Introduction

Malaysian livestock industry is generally classified into ruminant and non-ruminant sub-sectors. Ruminant sub-sector is consisted of cattle, buffalo, goat and sheep whilst poultry, eggs and pork are classified in the non-ruminant sub-sector. While swine and poultry industries have been able to transform themselves into modern and commercial industries, ruminant sub-sector is still not well developed and competitive enough compared to neighbouring countries like Thailand, Indonesia and Vietnam. Meat production in Malaysia generally increases around 3-5% each year, which is lower than the increase of annual domestic consumption. The industry could only supply between 28-30% of domestic demands and as such, Malaysia has to rely on import for milk, beef and mutton (Loh 2002; Ahmad Zairi et al., 2018). Low production and low adoption of high and modern technology have been identified as the main constraints besides general problems such as lack of available lands, pest and diseases as well as lack of labour (Ahmad Zairi et al., 2018). Feed production systems vary from extensive to semi intensive and depend on native pastures that are often supplemented with other locally available feed stuffs such as palm kernal cake, palm oil sludge, oil palm frond and soy waste (Loh, 2002). Transformation of ruminant sub-sector into an efficient and well developed industry requires participation and commitment from all relevant parties involved. One of the aspects that requires attention is the availability of high quality pasture and fodder grass.

Napier grass (*Pennisetum purpureum*) is native to Africa and was first introduced to Malaysia in the 1920's. Currently, Napier grass is one of most popular fodder grass in dairy and feedlot production systems. Some popular cultivars are Taiwan Napier, Dwarf Napier, King Grass and Red Napier. According to Halim et al (2013), there are morphological differences between the varieties and clearly divided into two distinct group based on the plant height which are greater than 139 cm or less than 95 cm. Taller varieties are King Grass, Common Napier, Red Napier, Taiwan Napier, Uganda Napier and Indian Napier. Dwarf varieties are Dwarf Napier, Dwarf 'Mott' and Australian Dwarf. Farmers can choose the tall varieties of Napier to obtain higher quantity of forage but when livestock has special needs for higher quality feed such as for dairy production then the shorter varieties will be a better choice because the short Napier varieties were leafier and also had higher nutritive quality than the tall varieties.

Generally, all the morphological and physiological characteristics of Napier were affected by the change in their atmospheric and soil condition environment. Green fodder yield, dry matter yield and crude protein CP for Dwarf 'Mott' are linearly increased with the increase in nitrogen fertilization rates (Zahid et al., 2002). Studied by Zailan *et al*. (2016) showed the nutritive value of Napier grasses decreases with advancing maturity. Also, the growth rate, degree of lignification and accumulated structural carbohydrate were differed between cultivars as the harvesting age increased. The recommended age to harvest Napier is at 6-8 weeks of growth to optimize the dry matter yield and nutritive value (Lounglawan *et al.*, 2014). McDonald et al. (2002), found the minimum requirement of CP for lactation and growth was 15%. The CP content for Dwarf Napier was closed to 16% at 6th weeks old harvesting which is required in optimizing the milk output of lactating animals (Imaizumi *et al*., 2010). The CP for Taiwan Napier was decreased with harvesting age which was 17% to 14% at 5th and 6th weeks respectively (Haryani *et al*., 2018). The dry matter yield for Dwarf Napier was approximately 11.58 t/ha and for Taiwan Napier was 11.12 t/ha at 8th weeks old harvesting (Halim *et al*., 2013).

Even though Napier grass is the most popular fodder grass in dairy or feedlot production systems, very little research on breeding program for improvement of Napier grass has been reported in Malaysia. Therefore, there is a high need to initiate a mutation breeding programme for Napier grass using acute and chronic gamma irradiations in order to improve its yield and nutritive values for livestock. Gamma radiation is a promising method to induce genetic variation in variable crops (van Harten, 1998). The ultimate aim of this study is to improve Napier grass through mutation breeding with targeted breeding traits such as high biomass, high protein content and less toxicity for ruminant consumption. These are among the important traits needed to produce high quality animal feed.

