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Radiosensitivity of Vetiver to Acute and Chronic Gamma Irradiation

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ABSTRACT

Nuclear technology has been widely applied in agriculture for crop improvement. In this study, the radiosensitivities of native Thai vetiver, *Chrysopogon zizaninoides*, the Kamphaeng Phet 2 and the Surat Thani ecotypes, and *Chrysopogon nemoralis*, the Ratchaburi ecotype, were investigated with acute and chronic gamma irradiation. Vetiver tillers of the Kamphaeng Phet 2 and the Surat Thani ecotypes were exposed to acute irradiation with gamma radiation from Cs-137 at doses of 0, 10, 20, 30, 40, 50, 60, 80 and 100 Gy. For chronic irradiation with a Co-60 source, the Kamphaeng Phet 2 tillers were exposed to 0, 65, 104, 116, 157, 182 and 205 Gy whereas the Ratchaburi tillers were exposed to 0, 63, 87, 127, 150, 173 and 213 Gy. The survival rate and growth performance measured by plant height, shoot and root dry weight of vetiver at 90 d after irradiation were recorded for median lethal dose ($LD_{50/90}$) and 50% growth reduction dose ($GR_{50/90}$) determination.

Gamma radiosensitivity differences were observed between the irradiation methods and between the vetiver ecotypes. Acute irradiation caused higher radiosensitivity of vetiver than chronic irradiation. The $LD_{50/90}$ values of the Kamphaeng Phet 2 ecotype to acute and chronic irradiation were 82 and 100 Gy, respectively. In general, the survival rate and growth of vetiver decreased with an increase in the gamma radiation doses. However, chronic irradiation of the Kamphaeng Phet 2 ecotype at 65 Gy gave higher shoot and root dry weights than the control treatment (0 Gy). The $GR_{50/90}$ values of the Kamphaeng Phet 2 and the Surat Thani ecotypes to acute irradiation were 48 and 75 Gy for plant height and were 30 and 43 Gy for total dry weight, respectively. For chronic irradiation, the $GR_{50/90}$ values of the Kamphaeng Phet 2 and the Ratchaburi ecotypes were 118 and 109 Gy for plant height and 121 and 67 Gy for total dry weight, respectively. This information will be useful for radiation-induced mutations in vetiver research.

Keywords: acute irradiation, chronic irradiation, lethal dose, growth reduction dose

INTRODUCTION

Nuclear technology has been greatly utilized for the global benefit of mankind, not only in the field of medical science, but also in agriculture for food preservation, pest and disease control, soil fertility and plant breeding (IAEA, 2010). Nuclear technology involving the use of ionizing radiation for mutation induction has been successfully applied worldwide in developing new varieties of crop plants. Some mutations might be beneficial and have high economical value.

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A wide range of plant characteristics have been improved through nuclear technology including: yield, flowering, maturity, quality and tolerance to biotic and abiotic stress. Several researchers have successfully developed new plant varieties using this technique. Gustafsson *et al.* (1971) revealed that seven barley varieties, originating from mutations induced by X-rays, have been officially approved in Sweden. The new varieties were superior to their mother varieties in some characteristics like stiffness of straw, early maturity and high protein content. Wongpiyasatid *et al.* (1999) reported an improvement in mungbean resistance to powdery mildew, *Cercospora* leaf spot and cowpea weevil through gamma radiation-induced mutation. Khatri *et al.* (2005) created three high grain yielding and early maturing mutants of *Brassica juncea* L. by gamma radiation and EMS (ethyl methanesulfonate) treatments. In addition, Singh and Datta (2010) have recently shown that low doses of gamma radiation could potentially be exploited to improve plant vigor and grain productivity in wheat.

