand and short-wave radio frequencies emitted by antennae and the bombardment of cosmic rays occurring in orbital flight (Sparrow et al. 1974).

Recent studies have shown that the sensitivity of the *Tradescantia* to the effects of radiation serve as a way of connecting gamma radiation dosage rates to which it was submitted to the mutational frequency from low dosage rates (Santos et al. 2005), using the micronuclei as a methodology. The answers acquired from the biosensor, *Tradescantia Pallida Purpurea*, results is an advantage, when using the present methodology, as one can observe a high quantity of mutational alterations in a short period of time, as such one is capable of anticipating the effects caused on the environment and as such on the human being, as a result of the level of occupation. It is a recommended, therefore, the use of this method for periodic monitoring, as the biosensor can be introduced into the environment, due to its ease of planting, propagation and excellent acclimatization, and may also be used in the prevention of radiological accidents.

The main objective of this study is to apply the high sensitivity of a botanical mutagenicity test (*Tradescantia* stamen-hair mutation bioassay), in "in situ" bioassays to determine the responses of five *Tradescantia sp.* available in Malaysia induced by the exposure to low levels of ionizing radiation.

MATERIALS AND METHODS

Plant Materials

Five species of *Tradescantia* (Figure 1) that will be used for this study were maintained in Agro Green System facility at Kompleks MINT Tech-Park Jalan Dengkil with climate control system (humidity and temperature), equipped with misting and sprinkler system. These varieties are diploid hybrids (2n = 12). Healthy plants with normal morphological characteristics were selected and unnecessary plant parts including dried leaves, diseased leaves, flowers and weeds were removed before irradiation.

Tradescantia species:
1. *Tradescantia Pallida Purpurea* / Purple Heart / Wondering Jew
2. *Tradescantia Spathaceae* Discolor
3. *Tradescantia rhoeo spathaceae* ground cover
4. *Tradescantia Spathaceae* Purple Heart Ground Cover
5. *Tradescantia (Rhoeo) spathaceae* Bermudensis Variegata (Pink)

Method of Irradiation

Irradiation of plant samples were carried out using Gamma Greenhouse located at Kompleks MINT Tech-Park Jalan Dengkil. It is an outdoor irradiation facility with Caesium-137 as the main radioactive source for chronic gamma irradiation. There were 15 rings available inside the Gamma Green House with different dose rate for each ring. Five different rings with low dose-rate were selected for chronic gamma irradiation; Ring 6 (0.7 Gy/h), ring 8 (0.4 Gy/h), ring 10 (0.3 Gy/h), ring 12 (0.2 Gy/h) and ring 14 (0.1 Gy/h). For each dose-rate, 10 replicates of each *Tradescantia species* in plastic pots were placed and organized on the respective coordinate of each ring. Irradiation time per day was 7 hours, starting from 10.00 am in the morning until 5.00 pm in the evening. As a control experiment, an equal number of pots from five species of *Tradescantia* were placed at 2 different locations; outside concrete shielding wall (Control 1:30 meters from the source) and inside Agro Green System (Control 2: 200 meters from the source).

Analysis of mutation

For scoring of somatic mutation (occurrence of pink cells induced on the stamen hairs), all the flowers that opened after 13 days of exposure to gamma radiation were collected. The methods used for scoring pink mutations in stamen hairs in the present study were similar to those described earlier by Ichikawa (1992). Flowers from each ring were collected and placed in an ice box to maintain their freshness and kept in a refrigerator before scoring is done. Ten flowers were selected from each ring for analysis of mutation. Six stamens from each flower were removed and placed on a slide followed by the addition with a few drops of distilled water, without any staining. The slide was covered with a cover slip and observed under stereo microscope with a white fluorescence lamp at magnification of 20 times. The number of stamen hairs with pink cells was recorded for each flower.

