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EFFECTS OF CHRONIC GAMMA IRRADIATION ON THE GROWTH OF LOCAL TARO VARIETY (*Colocasia Esculenta L.* WANGI)

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ABSTRACT

Mutation breeding has extensively been utilised for many years to enhance the agronomic qualities of crops. Taro (Colocasia esculenta L.) is an economically important tuber plant, hence induced mutation offers the best way to induce genetic variations for its breeding programme. The purpose of this study was to determine the effects of chronic gamma irradiation in local taro cultivar, Wangi. This research was conducted at the chronic gamma irradiation facility (Gamma Green House), Nuklear Malaysia, Selangor from 12th July until 18th August 2019. Suckers of taro (Wangi variety) were exposed to chronic gamma irradiation (low dose, long period) at 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), 8 (0.04 Gy/h), and 10 (0.03 Gy/h), in which ring 2 is the nearest to the radiation source whilst ring 10 is the farthest. The effects of gamma irradiation on growth traits were measured after 35 days of irradiation by measuring plant height, petiole height, width and length leaf, number of leaf and survival rate. The results showed that the LR_{25} (Leaf Reduction) occurred at 245.90 Gy. Leaf shapes variation (variegated and wrinkled) were observed prominently at accumulated dose 120.12 Gy (ring 2) and 268.28 Gy (ring 3), respectively. Plant and petiole height, leaves width and number of leaves were affected at all doses of irradiation. The findings in this study showed the potential of using chronic gamma irradiation to create genetic variations in taro genotypes.

Keywords: Chronic gamma irradiation, *Colocasia esculenta*, Gamma Green House, taro, wangi variety

INTRODUCTION

Taro (*Colocasia esculenta* L.) is among the major root and tuber food crops around the world. The plants are native to Asian region, although it is mostly grown and become the primary source of carbohydrate in West Africa (Rao et al., 2010). Globally, taro ranks fourteenth among staple crops, with 9 million tons produced globally on some 1,730,000 hectares of land, out of which 74.9% of taro is produced in Africa whereas 21.7% is produced in Asia (FAOSTAT, 2017). In Malaysia, taro (*C. esculenta* L.) is one of the crops that are consumed across the country. However current taro production in Malaysia is very low (4.1 t/ha), compared to the other countries (DOA, 2018).

Previously, conventional breeding has been widely used to develop new taro cultivars for various aspects such as disease resistance (Brooks, 2005; Deo et al., 2009). The most common methods of conventional breeding are hybridization and selection (Borojevic, 2002). However, this technique takes a longer time and much cost to produce the desired new variety. Along with the rising of the population, demands for carbohydrates source, including taro, continue to increase year by year. Therefore, it is crucial to utilise breeding approaches that can produce new taro cultivar rapidly. Mutation breeding has long been used by breeders (Pathirana, 2011). It has several advantages, being the main benefit is to improve a defect in an otherwise elite cultivar, without losing its agronomic and quality characteristics. For vegetative crop improvement, mutation breeding is the direct alternative that can be considered (Sahoo et al., 2015).

For countries that primarily consume taro as their main source of carbohydrates such as Africa and Hawaii, mutation breeding programme have been extensively practiced. Seetohul et al. (2008) used mutation breeding programme to enhance the genetic properties of *Colocasia esculenta* var. esculenta for resistance against Taro Leaf Blight (TLB). Plantlets of taro were exposed to acute gamma radiation with 0, 2, 4, 6, 8, 10, 12, 14, 16, 20, 40 and 60 Gy. Sahoo et al. (2015) used the seeds *of Colocasia esculenta* L. exposed to 10 Gy gamma irradiation for testing against the same disease.

In addition, Fadli et al. (2018) used mutation breeding on taro white (*Xanthosoma Sagittifolium* L.) by exposing their shoots with 0, 30, 60, 90, and 120 Gy doses of gamma radiation. The plant samples are then evaluated for their changes in morphology characters. Unfortunately, to date there is no reported research on taro mutation breeding programme in Malaysia. Therefore, this study was carried out as an attempt to use induce mutation technique to determine the radiosensitivity and lethal doses as well as to investigate the effects of chronic gamma irradiation on growth traits of taro wangi cultivar in Malaysia.