Vetiver has been used in many countries and shown to be a simple and economical method for soil and water conservation especially for slope stabilization and erosion protection. A vetiver plantation could improve soil fertility and preserve soil moisture.(Roongtanakiat *et al*., 2000). Moreover, it was also suitable for phytoremediation applications (Roongtanakiat, 2009). Many researchers demonstrated that vetiver could be used for decontamination of several pollutants such as: heavy metals (Singh *et al*., 2007; Roongtanakiat 2009) petroleum (Brandt *et al*., 2006), 2,4,6-trinitrotoluene (Markis *et al.*, 2007a, 2007b), phenol (Singh *et al.*, 2007) and radioactive nuclides (Singh *et al*., 2008; Roongtanakiat *et al.*, 2010).

The purpose of this study was to investigate the effect of gamma radiation on the survival rate, tillering, plant height, shoot dry weight, root dry weight and total dry weight of Thai vetiver after acute and chronic irradiation. Estimates of the 50% lethal dose (LD_{50}) and 50% growth reduction dose (GR_{50}) were used as indicators for vetiver radiosensitivity. The results obtained from this study would be useful for large scale mutation breeding work.

MATERIALS AND METHODS

The study was carried out at the Gamma Irradiation Service and Nuclear Technology Research Center, Kasetsart University. The native Thai vetiver, *Chrysopogon zizaninoides*, the Kamphaeng Phet 2 and the Surat Thani ecotypes, and *Chrysopogon nemoralis*, the Ratchaburi ecotype, were used as test plants. Twenty vetiver tillers of each ecotype were prepared for each irradiation method.

In acute irradiation, tillers of the Kaphaeng Phet 2 and the Surat Thani ecotypes were irradiated with gamma radiation from Cs-137 using a research irradiator (Mark I-30; J.L. Shepherd & Associates; San Fernando, CA, USA) at doses of 0 (control), 10, 20, 30, 40, 50, 60, 80 and 100 Gy. For chronic irradiation, the Kamphaeng Phet 2 and the Ratchaburi tillers were planted in plastic bags containing 30 g of potting soil one week before irradiation as the irradiation time could be as long as two weeks. Vetiver tillers were irradiated in a gamma room at a two meter distance from a Co-60 source with seven different periods of irradiation time. The process resulted in the Kamphaeng Phet 2 tillers receiving gamma radiation doses of 0, 65, 104, 116, 157, 182 and 205 Gy, while the Ratchaburi tillers received 0, 63, 87, 127, 150, 173 and 213 Gy, respectively.

After irradiation, the irradiated tillers were planted in clay pots containing 8 kg of potting soil with four tillers per pot. Watering was carried out daily and was uniform for all the treatments; weeding was done as necessary. The number of survival tillers, plant height and the number of new tillers were recorded before harvesting at 90 d after planting. Vetiver shoots (aerial part) and roots were separated and washed until free from the soil. Afterward, these plant parts were placed in brown paper bags and oven dried at 70 ^oC until a constant weight was obtained for dry matter weight determination. The data were calculated as a percentage of the control. The 50% lethal dose (LD_{50}) and 50% growth reduction dose (GR_{50}) were estimated using regression analysis.

RESULTS

Acute irradiation

Tillering of vetiver was determined as the number of new tillers at 90 d after planting. The Surat Thani vetiver ecotype had better tillering than the Kamphaeng Phet 2 ecotype (Table 1). The number of new tillers and the survival percentage of irradiated vetiver decreased as the gamma dose increased and doses of 80 and 100 Gy inhibited tillering of both vetiver ecotypes. The correlation between survival percentage and gamma dose is shown in Figure 1. $LD_{50/90}$ values (the gamma radiation dose expected to cause death in 50% of an exposed population within 90 d) estimated from the linear regression equation were 82 Gy for the Kamphaeng Phet 2 ecotype and 73 Gy for the Surat Thani ecotype.