RESULTS AND DISCUSSION

In this preliminary study, mutation event is considered as the occurrence of pink hairs on the stamens of each flower that was scored. It was observed that only *Tradescantia Pallida Purpurea* and *Tradescantia Spathaceae* Discolor produced flowers after 13 days of exposure to chronic gamma irradiation. The flowers from each replicates were collected after 13 days of irradiation. Day-13 was chosen because according to Rodrigues et al. (1999), the set of daily data showing the highest mutation frequency during the period between 10-13 days after exposure. It represented the stamen hairs that were actively forming on the day of exposure and considered as valid for scoring. In other words, the day of maximum mutation frequency was the one in which the stamen hairs forming during exposure appeared in the opened flowers. However, out of 4 *Tradescantia spathaceae* varieties, only *Tradescantia Spathaceae* Discolor produced flowers. The other three species of *Tradescantia spathaceae*; *Tradescantia rhoeo spathaceae* ground cover, *Tradescantia Spathaceae* Purple Heart Ground Cover, *Tradescantia (Rhoeo) spathaceae* Bermudensis Variegata (Pink) did not produce any flower even though

they were kept outside Gamma Greenhouse for more than 30 days. It was observed that they are non-flowering *Tradescantia species*.

Observation under stereomicroscope, pink cells appeared on *Tradescantia Pallida Purpurea* stamen hairs after exposure to different doses of gamma rays (Figure 3a and 3b). Table 1 showed that the highest number of stamens with pink cells was recorded from ring 6, irradiated with the highest dose of 6.37Gy with 0.07Gy/h of dose rate. The lowest number of stamens with pink cells was recorded from ring 14 with an average of 0.57, irradiated with the lowest dose of 0.91Gy with 0.01Gy/h of dose rate. There were no pink cells observed on the stamens of *Tradescantia Pallida Purpurea* flowers collected from Control 1 and 2 (Figure 3e and 3f). Figure 2 showed the relationship between gamma radiation exposure and mutation rate of both *Tradescantia Pallida Purpurea* and *Tradescantia Spathaceae* Discolor. Unfortunately, there was no linear increase in mutation rate as the radiation dose increases. Nonlinear relationship between gamma radiation exposure and mutation rate may be due to several factors such as shielding and exposure continuity. Furthermore, during this experiment, Gamma Green House did not operate for about 4 to 5 days due to technical problem and the plants may undergo DNA repair mechanism during this period.

There were no pink cells observed on *Tradescantia Spathaceae* Discolor after exposure to different doses of gamma rays (Figure 3c and 3d). This situation is due to the difference in *Tradescantia species* sensitivity to the genetic effects of radiation and chemical agents (Ichikawa 1992; Rodrigues et al. 1997). According to Ichikawa (1992) and Sparrow et al. (1972), there were linear increases in mutation rate as the radiation dose increases. Similar negative results were observed with the control plants of both *Tradescantia Pallida Purpurea* and *Tradescantia Spathaceae* Discolor. The principal cells in this assay are the mitotic stamen hair cells developing in the young flower buds. After irradiation, the heterozygous dominant blue character of the stamen hair cell is prevented, resulting in the appearance of the recessive pink colour (Underbrink et al. 1973). The normal color of the stamen hair cells is blue and if either dominant allele for the blue color is deleted or mutated, the recessive allele determines that the daughter cells are pink. This color change also occurs by non-disjunction and mitotic crossing over.

For non-flowering plants or flowering plants whereby somatic mutation (occurrence of pink cells) induced on stamen hairs cannot be detected, there is an alternative method called Fluorescence *In Situ* Hybridazition (FISH) which can be used to analyze the biological mutational effects caused by low doses of ionizing radiation. FISH is a cytogenetic technique developed by Christoph Lengauer that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes in terms of chromosome aberration and chlorophyll mutation. However, FISH has its major disadvantages such as incomplete assessment of chromosomal complement and manual scoring and signal enumeration is difficult (due to truncation of nuclei on tissue sections and overlapping of nuclei). Furthermore, it requires the preparation of a probe, defined as sequences of single-stranded DNA, which needs to be labeled with fluorescent dyes. The process is complex due to the fact that it is necessary to tailor the probes to identify specific sequences of DNA and requires a powerful and expensive Fluorescent microscope or scanning laser microscope for analysis.

It was observed that low mutation frequency was observed on *Tradescantia Pallida Purpurea* in this preliminary experiment. This could be due to the low number of plants per treatment and low number of flowers scored for pink mutation. Both varieties produced flowers that could last only within a week, however pink mutation can only be detected on *Tradescantia Pallida Purpurea*. Therefore, evaluation should be carried out 13 days after exposure to radiation and scoring for pink mutation in the stamen hairs should be done daily. In this case, more number of flowers should be evaluated for mutation frequency.

