

EFFECTS OF GAMMA IRRADIATION ON *IN VITRO* CULTURE OF VETIVER

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Abstract

In vitro mutagenesis is an established method for mutation breeding and has played a role in the development of many valuable mutant varieties. In this study, the effects of acute and chronic gamma irradiation on vetiver *in vitro* cultured plantlets were investigated. The sterilized shoots of native Thai vetiver, *Chrysopogon nemoralis*, the Nakorn sawan ecotype and *Chrysopogon zizanioides*, the Song khla3 ecotype were cultured on the MS medium, their plantlets were irradiated with gamma rays for both acute and chronic treatments of 0, 15, 30, 45, 60, 75 and 90 Gy (acute) and 0, 33.6, 74.1, 107.8, 141.2, 174.8 and 215.3 Gy at a dose rate of 0.84 Gy/hr (chronic). The number of surviving plantlets and the number of new shoots 60 days after irradiation were recorded for 50% lethal dose ($LD_{50/60}$) and median growth reduction dose ($GR_{50/60}$) determination.

The $LD_{50/60}$ and the $GR_{50/60}$ values of the Nakorn sawan and Song khla3 ecotypes were evaluated after acute irradiation of which the $LD_{50/60}$ values indicated 41.5-42.7 and 41-45.6 Gy, respectively, and the $GR_{50/60}$ values were 50.2-57.3 and 25.5-26 Gy, respectively. It was not possible to calculate the $LD_{50/60}$ for the chronic irradiation of the Song khla3 ecotype as the survival rates were 100% for 0, 33.6 and 74.1 Gy treatments, and 0% for other treatments, whereas the $LD_{50/60}$ of the Nakorn sawan ecotype was 107.7-120.1 Gy. The calculated $GR_{50/60}$ of the Nakorn sawan and Song khla3 ecotypes after chronic irradiation were 106.4-122 and 65.3-72 Gy, respectively. Observation of the $LD_{50/60}$ and the $GR_{50/60}$ not only implied the radiosensitivity difference in the irradiation methods but also the vetiver ecotypes. Gamma irradiation stimulated the new tillers in M_1V_2 generation of the Nakorn sawan ecotype at the doses of 141.2 and 174.8 Gy. This study provided the information of $LD_{50/60}$ and $GR_{50/60}$ values combined with tissue culture technique could contribute potentially towards crop improvement in vetiver.

Keywords: vetiver, *in vitro* culture, gamma irradiation, lethal dose, growth reduction dose

Introduction

Vetiver grass, a perennial native to Thailand, is an excellent grass in the aspect of water and soil conservation due to its unique characteristics, such as massive and deep roots, stiff stalk and dense hedgerows. In the process of vetiver's dissemination and application for erosion control, it is found to have a strong resistance to such adverse conditions as infertility, strong acidity or alkalinity, organic pollutants and heavy metals. This makes it an excellent plant in the aspects of environmental amelioration and ecological restoration. Since vetiver has so many fine qualities, it has been spread and used in over 100 countries and regions of the tropics and subtropics so far. (Truong and Baker, 1998; and Xia *et al.*, 1998).

Nuclear technology organized with *in vitro* mutagenesis has been widely applied in developing new varieties of crop plants. A wide range of plant characteristics have been improved through nuclear technology especially from ionizing radiation such as gamma rays which transferred energy to molecules in the cells of plants, especially the DNA molecules that contain the genetic information, resulting in point mutations (Lamseejan *et al.*, 2000). Roongtanakiat *et al.* (2012) reported the effects and radiosensitivity of acute and chronic gamma radiation on native Thai vetiver ecotypes, *Chrysopogon zizanioides*, the Kamphaeng Phet 2 and the Surat Thani ecotypes, and *Chrysopogon nemoralis*, the Ratchaburi ecotype and suggested that the acute irradiation caused higher radiosensitivity of vetiver than chronic irradiation, however, chronically exposure gave higher shoot and root dry weights than the control treatment (0 Gy). Kresovich *et al.* (1994) reported that the genome of vetiver within the same clones generated stable RAPD (Random Amplified Polymorphic DNA) patterns but they were able to distinguish between various vetiver accessions. In contrast, Adams and Dafforn (1998) found that 86% of 121 accessions of vetiver appeared to be from a single clone. Hence, the utilization of *in vitro* mutagenesis of vetiver can provide genetic variation contributing potentially towards crop improvement in vetiver. The objective of this research was to study the effect of both acute and chronic gamma irradiation on the plant survival and proliferation rate as well as the effect of mutagenesis to the native Thai vetiver, *Chrysopogon nemoralis*, the Nakorn sawan ecotype and *Chrysopogon zizanioides*, the Song khla3 ecotype plantlets grown in tissue culture.