In general, increasing the gamma dose caused a negative effect on plant development as indicated by the reduction in plant height, shoot dry weight and root dry weight of both vetiver ecotypes (Figures 2–3). The $GR_{50/90}$ values based on the plant height of the Kamphaeng Phet 2 and the Surat Thani ecotypes were 75 and 40 Gy, respectively. The results indicated that the Surat Thani ecotype had higher radiosensitivity than the Kamphaeng Phet 2 ecotype. A similar trend was obtained from the vetiver biomass data. The $GR_{50/90}$ values of the Kamphaeng Phet 2 ecotype for shoot dry weight, root dry weight and total dry weight were 40, 41 and 43 Gy, respectively, while the $GR_{50/90}$ value of the Surat Thani ecotype for shoot dry weight was 30 Gy, which was equal to that for root dry weight and total dry weight.

Chronic irradiation

Chronic gamma irradiation caused adverse and inhibitory effects on vetiver as shown in Table 2. However, the number of new tillers of the Kamphaeng Phet 2 ecotype after gamma irradiationd with 63 Gy was higher than in the non-irradiated plants and then decreased when the radiation doses were increased further. There

Table 1 Total number of new tillers, survival rate (% of control) of the Kamphaeng Phet 2 and the Surat Thani vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 d after planting.

Gamma dose (Gy)	Kamphaeng Phet 2 ecotype		Surat Thani ecotype	
	No. of new tillers	Survival (% of control)	No. of new tillers	Survival (% of control)
0	27	100	53	100
10	24	80.0	51	100
20	18	80.0	23	94.1
30	9	80.0	47	88.2
40	9	73.3	29	76.5
50	4	73.3	14	64.7
60	2	66.7	5	52.9
80	0	53.3	0	47.1
100	0	33.3	0	47.1

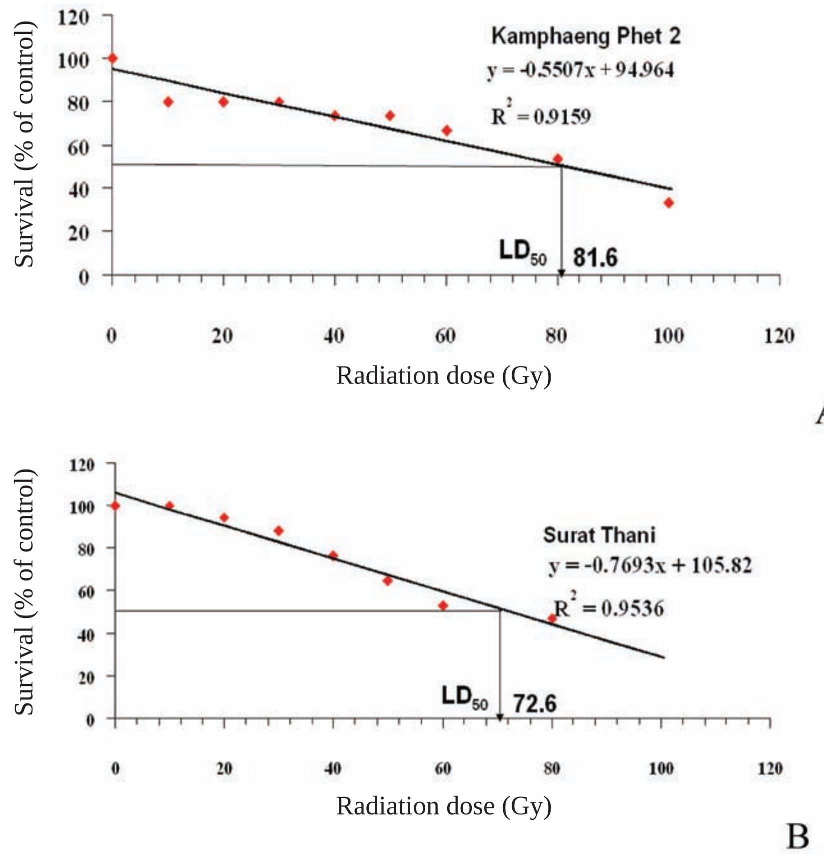

Figure 1 Data and regression equation for survival rate (as % of control) of the Kamphaeng Phet 2 (A) and the Surat Thani (B) vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 d after planting. (LD_{50} = 50% lethal dose.)