The *Tradescantia* stamen hair system encompasses the cytogenetic and somatic potential to make it a useful tool for mutagenicity monitoring of exposure to low dose ionizing radiation. This plant is uniquely adapted to field exposures, hardy enough to tolerate a broad range of environmental conditions, and requires no elaborate sterile culture conditions. The preliminary data presented demonstrate the high sensitivity of the system to low dose ionizing radiation and the relatively short time from start of exposure to definition of results (< 4 weeks). In the absence of hard genetic evidence for extrapolation from plants to man, at least this system can become part of a system of tests which can provide early warning of the potential health hazard of exposure to low dose ionizing radiation.

CONCLUSION

In this study of radiobiology, the system based on cells of *Tradescantia* is being considered. The sensitivity of the *Tradescantia* stamen hairs has been used widely and has demonstrated the relationship between radiation exposure and frequency mutational observed at low doses, and their consequences for human health. This system carries the advantage of observing meaningful data in a short period of time, being able to meditate

effects on human health and to prevent possible accidents, when adopted as periodical monitoring. *Tradescantia Pallida Purpurea* exhibits a noticeable quantity of cell alteration in a short time following radiation exposure. Hence, the effects caused on the environment might be anticipated, and by extension on the human being, as a result of its occupation exposition level. *Tradescantia Pallida Purpurea* is a good alternative for environmental bio-monitoring, as it is an excellent alternative tool in the studies of the effects of ionizing radiation on the environment, therefore can be recommended as a biosensor plant for exposure to low dose gamma radiation

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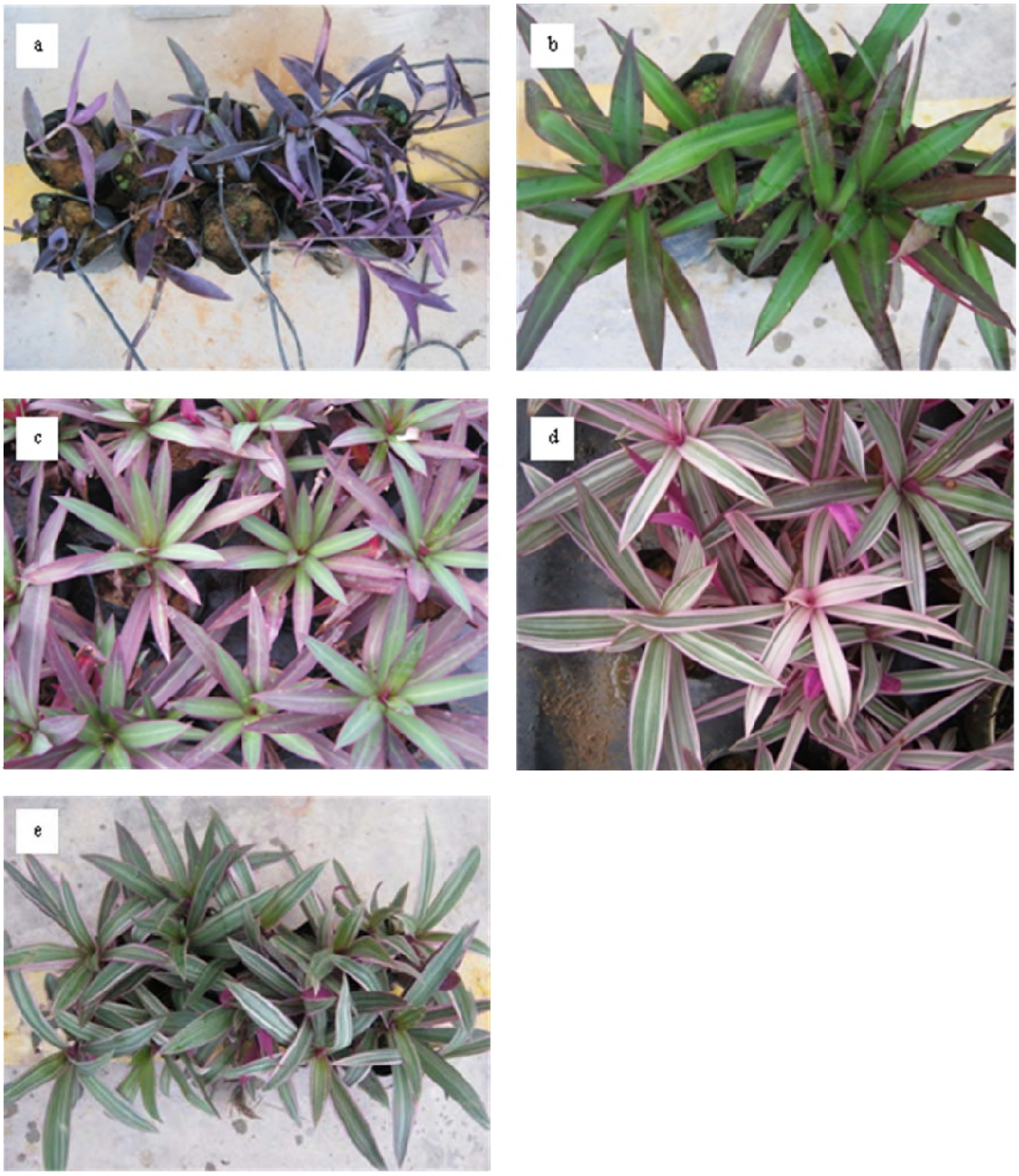

