Nuclear technology for vetiver variety improvement: Gamma radiosensitivity studies

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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 varieties were investigated with acute and chronic gamma irradiation. Vetiver tillers of the Kamphaeng Phet 2 and the Surat Thani ecotypes were acute irradiated with gamma radiation from Cs-137 at the doses of 0, 10, 20, 30, 40, 50, 60, 80 and 100 Gy. For chronic irradiation with 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 as plant height, shoot and root dry weight of vetiver at 90 days 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 irradiations were 82 and 100 Gy, respectively. In general, survival rate and growth of vetiver were decreased with an increase of 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 $GR_{50/90}$ of the Kamphaeng Phet 2 and the Surat Thani ecotypes to acute irradiation were 48 and 75 Gy for plant height, and they were 30 and 43 Gy for total dry weight, respectively. For chronic irradiation, the $GR_{50/90}$ of the Kamphaeng Phet 2 and the Ratchaburi ecotypes were 118 and 109 Gy for plant height and they were 121 and 67 Gy for total dry weight, respectively. This information will be useful in breeding of vetiver for environmental application.

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

Introduction

Nuclear technology has been greatly utilized for the global benefit of mankind. It is not only being used in the field of medical science, but also in agriculture for food preservation, pest and disease control, soil fertility and plant breeding. Nuclear technology involved the use of ionizing radiation for mutation induction has been successfully applied worldwide in developing new varieties of crop plants. Several mutations might be beneficial and have higher economical values. 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 been successfully developed new plant varieties using this technique. Gustafsson *et al.* (1971) revealed that seven barley varieties, originating from X-rays induced mutations, have been officially approved in Sweden. The new varieties were superior to their mother varieties in some characteristics like stiffness of straw, earliness in maturity and high protein content. Wongpiyasatid *et al.* (1999) reported an improvement of mungbean for 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 mutant of *Brassica juncea* L. by gamma radiation and EMS (ethyl methanesulfonate). In addition, Singh and Datta (2010) have recently shown that low dose of gamma radiation could be potential exploited for improving the 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 slope stabilization and erosion protection. Vetiver plantation could improve soil fertility and preserve soil moisture. Moreover, it was also suitable for phytoremediation application (Roongtanakiat, 2009). Many researchers demonstrated that vetiver could be used for decontamination of several pollutants such as; heavy metals (Roongtanakiat 2009; Singh *et al*., 2007) 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 and Roongtanakiat *et al.,* 2010).

The purpose of this study was to investigate the effect of gamma radiation on survival rate, tillering, plant height, shoot dry weight, root dry weight and total dry weight of Thai vetiver on acute and chronic irradiation. The 50% lethal dose (LD_{50}) and 50% growth reduction dose (GR_{50}) would be estimated and 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 Kampheng Phet 2 and the Surat Thani ecotypes, and *Chrysopogon nemoralis*, the Ratchaburi ecotype were tested plants. Twenty of vetiver tillers of each ecotype were prepared for each irradiation methods.

In acute irradiation, tillers of the Kaphaeng Phet 2 and the Surathani were irradiated with gamma radiation from Cs-137 using Mark I Research Irradiator at the doses of 0, 10, 20, 30, 40, 50, 60, 80 and 100 Gy. For chronic irradiation, the Kampheng 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 at a two meter distance from Co-60 source with seven different periods of irradiation time in a gamma room. The process resulted in the Kamphaeng Phet 2 tillers received 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 4 tillers per pot. Watering was daily done uniformly for all the treatments and weeding was done as necessary. Number of survival tillers, plant height and number of new tillers were recorded before harvesting at 90 days 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 ^{o}C until the constant weight was obtained for dry matter weight determination. The data were calculated as percent of 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 number for new tiller at 90 days after planting. The Surat Thani vetiver ecotype had better tillering than the Kamphaeng Phet 2 ecotype (Table 1). Number of new tiller and survival percentage of irradiated vetiver decreased as gamma dose increased and the 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 50 percent of an exposed population within 90 days, estimated from linear regression equation were 82 Gy for the Kampheng Phet 2 ecotype and 73 Gy for the Surat Thani ecotype.