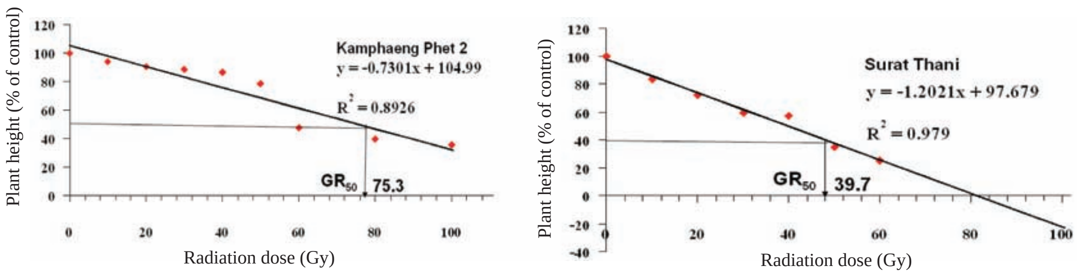

Figure 2 Data and regression equation for average plant height (as % of control) of the Kamphaeng Phet 2 (left) and the Surat Thani (right) vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 d after planting. (GR_{50} = 50% growth reduction dose.)

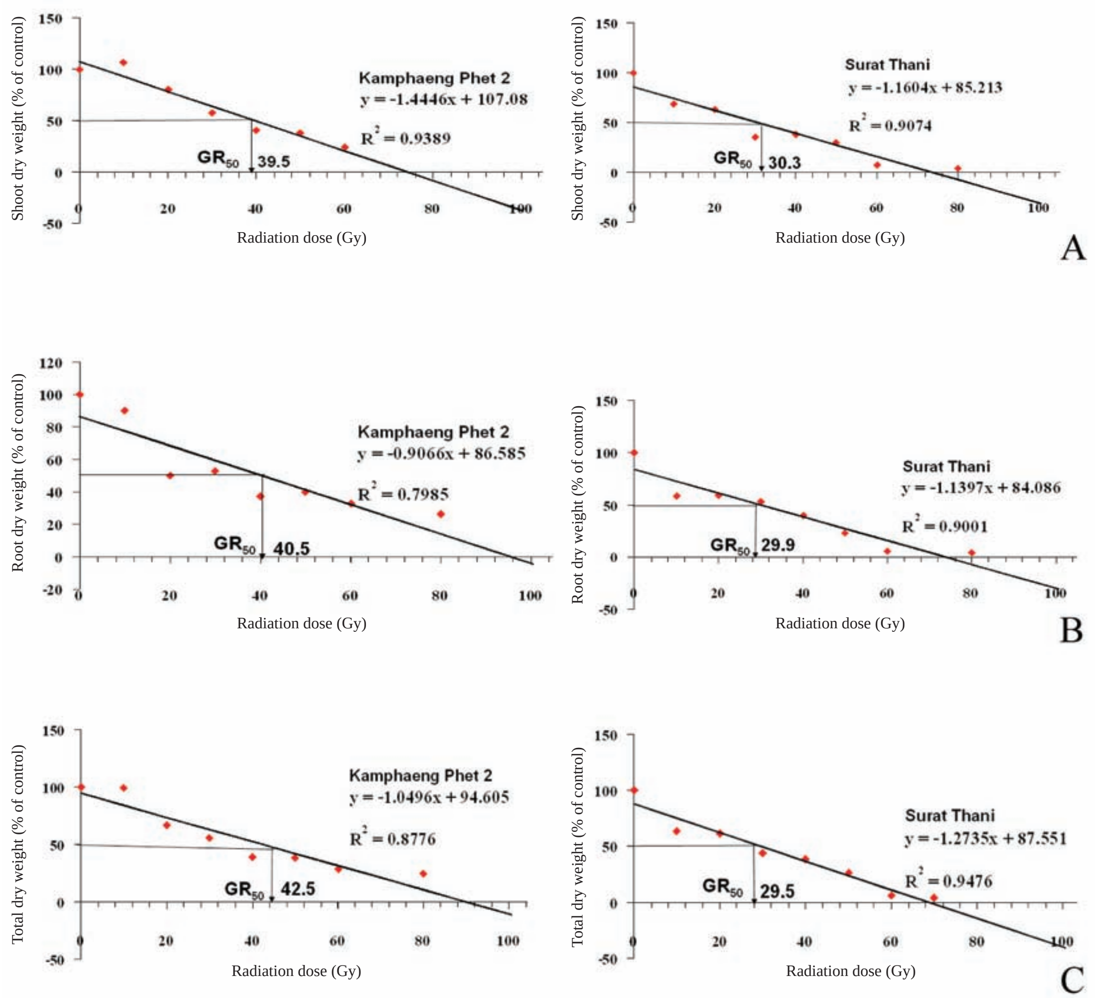

Figure 3 Data and regression equation for average shoot dry weight (A) , root dry weight (B) and total dry weight (C) (as % of control) of the Kamphaeng Phet 2 (left) and the Surat Thani (right) vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 d after planting. (GR_{50} = 50% growth reduction dose.)

Table 2 Total number of new tillers, survival rate of the Kamphaeng Phet 2 and the Ratchaburi vetiver ecotypes chronically exposed to different doses of gamma radiation at 90 d after planting.

Gamma dose (Gy)	Kamphaeng Phet 2 ecotype		Gamma dose (Gy)	Ratchaburi ecotype	
	No. of new tillers	Survival (% of control)		No. of new tillers	Survival (% of control)
0	19	100	0	153	100
65	27	87.5	63	62	100
104	21	62.5	87	1	100
116	19	37.5	127	1	100
157	0	0	150	0	100
182	0	0	173	0	100
205	0	0	213	0	100

was no tillering and no plant survival for the Kamphaeng Phet 2 ecotype at 157 Gy and above. Therefore, the survival rate data at 0–157 Gy were used for regression analysis, producing the $LD_{50/90}$ value of 100 Gy (Figure 4).