Figure 1. Five species of *Tradescantia* used for treatment with chronic gamma radiation: a) *Tradescantia Pallida Purpurea* / Purple Heart / Wondering Jew, b) *Tradescantia Spathaceae* Discolor, c) *Tradescantia rhoeo spathaceae* ground cover, d) *Tradescantia Spathaceae* Purple Heart Ground Cover and e) *Tradescantia (Rhoeo) spathaceae* Bermudensis Variegata (Pink)

Table 1. Scoring of pink mutation from *Tradescantia Pallida Purpurea* after 13 days of exposure to chronic gamma radiation

Treatment	Dose (Gy)	Dose-rate (Gy/h)	No of flowers scored	Average number of stamens with pink cells
Ring 6	6.37	0.07	10	4.60
Ring 8	3.64	0.04	10	4.37
Ring 10	2.73	0.03	10	2.96
Ring 12	1.82	0.02	10	3.27
Ring 14	0.91	0.01	10	0.57
Control 1	0	0	10	0
Control 2	0	0	10	0

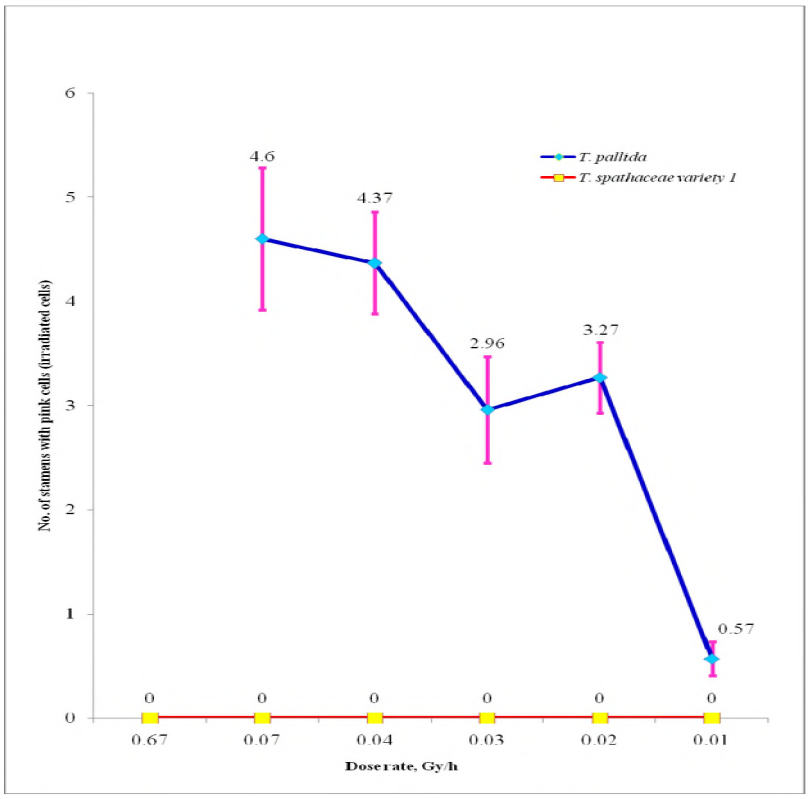

Figure 2. Average number of stamens with pink cells on *Tradescantia Pallida Purpurea* and *Tradescantia Spathaceae* Discolor ater 13 days of exposure to chronic gamma radiation.