The mutation breeding work was carried at Malaysian Nuclear Agency (Nuklear Malaysia) using two facilities for mutation induction in plants for both acute (high dose) and chronic (low dose) irradiation. Both irradiators use consist of Caesium-137 as the radioactive source. Biobeam GM8000 gamma cell is the acute gamma irradiation facility that produces high dose radiation whilst Gamma Greenhouse (GGH) is a chronic gamma irradiation facility that provides low dose radiation. Usually the biological samples will be exposed to radiation for over period of times depending on the nature and sensitivity of the plant species. For high dose radiation the biological samples can be exposed in a short period of time as compare to low dose radiation where samples can be exposed in the span of days, weeks, months or years. Chronic gamma irradiation also produces a wider mutation spectrum and useful for minimizing radiation damages towards obtaining new improved traits for research and commercial values. Both irradiation facilities are utilized for irradiation of tropical and subtropical plants and tissue culture materials since 2010. The scope of this study was to evaluate morphological and physiological characteristic of M_1V_5 population of Napier grass cv. Dwarf and cv. Taiwan that have been irradiated using acute and chronic gamma.

MATERIALS AND METHODS

Plant Material

Two varieties of Napier grass used in this study (cv. Dwarf and cv. Taiwan) were obtained from Malaysian Agricultural Research and Development Institute (MARDI) collection. The healthy and uniform size stems for each variety were cut into approximately 25 cm segments that contain 2 to 3 nodes. The cuttings were cleaned and sent for irradiation at Malaysian Nuclear Agency, Selangor.

Gamma Irradiation

Acute irradiation

Acute irradiation was carried out using Gamma Cell (Biobeam GM8000, Germany). The cuttings were packed and loaded into BB13-5 container of 29.2 cm height and 10 cm diameter. A total of eight dose treatments were used in this study which were 0 (control/non-irradiated), 10, 20, 30, 40, 60, 80, 100 Gy at the dose rate of 15.7 Gy/min. Each treatment was consisted of 25 cuttings. Irradiated cuttings were then planted into polybag for germination and subsequently cut for multiplication for several generations in the glasshouse.

Chronic irradiation

The cuttings were exposed to chronic radiation at Gamma Greenhouse (GGH). GGH is a circular greenhouse of 15-meter radius consisting of 15 isodose rings in which every ring has different dose rate per hour. For this study, ten treatments which were 0 (control/non-irradiated), ring 2 (0.66 Gy/h), 3 (0.33 Gy/h), 4 (0.17 Gy/h), 5 (0.11 Gy/h), 6 (0.07 Gy/h), 7 (0.05 Gy/h), 8 (0.04 Gy/h), 9 (0.03 Gy/h), 11 (0.02 Gy/h) and 12 (0.02 Gy/h) were involved. A total of 12 cuttings were planted in containers in each ring. The accumulated dose for each ring was calculated based on dose rate and time of exposure. Due to the low dose rate nature of chronic gamma irradiation, the cuttings were exposed for three consecutive months in GGH before being transferred to a regular glasshouse for germination and multiplication for mutant selection.

Morphological and physiological evaluation of potential mutant plants

Preliminary selections for variant lines of both cv. Taiwan and cv. Dwarf were done at M_1V_4 generation. At this stage, a total of 129 variant lines were selected based on morphological variation and vigorousness among the irradiated population. These selected variant lines were further planted at M_1V_5 generation for evaluation of their major morphological and physiological characteristics in the glasshouse. At M_1V_5 generation, mutant lines are considered phenotypically stable. The experiment was carried out using Random Complete Block Design (RCBD) with 5 replicates.

Data collection

Measurement on main morphological and physiological traits of the potential mutant lines were taken at 30 days after planting (DAP) of M_1V_5 generation. Morphology and physiology data were focused on the number of leaves, plant height, stem diameter, inter-node length, leaf area, leaf length, average leaf width, maximum leaf width and chlorophyll content. Plant height was measured from the base of the plant to the leaf tip of the longest shoot whilst the stem diameter and inter-node length was measured by using Vernier caliper. LI-3000C Portable Leaf Area Meter was used to measure leaf area, leaf length, average leaf width and maximum leaf width. For leaf chlorophyll content, Soil Plant Analysis Development (SPAD, Minolta Camera Co., Osaka, Japan) chlorophyll meter was used. This meter provides a rapid and non-destructive approach that enables users to measure chlorophyll content in the field.

All studied characters of the potentials mutants were analyzed and compared with the control. For plant height measurement, the height of each mutant line was measured and compared with the average height of the control and subsequently categorized into three groups according to their height;
1) shorter by more than 10 cm than the control,
2) within the range of 10 cm with the control,
3) taller by more than 10 cm than the control.
Since these data were measured using non-destructive approach, the total leaf area was estimated using the relationship of leaf area times the number of leaves for each plant.