Tillering of the Ratchaburi ecotype was affected by radiation similarly to the Kamphaeng Phet 2 ecotype, with no tillering at doses of 150 Gy and above. Growth development stopped completely at these exposures, but the plants still survived and remained in almost the same state as when they were irradiated. Therefore, the survival rate for each treatments was 100%; and the $LD_{50/90}$ values of the Ratchaburi ecotype could not be estimated.

Figures 5 and 6 show the progressive reduction in plant height, shoot dry weight root dry weight and total dry weight of both vetiver ecotypes with increasing doses of gamma radiation. However, the 63 Gy gamma-irradiated Kamphaeng Phet 2 vetiver plants had higher shoot dry weight and root dry weight than those in the control treatment. The $GR_{50/90}$ values for plant height of the Kamphaeng Phet 2 and Ratchaburi ecotypes were 118 and 109 Gy, respectively. In relation to the biomass, the $GR_{50/90}$ values for shoot dry weight, root dry weight and total dry weight of the Kamphaeng Phet 2 ecotype were 120, 125 and 121 Gy, respectively, and they were 68, 66 and 67 Gy, respectively, for the Ratchaburi

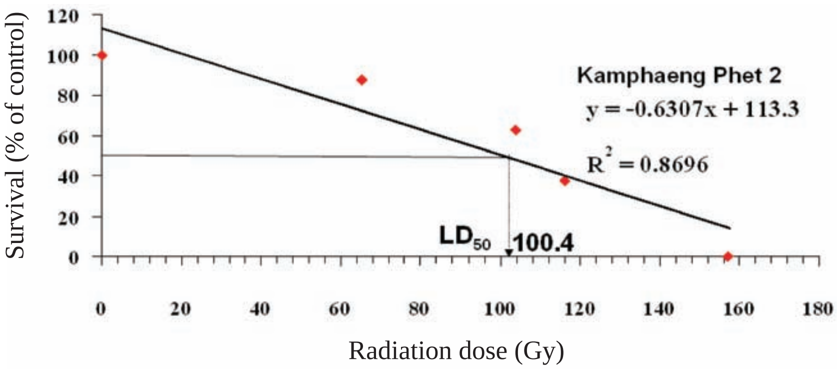

Figure 4 Data and regression equation for survival rate (% of control) of the Kamphaeng Phet 2 vetiver ecotype chronically exposed to different doses of gamma radiation at 90 d after planting. (LD_{50} = 50% lethal dose.)