MATERIALS AND METHODS

Plant Material

Local taro variety "Wangi" used in this study were obtained from Malaysian Agricultural Research and Development Institute (MARDI). The healthy and uniform size of the suckers consisted of 5 replicates were used in this study. The suckers were exposed to chronic gamma irradiation in Gamma Green House of Nuklear Malaysia (radiation source: Caesium-137).

Experimental Design

Each replicate consisted of 5 suckers. The planted suckers were placed in polybag at 7 different rings. The rings used were 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), 8 (0.04 Gy/h) and 10 (0.03 Gy/h) (Azhar and Wahab, 2014) The growing plants received dose rates ranging from 0.03 Gy/day (Ring 10, 10 m from the radioactive source) to 0.67 Gy/day (Ring 2, 2 m from the radioactive source) for 16 hours every day. Total radiation doses received by the plants were recorded for 35 days from 12th July to 18th August. Control plants (non-irradiated, 0 Gy) were placed in the shade house nearby the Gamma Green House.

Data Collection

Data on survival rate (%), plant and petiole height (cm), width (cm) and number of leaves (n), as well as leaf morphology were recorded on a weekly basis for the duration of eight weeks. Leaf morphological characters observed include wrinkled, variegated or normal leaf condition.

Determination of Effective Mutation Dose (LR_{25})

This experiment was carried out to determine the appropriate mutation dose (LR_{25}) which induce 25% reduction in the growth of the irradiated plants.

Data Analysis

Analysis of Variance (ANOVA) was performed using SPSS version 16 to determine the effect of gamma irradiation on growth traits of taro (Wangi variety). The mean differences between the doses were further tested using Tukey's method at 5% confidence interval.

RESULTS AND DISCUSSION

This study was carried out to determine the effect of chronic gamma irradiation on the growth of *C. esculenta* L., Wangi variety. Chronic gamma irradiation is the continuous exposure of low dosage of gamma rays to the target samples over long period of time (Ahmad et al., 2018). A total of 200 taro suckers from "Wangi" variety were exposed to caesium-137 as the radiation source for 35 days. Total accumulated dose from this experiment was 268.28 Gy (Ring 2), 120.12 Gy (Ring 3), 68.07 (Ring 4), 44.05 (Ring 5), 28.03(Ring 6), 16.02 (Ring 8) and 12.01 (Ring 10). Based from the result, *C. esculenta* L. Wangi showed tremendous effects of growth in terms of plant and petiole height, leaves width and leaves number, as well as the condition of the leaves against different doses of chronic gamma irradiation.

Lethal dose (LD) is an indicator on how much lethal effects that radiation can cause to the tested samples (Saganuwan, 2017). According to Fadli et al. (2018), the determination of optimal dosage in induction of mutation that produce the most mutants and give rise to diversity normally occurs at LD_{50}. However, in this study, all irradiated plant samples showed high survival rate (above 80%). Therefore, lethal dose of 50% deaths (LD_{50}) could not be extrapolated. Therefore, it is easier to consider leaf reduction doses, LR_{25}, which respectively means that growth parameters are assumed to be decreased by 25 percent over respective power. In the present study, growth reduction in LR_{25} was calculated and evaluated for the leaf width after the gamma irradiation. The estimation of these values was based on the quadratic formula obtained in a dose plot versus the leaves width characters (Figure 1). The values recorded for LR_{25} were 245.90 Gy.