Materials and Methods

Vetiver plantlets induction in tissue culture

The native thai vetiver explants, *Chrysopogon nemoralis*, the Nakorn sawan ecotype and *Chrysopogon zizanioides*, the Song kha3 ecotype from field-grown were surface disinfested as intact stems in 6% Sodium hypochlorite for 5 min 2 times, rinsed three times with sterile deionized water. The explants were cultured on MS medium (Murashige and Skoog, 1962).

Acute and chronic gamma irradiations

In acute gamma irradiation, plantlets of the Nakorn sawan and Song kha3 ecotypes raised *in vitro* were exposed to gamma radiation at 0 (control), 15, 30, 45, 60, 75 and 90 Gy from Cs-137 source using a MARK I Research Irradiator. *In vitro* plantlets of the Nakorn sawan and Song kha3 ecotypes were exposed to chronic gamma ray at a dose rate of 0.84 Gy/hr from a Co-60 source in the Gamma Room at the Nuclear Technology Research Center, Faculty of Science, Kasetsart University. The samples received 0 (control), 33.6, 74.1, 107.8, 141.2, 174.8 and 215.3 Gy.

Results and Discussion

Effects of acute irradiation on the plant survival and proliferation rate

The *in vitro* cultured plantlets of the Nakorn sawan and Song kha3 ecotypes were exposed with acute gamma irradiation of 0, 15, 30, 45, 60, 75 and 90 Gy. The number of survival plantlet and survival rate of irradiated vetivers decreased as the gamma dose increased (Table 1) and dose of 90 Gy completed survival plantlets of both vetiver ecotypes. The results from ANOVA indicated that there was significant difference between the control and the irradiated treatments. The correlation between survival rate and gamma dose is shown in Figure 1, 50% lethal dose ($LD_{50/60}$) was estimated from survival rate of 60 days plantlets and the linear regression equation were 41.5-42.7 Gy for the Nakorn sawan ecotype and 41-45.6 Gy for the Song kha3 ecotype. The result agreed with the report of Roongtanakiat (2012) that the tillering of native Thai vetiver, Kamphaeng Phet2 and Surat Thani ecotypes rapidly dropped after acute irradiation with gamma-rays. Tangpong (2009) also suggested the percentage of survival *in vitro* culture plantlets of *Anubias congensis* N.E. Brown was negatively correlated with the amount of gamma radiation received.

Table 1 Total number of irradiated plantlets, the number of survived plantlets, and survival rate (% of control) of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days after acutely exposed to *in vitro* culture plantlets with different doses of gamma-rays.

Gamma dose (Gy)	Total no. of irradiated plantlets	Nakorn sawan ecotype		Song kha3 ecotype	
		No. of survival plantlets	Survival (% of control)	No. of survival plantlets	Survival (% of control)
0	18	18	100 a ^{1/}	18	100 a
15	18	18	100 a	18	100 a
30	18	14	77.8 b	14	77.8 b
45	18	7	38.9 c	7	38.9 c

60	18	5	27.8 c	6	33.3 c
75	18	0	0 d	1	5.6 d
90	18	0	0 d	0	0 d
F-test			*		*
C.V. (%)			18.11		16.26