Table 1. Average of new tillers number, 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 days 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	51	100
20	18	80	23	94.1
30	9	80	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

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

Chronic irradiation

Chronic gamma irradiation caused adverse and inhibitory effect on vetiver as shown in Table 2. However, the average number of new tillers of the 63 Gy gamma irradiated Kamphaeng Phet 2 was higher than the unirradiated one, then decreased when radiation doses got higher. There were no tillering and plant survival of the Kamphaeng Phet 2 ecotype at 157 Gy and above. Therefore, the survival rate data at 0-157 Gy were used for regression analysis, giving the $LD_{50/90}$ at 100 Gy (Figure 4).

Tillering of the Ratchaburi ecotype was affected by radiation, similarly to that of the Kamphaeng Phet 2 ecotype; there was no tillering at the dose of 150 Gy and above. Their growth development completely stopped at these exposures, but the plants still survived and remained at almost the same state at which they were irradiated. Therefore, the survivals of all treatments were 100%; and the $LD_{50/90}$ of the Ratchaburi ecotype could not be estimated.

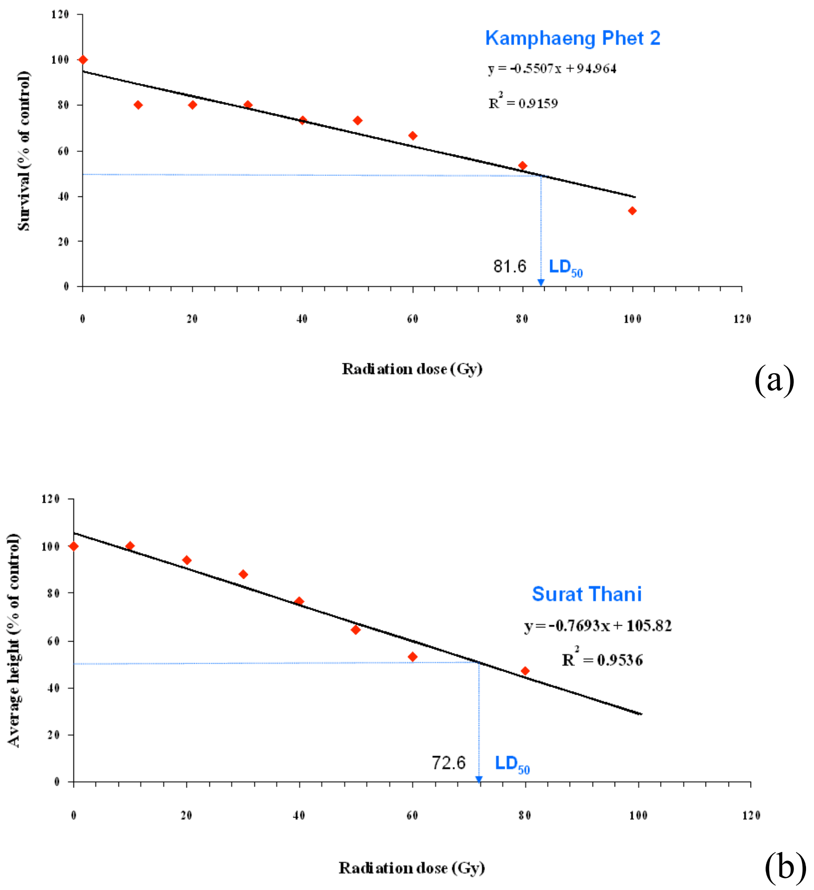

Figure 1. Survival rate (express as % of control) of the Kamphaeng Phet 2 (a) and the Surat Thani (b) vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 days after planting