RESULT AND DISCUSSION

Selection of potential mutant lines

Surviving plants after irradiation treatments were propagated until M_1V_4. Selections for possible mutation were made based on the visual sign of irradiation effect such as leaf chlorophyll mutation, plant height and vigorous growth performance. A total of 129 variant lines were generated and selected at M_1V_4 from the initial number of 668 irradiated cuttings from both varieties and irradiation types. Detailed numbers of the variant lines are as follows;
(1) cv. Taiwan irradiated with acute gamma - 35 lines
(2) cv. Taiwan irradiated with chronic gamma - 30 lines,
(3) cv. Dwarf irradiated with acute gamma - 34 lines
(4) cv. Dwarf irradiated with chronic gamma - 35 lines

These variant lines were further multiplied until M_1V_5 generation for specific morphological and physiological evaluations. At M_1V_5 stage, chimeric tissues have been mostly eliminated (Jankowicz-Cieslak and Till, 2016; Jankowicz-Cieslak et al., 2016) and mutant lines are considered phenotypically stable and suitable for morphological and physiological assessment. Meanwhile, leaf chlorophyll mutation is generally not stable and only appears in M_1V_1 and M_1V_2 generations. In this study, the mutant lines clearly showed some variations in their plant height.

Table 1 summarizes the number of cv. Dwarf mutant lines with height variation induced by acute and chronic gamma irradiation at M_1V_5. The height mutation rate was calculated based on the number of mutant plants with average height of more or less than 10 cm than the control at 30 DAP. In this study, the height mutation rate among the selected mutant lines of cv. Dwarf were between 40 to 100% for acute gamma and between 20 to 60% for chronic gamma. Overall, acute gamma irradiation generated a significantly higher percentage of height variation in irradiated population (82.4%) as compared to chronic gamma (43.3%). However, an interesting trend was also observed in which acute gamma irradiation tend to generate shorter mutant lines with 10 cm lower in height than the control, whereas chronic gamma tend to generate taller plants by more than 10 cm in height when compared to control (Table 1). Only one mutant line with a height of more than 10 cm than the control was observed in acute gamma induced cv. Dwarf population. In contrast, chronic gamma irradiation generated 12 mutant lines that were 10 cm taller than the control. A reduce in plant height is a common effect of gamma radiation and has been reported in many previous research (Marcu et al, 2013; Songsri et al. 2011), but this study on cv. Dwarf showed that chronic gamma radiation has the advantage over acute gamma in inducing taller than shorter mutants.

Table 1: Number of mutant lines and mutation rate based on height variation induced by acute and chronic gamma irradiation for cv. Dwarf.

Type of gamma irradiation	Dose (Gy)	Number of screening variant lines	Number of lines in height group			Number of mutant lines selected	Height mutation rate (%)
			≥ 10 cm from control	Within 10 cm	≤ 10 cm from control		
Acute	10	4	0	1	3	3	75
	20	5	1	0	4	5	100
	30	5	0	3	2	2	40
	40	5	0	1	4	4	80
	60	5	0	0	5	5	100
	80	5	0	0	5	5	100
	100	5	0	1	4	4	80
	Total	**34**	**1**	**6**	**27**	**28**	**82.4**
Chronic	915.10 (Ring 2)	5	1	4	0	1	20
	409.74 (Ring 3)	5	1	4	0	1	20
	232.19 (Ring 4)	5	3	2	0	3	60
	150.24 (Ring 5)	5	3	2	0	3	60
	95.61 (Ring 6)	5	2	2	1	3	60
	54.63 (Ring 8)	5	2	3	0	2	40
	Total	**30**	**12**	**17**	**1**	**13**	**43.3**

Meanwhile, Table 2 summarizes the data on height variation among the screening lines for cv. Taiwan. Similar to cv. Dwarf, average height of each screening variant line was measured, then compared with the control variety, and subsequently categorized into its respective plant height group as discussed in the Materials and Methods. In this study, the mutation rate in terms of plant height variation among the screening mutant lines was between 20 to 80% for acute gamma and between 40 to 60% for chronic gamma. Unlike cv. Dwarf, both types of gamma irradiation showed almost similar capacity in inducing height variation on cv. Taiwan plants where an overall average of 54.3% height mutation rate was observed in acute gamma population and 50% in chronic gamma population.