* significant at 5% level

1/ data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT

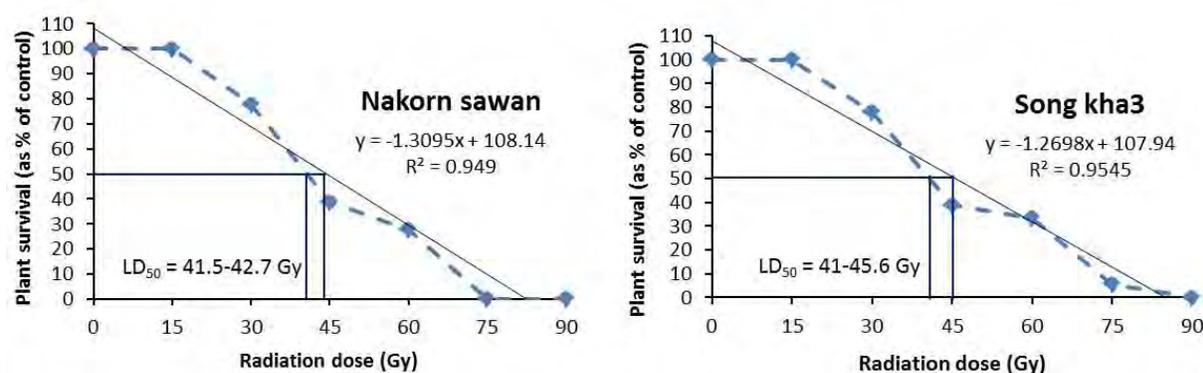


Figure 1 Survival rate (as % of control) and regression equation of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days after acutely exposed to different doses of gamma irradiation. (LD_{50} = 50% lethal dose.)

The effects of acute irradiation on the number of new tillers and proliferation rate of the M_1V_1 plantlets are summarized in the Table 2. The gamma dose caused a negative effect on plant development as indicated by the reduction of the new tiller of both vetiver ecotypes and dose of 75 - 90 Gy inhibited the new tillers of Nakorn sawan ecotype while dose of 45 to 90 ceased of the new tillers of Song kha3 ecotype. (Table 2). The 50% Growth reduction ($GR_{50/60}$) was estimated from plant proliferation rate of 60 days plantlets and the linear regression equation were 50.2-57.3 Gy for the Nakorn sawan ecotype and 25.5-26 Gy for the Song kha3 ecotype. This corresponded with the report of Roongtanakiat (2012) revealed the increasing of the gamma dose cause reduction in growth performances such as plant height, shoot dry weight and root dry weight of two native Thai vetivers.

Table 2 The number of new tillers, and proliferation rate (% of control) of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days after acutely exposed to *in vitro* culture plantlets with different doses of gamma-rays.

Gamma dose (Gy)	Nakorn sawan ecotype		Song kha3 ecotype	
	No. of new tillers	Proliferation (% of control)	No. of new tillers	Proliferation (% of control)
0	1.38	100 a	1.16	100 a
15	1.21	87.7 ab	1.44	124.1 a
30	1.33	96.4 a	0.16	13.8 b
45	0.88	63.8 ab	0	0 b
60	0.66	47.8 a	0	0 b
75	0	0	0	0 b
90	0	0	0	0 b
F-test		*		*
C.V. (%)		44.77		48.67