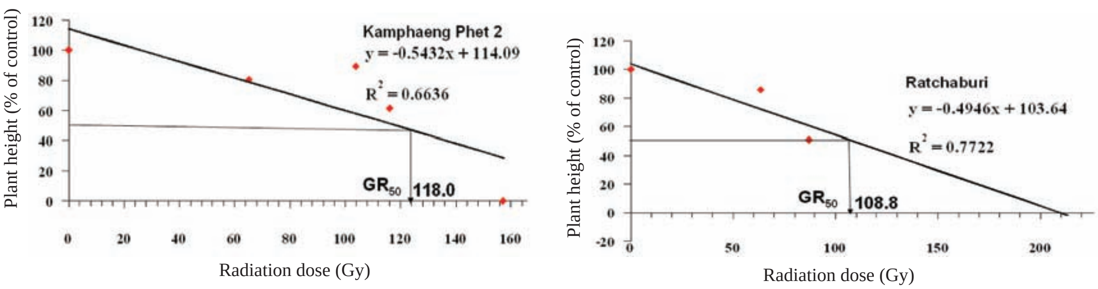

Figure 5 Data and regression equation for average plant height (% of control) of the Kamphaeng Phet 2 (left) and the Ratchaburi (right) vetiver ecotypes chronically exposed to different doses of gamma radiation at 90 d after planting. (GR_{50} = 50% growth reduction dose.)

ecotype. The results indicated that with chronic irradiation, the Ratchaburi ecotype was onefold more sensitive to gamma radiation than the Kamphaeng Phet 2 ecotype.

DISCUSSION

After vetiver tillers were acutely and chronically irradiated with gamma radiation at various doses, their growth performance (tillering, plant height, shoot dry weight and root dry weight) was inversely correlated with the gamma dose and was inhibited at the higher doses. This might have been due to blocking the cellular DNA; which caused plant growth to stop or slow (Omar, *et al.*, 2008). The effect of gamma irradiation is referred to as radiation injury and may be manifested in several forms including a reduction in sprouting ability, survival, plant height and the number of plant organs (Nwachukwu *et al.*, 2009). The