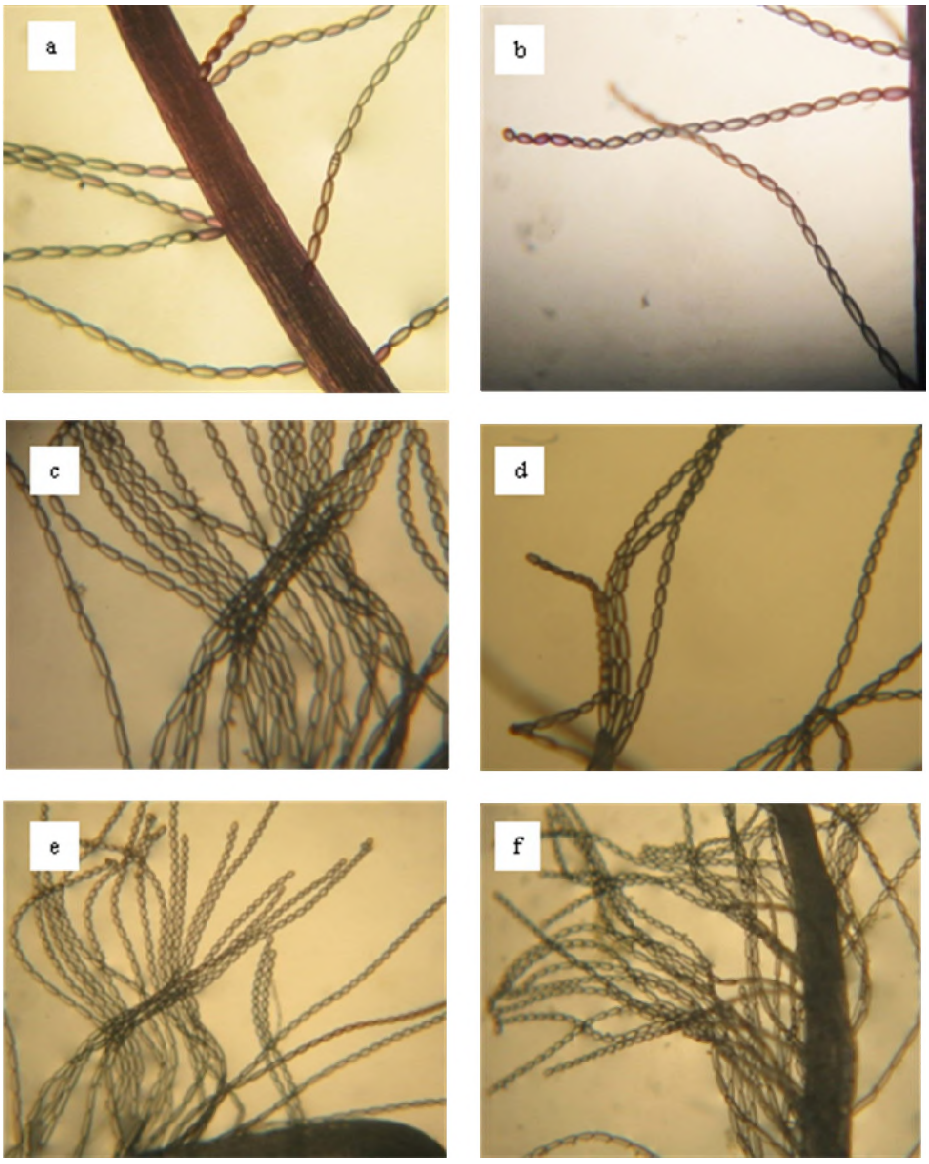

Figure 3. Observation of pink cells under stereomicroscope after 13 days of exposure to chronic gamma radiation and control plants of *Tradescantia Pallida Purpurea* and *Tradescantia Spathaceae* Discolor: a) Pink cells of *Tradescantia Pallida Purpurea* after treatment with high dose of 6.37 Gy at 0.07 Gy/h, b) Pink cells of *Tradescantia Pallida Purpurea* after treatment with low dose of 0.91 Gy at 0.01 Gy/h, c) Blue cells of *Tradescantia Spathaceae* Discolor after treatment with high dose of 6.37 Gy at 0.07 Gy/h, d) Blue cells of *Tradescantia Spathaceae* Discolor after treatment with low dose of 0.91 Gy at 0.01 Gy/h, e) Blue cells of *Tradescantia Pallida Purpurea*, from Control 1 and f) Blue cells of *Tradescantia Pallida Purpurea*, from Control 2.