* significant at 5% level

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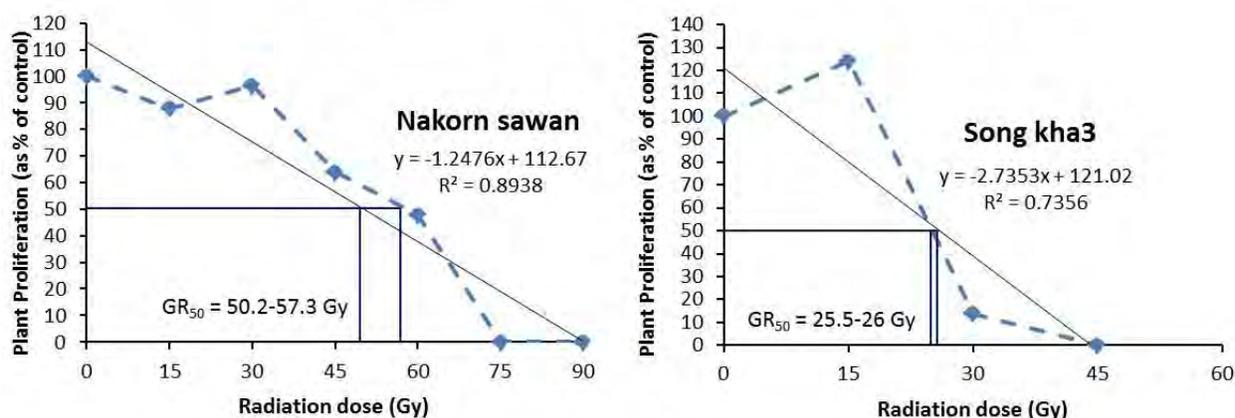


Figure 2 Proliferation rate (as % of control) and regression equation of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days acutely exposed to different doses of gamma irradiation. (LD₅₀ = 50% lethal dose.)

Effects of chronic irradiation on the plant survival and proliferation rate

The effects of chronically exposed of the number of survival plantlets and survival rate in the M₁V₁ are summarized in the Table 3. The gamma dose caused a negative effect on plant survival as indicated by the reduction of the number of survival plantlets and dose of 215.3 Gy inhibited the new tillers of the Nakorn sawan ecotype while the plantlets of the Song kha3 exposed to radiation from 0 to 74.1 Gy did not have any statistically significant difference from the control regarding to the parameter of number of survival plantlets. The result agreed with the

report of Tangpong (2009) that the plantlets of *A. congensis* N.E. Brown chronically exposed to radiation from a dose of 0 – 91.69 Gy did not show significant difference from control in the parameters of number of new shoots, number of new leaves, leaf width or root length. Samples that were exposed to radiation at a dose of 107.7 to 215.3 Gy ceased of the survival plantlets of Song kha3 ecotype (Table 3). The results from ANOVA indicated the significant difference between the control and irradiated treatments of the Nakorn sawan ecotype. The correlation between survival percentage and gamma dose is shown in Figure 3, 50% lethal dose (LD_{50/60}) was estimated from survival rate of 60 days plantlets and the linear regression equation suggested 107.7-120.1 Gy.

Table 3 Total number of irradiated plantlets, the number of survived plantlets, and survival rate (% of control) of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days after chronically exposed to *in vitro* culture plantlets with different doses of gamma-rays.

Gamma dose (Gy)	Total no. of irradiated plantlets	Nakorn sawan ecotype		Song kha3 ecotype	
		No. of survival plantlets	Survival (% of control)	No. of survival plantlets	Survival (% of control)
0	18	18	100 a	18	100 a
33.6	18	18	100 a	18	100 a
74.1	18	18	100 a	18	100 a
107.7	18	9	50 b	0	0 b
141.2	18	6	33.3 bc	0	0 b
174.8	18	3	16.7 bc	0	0 b
215.3	18	0	0 c	0	0 b
F-test			*		*
C.V. (%)			39.75		32.87

* significant at 5% level

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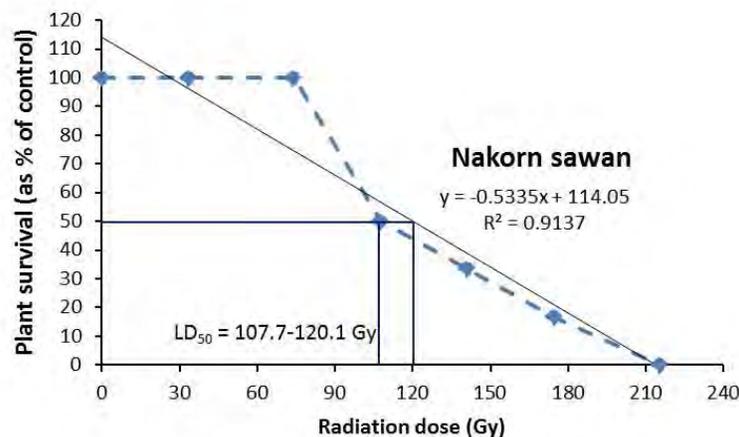


Figure 3 Survival rate (as % of control) and regression equation of the Nakorn sawan vetiver ecotype at 60 days after chronically exposed to different doses of gamma irradiation. (LD₅₀ = 50% lethal dose.)