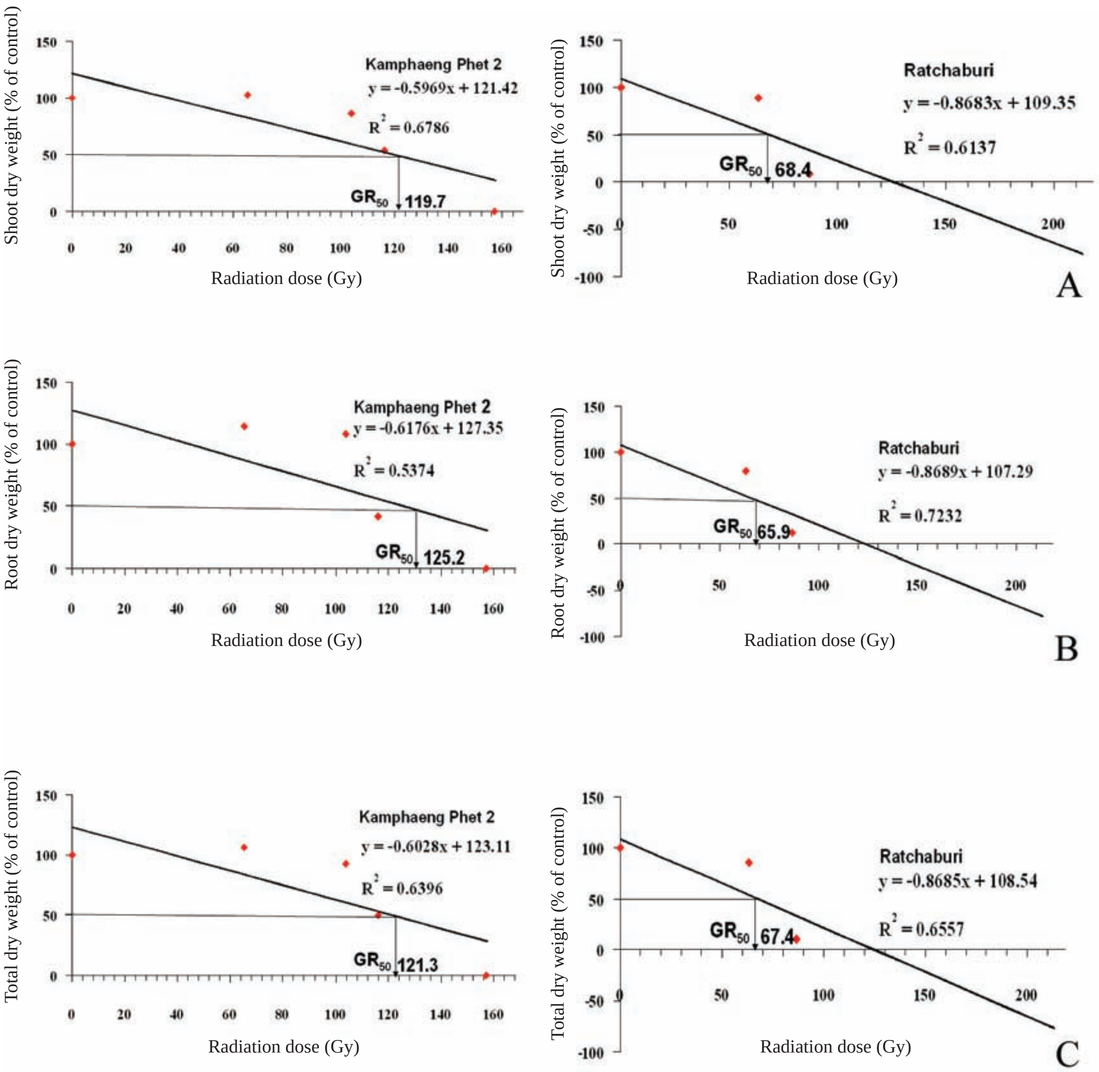

Figure 6 Data (% of control) and regression equation for average shoot dry weight (A) , root dry weight (B) and total dry weight (C) of the Kamphaeng Phet 2 (left) and the Ratchaburi (right) vetiver ecotypes chronically exposed to different doses of gamma radiation at 90 d after planting. (GR_{50} = 50% growth reduction dose.)

observed growth reduction caused by gamma radiation was consistent with the findings of Kon *et al.* (2007) who revealed that germination, plant height, survival, root length, root dry weight and shoot dry weight of long bean decreased with increasing doses of gamma rays. Similar results were observed by Ramachandran and Goud (1983) in safflower, Benerji and by Datta (1992) and Lamseejan *et al.* (2000) in Chrysanthemum, Nwachukwu *et al*. (2009) in yam, Omar *et al.* (2008) in chili and Joompuk *et al.* (2009) in torch ginger.

Even though growth reduction was the main effect of gamma radiation in vetiver, it was noticed that the Kamphaeng Phet 2 ecotype when chronically gamma irradiated at 63 Gy had higher shoot and root dry weight than non-irradiated plants. This information supported the finding of Veeresh *et al*. (1995) who observed an increase in the shoot fresh weight of winged bean at lower doses. Further, Majeed and Muhammad (2010) indicated that the fresh and dry weight of *Lepidium sativum* L. increased due to gamma radiation at 20 Gy, then gradually decreased when the dose increased. A growth stimulation effect has been found with both acute and chronic irradiation in several plants (Bari, 1971). It was pronounced at low doses of chronic irradiation in durum and bread wheat (Donini *et al*., 1964), in grapevine, *Vitis vinifera,* (Charbaji and Nabulsi, 1999), in pea, *Pisum sativum*, (Zaka *et al.*, 2004) and in hard wheat (Milki and Marouani, 2010).