However similar to the effect on cv Dwarf, acute gamma radiation was observed to generate more mutant plants with shorter height than the control (12/35 or 34.3%) compared to taller plants (7/35 or 20%). For chronic gamma, the percentage of taller and shorter plants was 30% (9/30) and 20% (6/30), respectively. Therefore, acute gamma was also relatively good in inducing shorter plants and chronic gamma for taller Napier plants.

Table 2: Number of mutant lines and mutation rate based on height variation induced by acute and chronic gamma irradiation for cv. Taiwan.

Type of gamma irradiation	**Dose (Gy)**	**Number of screening variant lines**	**Number of lines in height group**			**Number of mutant lines selected**	**Height mutation rate (%)**
			≥ 10 cm from control	**Within 10 cm**	**≤ 10 cm from control**		
Acute	10	5	1	3	1	2	40
	20	5	3	2	0	3	60
	30	5	2	2	1	3	60
	40	5	1	4	0	1	20
	60	5	0	3	2	2	40
	80	5	0	1	4	4	80
	100	5	0	1	4	4	80
	Total	**35**	**7**	**16**	**12**	**19**	**54.3**
Chronic	178.93 (Ring 5)	5	3	2	0	3	60
	113.87 (Ring 6)	5	1	2	2	3	60
	81.33 (Ring 7)	5	2	2	1	3	60
	65.07 (Ring 8)	5	2	3	0	2	40
	48.80 (Ring 9)	10	1	6	3	4	40
	Total	**30**	**9**	**15**	**6**	**15**	**50.0**

Besides plant height, total leaf area is another character of interest in this study. Leaf is the main organ of plant to carry out the process of photosynthesis by the conversion of carbon dioxide, water, and UV light into glucose for energy or to produces food for the whole plant (Tsukaya, 2013; BiologyDictionary.net Editors, 2017). In a simple way of understanding, more leaves contribute to more energy for plant to grow. Increasing in plant growth will lead to a higher production of plant biomass (Demura and Ye, 2010). Estimated total leaf area in this study was used to compare mutant lines with the control plant. This was based on the assumption that plants with higher total leaf area value may eventually produce higher biomass yield. Therefore, based on the total leaf area, a total of 21 potential higher biomass mutant lines were eventually selected from the overall 129 variant lines tested. Detailed morphology and physiology characteristic of the 21 potential higher biomass mutant lines of cv. Dwarf and cv. Taiwan are summarized in Table 3 and Table 4, respectively.

Napier cv. Dwarf produced 15 mutants (or 71.4%) from the total of 21 potential higher biomass mutant lines and the rest are from cv. Taiwan (28.6%). In terms of irradiation types, both acute and chronic gamma irradiation produced approximately the same number of mutant lines, 11 and 10 mutant lines, respectively. In this study, Soil Plant Analysis Development (SPAD) chlorophyll meter were also used to measure chlorophyll content in the field. This meter provides a rapid reading with non-destructive approaches and the values are given as a relative unit. SPAD meter is also widely used in other research areas because of its quick and non-destructive testing features that not only allow direct measuring of leaf chlorophyll or "greenness" but also can indirectly assess the proportional parameter of leaf, plant nitrogen status and finally, grain yield in coffee and rice (Netto et al., 2005; Gholizadeh et al., 2011). Spano et al. (2003), used SPAD meter to measure the relative chlorophyll contents of `stay green' mutants in durum wheat leaves.

In this study, SPAD measurement showed various leaf chlorophyll content or "greenness" within the mutant lines, where the lowest was recorded in cv. Dwarf irradiated with acute gamma at 80 Gy (23.8) and the highest was Cv. Taiwan irradiated with chronic gamma irradiated with cumulative dose of 178.93 Gy. An interesting trend was also observed in which chronic gamma radiation gave higher reading of chlorophyll content for both irradiated Napier varieties than acute gamma. The generation of mutant lines with better plant height and higher SPAD values in plants irradiated with chronic gamma probably strengthen the findings that chronic gamma radiation has the ability to induce a wider mutation spectrum than acute gamma irradiation as reported by Okamura et al. (2006) and Faiz et al. (2018).

The ultimate aim of this study is to improve Napier grass through mutation breeding with targeted breeding traits such as high biomass, high protein content and less toxicity for ruminant consumption. These traits are really needed in order to produce high quality animal feed. In this study, the promising mutant lines with high biomass potential (21 lines) have been identified. Therefore, the step forward is to plant these promising mutant lines for several additional generations to analyze their nutritional quality, toxicity and overall yield to establish new mutant Napier varieties for ruminant industry.