The effects of chronically exposed of the number of new tillers and proliferation rate in the M₁V₁ shown the irradiation affected the growth of the M₁V₁ generation. The results from ANOVA indicated the significant difference between the control and the irradiated treatments (Table 4). The correlation between survival percentage and gamma dose is shown in Figure 4, 50% growth reduction (GR_{50/60}) was estimated from plant proliferation rate of 60 days tillering and the linear regression equation were 106.4-122 Gy for the Nakorn sawan ecotype and 65.3-72 Gy for the Song kha3 ecotype. The effect of gamma irradiation is referred to as radiation injury and may be manifested in several forms including a reduction in sprouting ability and the number of plant organs (Mwachukwu et al., 2009). Similar results were observed by Lamseejan *et al.* (2000) in Chrysanthemum, Ramachandran and Goud (1983) in safflower, Benerji and by Datta (1992) and Joompuk *et al.* (2009) in torch ginger.

Table 4 The number of new tillers, and proliferation rate (% of control) of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days after chronically exposed to *in vitro* culture plantlets with different doses of gamma-rays.

Gamma dose (Gy)	Nakorn sawan ecotype		Song kha3 ecotype	
	No. of new tillers	Proliferation (% of control)	No. of new tillers	Proliferation (% of control)
0	1.51	100 a	1.77	100 a
33.6	1.25	82.8 a	1.72	97.17 a
74.1	0.94	65.3 ab	0.83	46.89 b
107.7	0.38	25.2 bc	0	0 c
141.2	0.33	21.9 bc	0	0 c
174.8	0.11	7.3 c	0	0 c

215.3	0	0 c	0	0 c
F-test		*		*
C.V. (%)		51.90		29.85

* significant at 5% level

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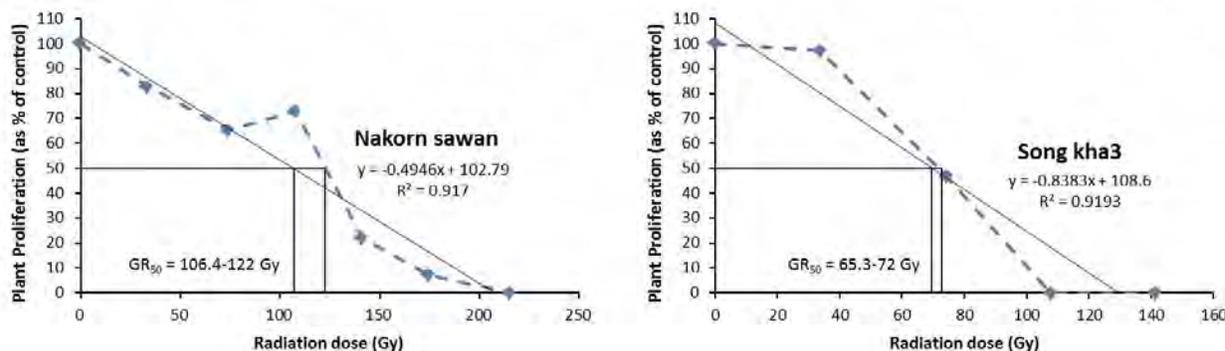


Figure 4 Proliferation rate (as % of control) and regression equation of the Nakorn sawan and Song kha3 vetiver ecotypes at 60 days after chronically exposed to different doses of gamma irradiation. (LD₅₀ = 50% lethal dose.)