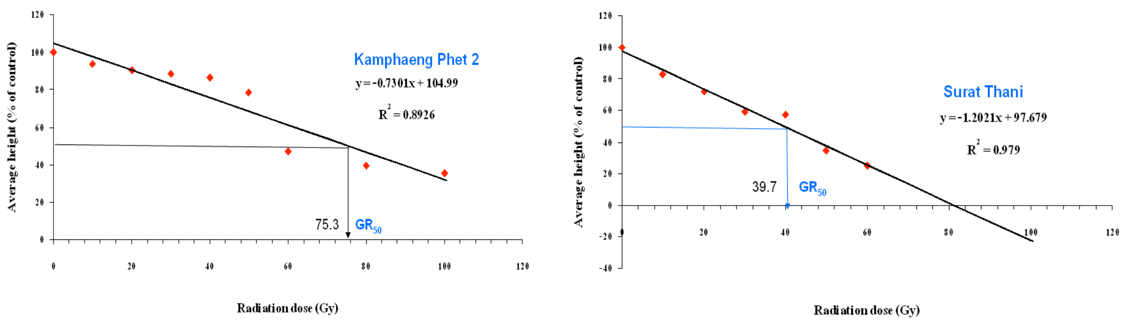

Figure 2. Average plant height (express as % of control) of the Kamphaeng Phet 2 (left) and the Surat Thani (right) vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 days after planting