Pitirmovae (1979) reported that the stimulation of plant growth caused by low doses of gamma irradiation may have been due to stimulation of cell division or cell elongation, producing an alteration of the metabolic process that affects the synthesis of phytohormones or nucleic acids (cited in Nassar *et al.,* 2004). In the present study, the improvement in shoot and root dry weight of the Kamphaeng Phet 2 ecotype following low dose chronic irradiation will help vetiver to be more efficient in phytoremediation and carbon sequestration applications by increasing pollutant total uptake.

The acute irradiation caused more damage to the vetiver plant than the chronic irradiation as indicated by LD_{50} and GR_{50} values. For example, the $LD_{50/90}$ values of the Kamphaeng Phet 2 ecotype to acute and chronic irradiation were 82 and 100 Gy; its GR_{50} values for plant height were 75 and 118 Gy, and those for total dry weight were 43 and 121 Gy, respectively. The results agreed well with the observation that acute exposure is more injurious than chronic exposure (Sparrow and Woodwell, 1962; Amino, 1989; Tangpong *et al*., 2009). The recovery mechanism from radiation damage during a long, chronic, irradiation process could be used to explain this phenomenon.

When the data on vetiver survival and growth response to gamma radiation were plotted against dosages (Figures 1–6), some insubstantial data at high doses caused by the inhibiting effect were discarded. The determination coefficient (r^2) of regression equations ranged between 0.74 and 0.95 for acute irradiation and between 0.57 and 0.87 for chronic irradiation. The low correlation of the chronic experiment might have been due to the influence of the external and internal environment of the plant as mentioned by Sparrow and Gunckel (1956) (cited in Bari, 1971)

Considering the LD_{50} and GR_{50} values as the criteria of radiosensitivity, they varied not only between irradiation methods but also varied between vetiver varieties or ecotypes. Considering acute irradiation with two vetiver (*Chrysopogon zizanioides)* ecotypes, the Kamphaeng Phet 2 ecotype (LD_{50} = 82 Gy and GR_{50} = 41–75 Gy) had higher resistance to gamma radiation than the Surat Thani ecotype (LD_{50} = 73 Gy and GR_{50} = 30–40 Gy). For chronic irradiation, the Kamphaeng Phet 2 ecotype still showed good resistance to radiation with LD_{50} = 100 Gy and GR_{50} = 118–121 Gy, which were better than for the Ratchaburi ecotype (*Chrysopogon nemoralis*) with GR_{50} =

67–109 Gy, while its LD_{50} could not be determined since survival was 100% for all treatments. The results clearly indicated that different varieties were differently sensitive to gamma radiation as mentioned by Datta (1992). Compared with other plant species, the GR_{50} value was 21 Gy for the canes of the Amasya grape variety (Semun *et al.*, 2003) and 16.65 Gy for the young plantlets of torch ginger (Jompuk *et al.*, 2009), which showed much lower sensitivity than vetiver. This indicated that vetiver is rather resistant to radiation and could be suitable for phytoremediation by radionuclide application.

CONCLUSION

The experimental results indicated that vetiver radiosensitivity depends on both the irradiation method and the vetiver variety. In general, increasing gamma doses from both acute and chronic irradiation levels decreased the survival rate and growth performance of vetiver. The LD_{50} and GR_{50} values of vetiver ranged between 73–100 Gy and 30–121 Gy, respectively. For environmental application, a vetiver variety with high biomass is usually needed. Thus, the recommended gamma dose treatment for efficient induced mutation in vetiver should not be more than the GR_{50} value on a dry biomass basis for each vetiver ecotype.

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