CONCLUSION

Based on the study, both acute and chronic gamma irradiation were useful in inducing height variation on Napier grass cv. Dwarf and cv. Taiwan. Acute gamma was seen to induce more short plants whilst chronic gamma seemed to generate more tall plants. At M_1V_5 stage, a total of 21 potential mutant lines have been found to produce higher biomass than the control. Since the ultimate aim was to improve Napier grass with targeted breeding traits such as high biomass, high protein content and less toxicity for ruminant consumption, the way forward is to plant these potential mutant lines for several additional generations to evaluate their nutritive values, toxicity and total yield. These studies are really needed in order to establish new mutant varieties for high quality animal feed.

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Table 3: Summary of major morphological and physiological characteristic of M_1V_5 cv. Dwarf mutant lines at 30 days after planting (DAP)

No.	Mutant lines	Dose (Gy)	Plant Height (cm)	Stem Diameter (mm)	Internode Length (mm)	Number of Leaf (a)	Leaf Area (b)	Estimated Total Leaf Area (a x b)	Leaf Length (cm)	Average Leaf Width (cm)	Maximum Leaf Width (cm)	SPAD
-	**Control**	0	95.35	7.05	26.47	6	93.80	562.8	55.6	1.6	2.5	27.5
	Acute Gamma											
1	DA10.3	10	77.5	9.55	1	7	90.24	631.68	57.7	1.5	2.4	24.8
2	DA10.4	10	85	8.29	1	8	91.3	730.4	57.9	1.5	2.3	26.3
3	DA30.2	30	79	8.46	1	7	85.23	596.61	56.0	1.5	2.3	26.8
4	DA30.5	30	71.5	8.24	1	7	93.95	657.65	56.6	1.6	2.5	24.3
5	DA40.4	40	80	10.34	1	8	93.72	749.76	55.1	1.7	2.5	28.5
6	DA80.2	80	72.5	11.59	1	8	85.56	684.48	48.5	1.7	3.5	23.8
7	DA100.2	100	78	10.14	1	9	105.98	953.82	51.5	2.0	2.9	26.4
8	DA100.5	100	73.5	9.03	1	7	88.56	619.92	46.2	1.9	2.6	26.8
	Chronic Gamma											
1	DC54.1	54.63	118	11.1	16.72	5	112.4	562.0	60.4	1.8	2.7	31.6
2	DC95.2	95.61	130	5.83	37.86	5	154.04	770.2	72.2	2.1	3.1	30.9
3	DC95.3	95.61	141	10.84	23.04	5	117.94	589.7	62.6	1.8	2.7	32.4
4	DC150.4	150.24	114	8.2	13.04	6	108.80	652.8	60.4	1.8	2.6	30.7
5	DC150.5	150.24	109	8.98	11.8	6	106.28	637.68	59.6	1.7	2.8	30.5
6	DC232.3	232.19	114	8.46	15.31	5	119.44	597.2	61.8	1.9	2.9	31.5
7	DC409.5	409.74	107	8.54	19.11	6	99.81	598.86	52.2	1.9	2.7	30.5

Table 4: Table 3: Summary of major morphological and physiological characteristic of M_1V_5 cv. Taiwan mutant lines at 30 days after planting (DAP)

No	Mutant lines	Dose (Gy)	Plant Height (cm)	Stem Diameter (mm)	Internode Length (mm)	Number of Leaf (a)	Leaf Area (b)	Estimated Total Leaf Area (a x b)	Leaf Length (cm)	Average Leaf Width (cm)	Maximum Leaf Width (cm)	SPAD

-	**Control**	0	122.9	9.43	28.76	6	107.56	645.36	69.3	1.5	2.4	30.3
	Acute gamma											
1	TA10.3	10	153	8.31	47.53	6	127.56	765.36	64.1	1.9	2.9	27.2
2	TA20.5	20	144	7.76	46.87	6	125.16	750.96	72.2	1.7	2.6	30.4
3	TA30.3	30	150	9.23	50.74	5	132.33	661.65	74.1	1.7	2.7	27.9
	Chronic gamma											
1	TC65.1	65.07	157	11.35	93.77	5	142.45	712.25	78.2	1.8	2.6	31.9
2	TC178.3	178.93	158	8.61	78.89	5	148.39	741.95	81.9	1.8	2.9	37.9
3	TC178.4	178.93	153	10.74	62.29	5	144.82	724.1	85.2	1.6	2.6	39.9

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