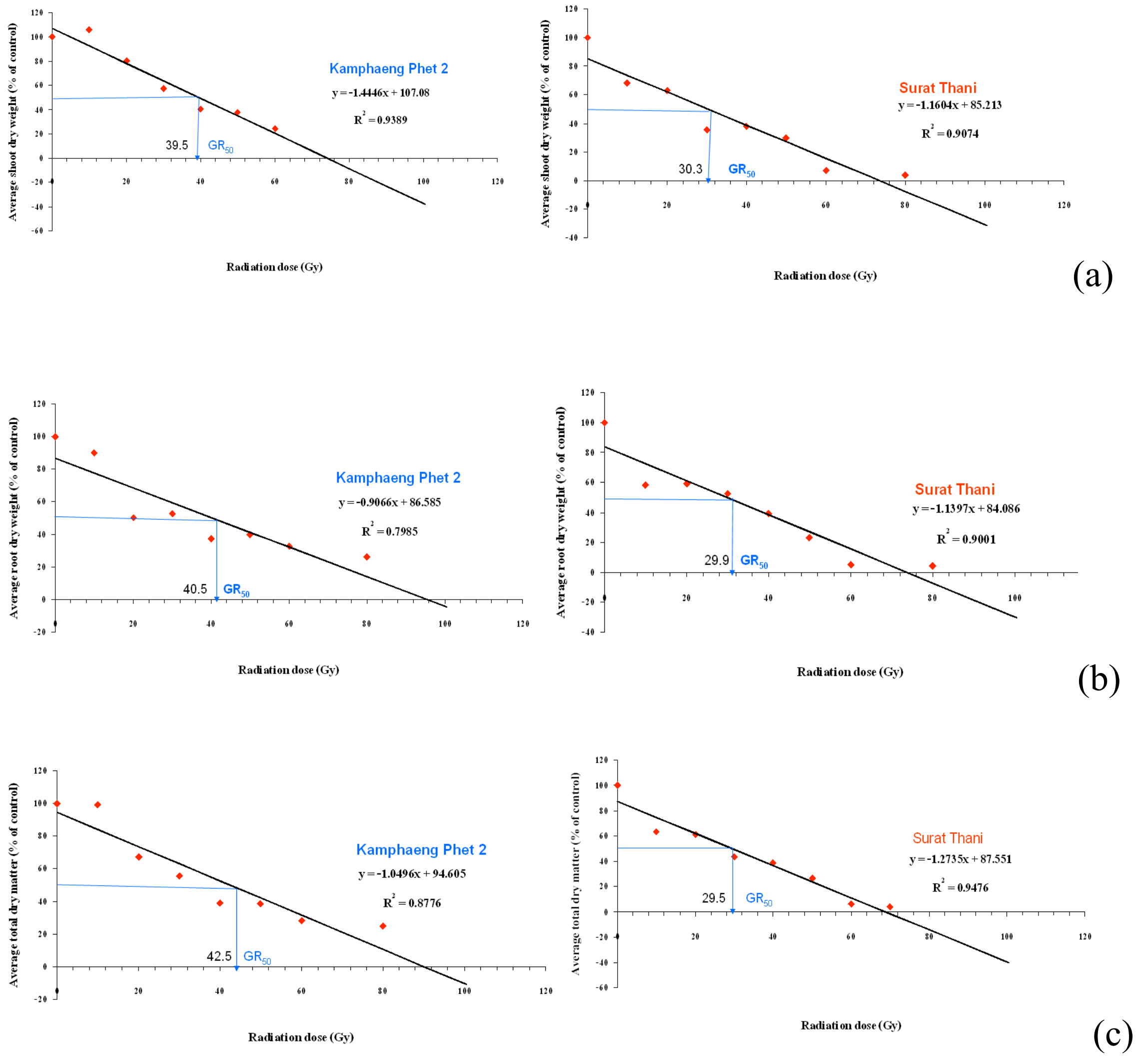

Figure 3. Average shoot dry weight (a) , root dry weight (b) and total dry weight (c) (express as % of control) of the Kamphaeng Phet 2 (left) and the Surat Thani (right) vetiver ecotypes acutely exposed to different doses of gamma radiation at 90 days after planting

Figures 5 and 6 showed progressive reduction in plant height, shoot dry weight and root dry weight of both vetiver ecotypes with increasing doses of gamma radiation. However, the 63 Gy gamma irradiated Kamphaeng Phet 2 vetiver plant 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 ecotype. The result indicated that with chronic irradiation the Ratchaburi ecotype was one-fold more sensitive to gamma radiation than the Kamphaeng Phet 2.

Table 2. Average 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 days after planting

Gamma dose (Gy)	Kampheng Phet 2 ecotype		Gamma dose (Gy)	Surat Thani ecotype	
	No. of new tillers	Survival (% of control)		No. of new tillers	Survival (% of control)
0	19	100	0	153	100
63	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

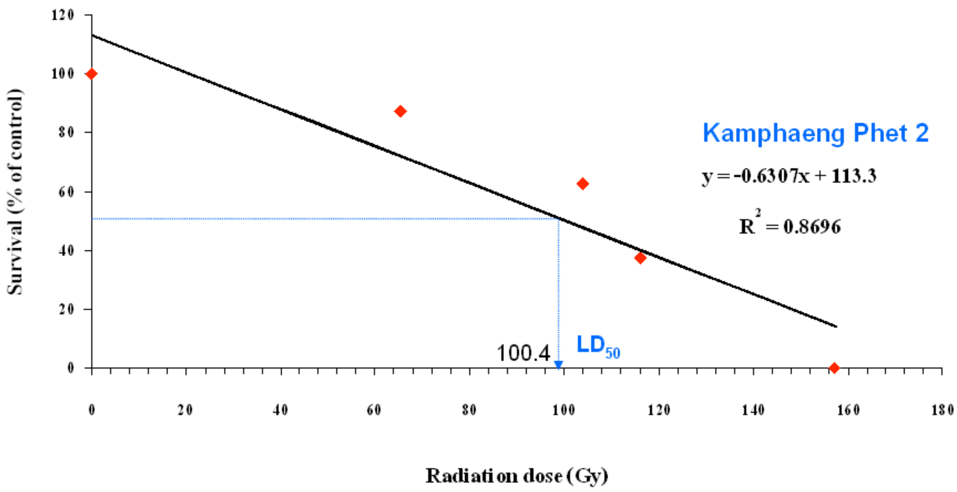

Figure 4. Survival rate (% of control) of the Kamphaeng Phet 2 vetiver ecotype acutely exposed to different doses of gamma radiation at 90 days after planting