Growth performance in M₁V₂ generation

The comparative growth performance on the tillering character of the M₁V₂ generation was summarized in the Table 5 and 6. Tillering of the Nakorn sawan ecotype was affected by acute irradiation similarly to the Song kha3 ecotype, of which in M₁V₂ generation the number of new tillers at 60 days plantlets revealed significantly lower than the control (Table 5), however, the doses of 15 – 45 Gy did not effected the 45 days plantlets of the Nakorn sawan ecotype. The lower dose rate of radiation probably caused little damage to the plants' genetic material so that the cells could repair themselves (Neary *et al.*, 1957). Moreover, the radiation associated with *in vitro* culture could induce mutation in short time, given novel characters; however, plant growth caused by low doses of gamma irradiation may have been either due to stimulation of cell division or cell elongation nor DNA repair mechanism.

Table 5 Comparative growth performance, number of new tillers after acute gamma irradiation at 0-60 Gy of the Nakorns awan and Song kha3 vetiver ecotypes in the M₁V₂ generation at 15, 30, 45 and 60 days after transplanting.

Gamma dose	Nakorn sawan ecotype	Song kha3 ecotype
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(Gy)	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
0	0	0.88 a	1.27 a	1.88 a	0	1.00 a	2.38 a	2.61 a
15	0	0.33 c	0.77 a	1.33 c	0	0.61 ab	0.88 b	1.44 b
30	0	0.16 d	0.44 a	0.88 d	0	0.35 b	0.71 c	1.00 c
45	0	0.16 d	0.61 a	0.83 d	-	-	-	-
60	0	0.50 b	1.00 b	1.50 b	-	-	-	-
F-test	ns	*	*	*	ns	*	*	*
C.V. (%)	0	11.01	47.63	6.97	0	44.09	0	1.87

ns non-significant

* significant at 5% level

^{1/} data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT

The number of new tillers of M₁V₂ generation of the Song kha3 ecotype after chronic irradiation shown significantly lowers than control opposed to the Nakorn sawan ecotype. At the doses of 141.2 and 174.8 Gy stimulated tillering of the Nakorn sawan ecotype and demonstrated significantly higher than non-irradiation plants (Table 6). Physiological changes in a large range of plants exposed to gamma rays have been described by many researchers, the morphological frequently observed in the low- or high-dose irradiated plants are enhancement or inhibition of germination, seedling growth, and other biological responses (Kim *et al.*, 2000; Wi *et al.*, 2005), affected in generating variability in tiller number and plant height (Silveira *et al.* 2014). The potential mutants of the native Thai vetivers in this research were the tillering efficiency.

Table 6 Comparative growth performance, number of new tillers after chronic irradiation at 0-174.8 Gy of the Nakorn sawan and Song kha3 vetiver ecotypes in the M₁V₂ generation at 15, 30, 45 and 60 days after transplanting.

Gamma dose (Gy)	Nakorn sawan ecotype				Song kha3 ecotype			
	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
0	0	0.38 b	0.55 ab	0.66 b	0	0.88 a	1.22 a	1.38 a
33.6	0	0.22 de	0.27 b	0.33 c	0	0.16 c	0.61 c	0.72 b
74.1	0	0.27 cd	0.66 ab	0.72 b	0	0.55 b	0.77 b	0.77 b
107.7	0	0.10 e	0.50 ab	0.70 b	-	-	-	-
141.2	0	0.33 bc	1.00 a	1.16 a	-	-	-	-
174.8	0	0.50 a	0.27 b	1.00 a	-	-	-	-
F-test	ns	*	*	*	ns	*	*	*
C.V. (%)	0	16.95	42.08	40.74	0	5.96	3.64	3.30

ns non-significant

* significant at 5% level

^{1/} data within columns, means followed by a common letter are not significantly different at the 5% level by DMRT

Conclusion

The experimental results of the LD_{50/60} and the GR_{50/60} not only implied the radiosensitivity difference in the irradiation methods but also the vetiver ecotypes. The potential mutants of the native Thai vetivers in this research were the tillering efficiency of the Nakorn sawan ecotypes. This study provided the information of LD_{50/60} and GR_{50/60} values combined with tissue culture technique could contribute potentially towards crop improvement in vetiver.

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