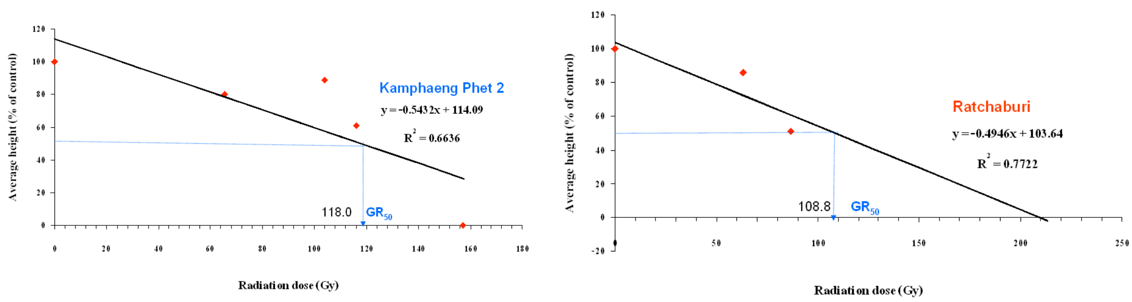

Figure 5. Average plant height (% of control) of the Kamphaeng Phet 2 (left) and the Ratchaburi (right) vetiver ecotypes chronically exposed to different doses of gamma radiation at 90 days after planting

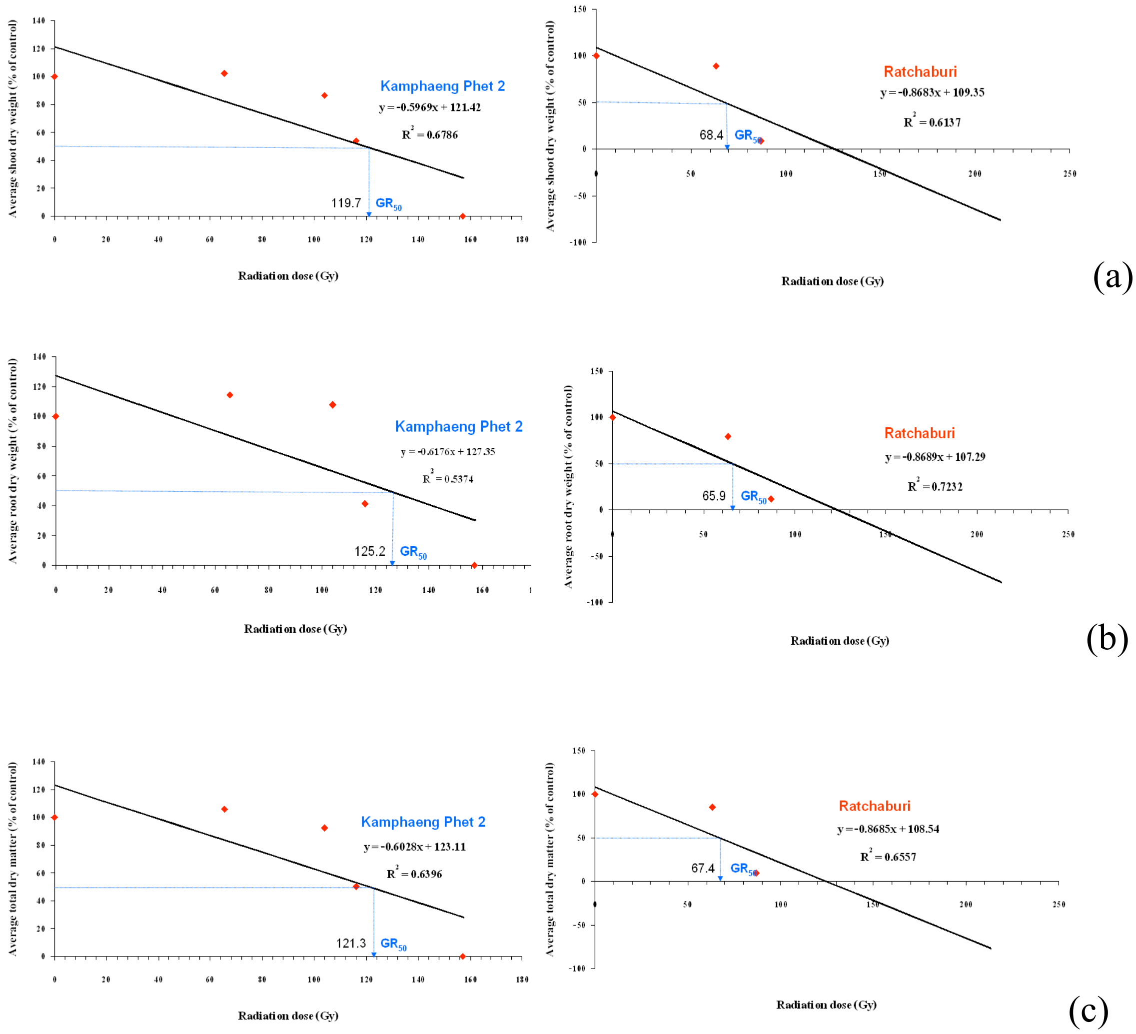

Figure 6. Average shoot dry weight (a) , root dry weight (b) and total dry weight (c) (% of control) of the Kamphaeng Phet 2 (left) and the Ratchaburi (right) vetiver ecotypes chronically exposed to different doses of gamma radiation at 90 days after planting

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) showed inversely correlated with gamma dose, and was inhibited at the higher doses. This might due to blocking cellular DNA; causing plant growth stop or slow (Omar, *et al.,* 2008). The effect of gamma irradiation is referred to radiation injury and may manifest in several forms including reduction in sprouting ability, survival, plant height and number of plant organs (Nwachukwu *et al*., 2009). Growth reduction caused by gamma radiation was in harmony the finding 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 dose of gamma rays.

Similar results were observed by Ramachandran and Goud (1983) in safflower, Benerji and 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 growth reduction was the main effect of gamma radiation in vetiver, it was noticed that the Kamphaeng Phet 2 ecotype chronically gamma irradiated at 63 Gy had higher shoot and root dry weight than those non-irradiated one. This information supported the finding of Veeresh *et al* (1995) who observed an increase in shoot fresh weight of winged bean at lower dose. Besides, Majeed and Muhammad (2010) indicated that fresh and dry weight of *Lepidium sativum* L. increased due to gamma radiation at 20 Gy, then gradually decreased when dose increased. The growth stimulation effect has been found with both acute and chronic irradiations in several plants (Bari, 1971). It was pronounced with low dose 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 simulating effect on low doses of gamma irradiation on plant growth may be due to stimulation of cell division or cell elongation, alteration of metabolic process that affect synthesis of phytohormones or nucleic acids (cited in Nassar, *et al.,* 2004). In this 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.

In the present studies, the acute irradiation caused damage on vetiver plant more 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 irradiations 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 result agreed well with the observation that acute exposure is more injurious than chronic one (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 for explanation of this phenomenon.

When the data on vetiver survival and growth responses to gamma radiation were plotted against dosages (Figures 1-6), some insignificant data at high doses caused by inhibiting effect were discarded. The determination coefficient (r^2) of regression equations ranged between 0.74-0.95 for acute irradiation and 0.57-0.87 for chronic irradiation. The low correlation of the chronic experiment might due to influence of 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} as the criteria of radiosensitivity, they varied not only between irradiation methods but also varied between vetiver varieties or ecotypes. The 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 was better than the

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

Conclusion

The experimental results indicated that vetiver radiosensitivity depends on both irradiation methods and vetiver varieties. In general, increasing gamma doses from both acute and chronic irradiations decreased in survival rate and growth performance of vetiver. The LD_{50} and GR_{50} values of vetiver ranged between 73-100 Gy and 30-125 Gy, respectively. For environmental application, vetiver variety with high biomass is usually needed. Thus, the recommended gamma dose treatment for efficient induced mutation in vetiver should not more than GR_{50} value on dry biomass of each vetiver ecotypes.

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