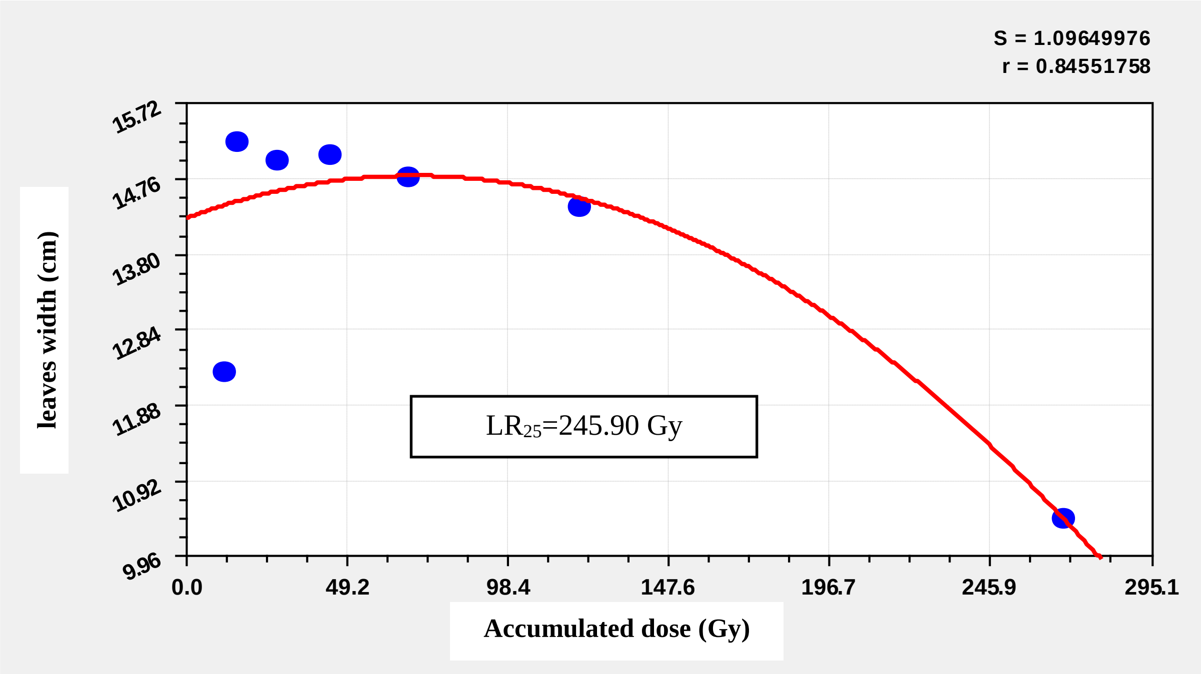

Figure 1: Effect of gamma irradiation on the leaves reduction of *C. esculenta*. Value of width reduction (LR) is determined by using CurveExpert software. There was a quadratic fit Y= a + bx + cx^2 with a = 1.427E+001, b = 1.544E-002, c = -1.109E-004

Gamma rays is a physical mutagen that have ionizing radiation. Ionizing radiation have enough energy to detach electrons from atoms and create free ions. The energy absorbed from ionizing radiation can trigger changes in plants at molecular level. The changes caused is usually resulting from the formation of free radicals within the plant molecular composition (Spencer-Lopes, 2018). Free radicals are any reactive substance that able to exist independently, that contains one or more impaired electron (Phaniendra et al. 2015). Free radicals are formed resulting from the ionization process from the gamma irradiation towards the plant cells. This occurs relatively to the water content of the irradiated samples. This is because water molecules (H_2O) are polar molecules that are easily reacted towards the ionizing energy of gamma radiation. Therefore, higher content of water in the irradiated plants will cause higher number of free radical formed. This free radical will in turn react to any molecule in the irradiated plant cells to form stable products, hence causing changes in molecular structure of the cell. This will lead to mutation. According to Aisyah (2006), higher number of free radicals formed will increase the sensitivity of plants. However, in the case of chronic irradiation, the doses given to the irradiated plants are relatively low, therefore the ionizing energy might not be strong enough to form high number of free radicals, thus causing most of the irradiated plants to survive the irradiation (Caplin and Willey, 2018).

Table 1 showed the growth parameter observed in *C. esculenta* L. against different doses of gamma irradiation. Generally, increasing doses of gamma irradiation resulted in the decrease of growth in *C. esculenta* L. Irradiated taro plants that received an accumulated dose of 68.07 Gy (Ring 4) recorded the highest plant and petiole height. Irradiated plants that received 44.05 Gy (Ring 5) showed the highest leaves width and leaves number. While irradiated plants that received 268.28 Gy (Ring 2) showed lower plant and petiole height, as well as leaf width and number of leaves. Although there is no definitive study on chronic gamma irradiation on similar plant, the results from studies conducted by Nur Aziliana et al. (2015) showed similar pattern on the growth of rice (*Oryza sativa).* Irradiated rice plants located in Ring 4 (120.12 Gy of cumulative dose) and 5 (68.07 Gy) were found to be taller as compared to the plants in located in Ring 2 (268.28 Gy).

Table 1: Effect of chronic gamma irradiation on the growth parameters of *C. esculenta* L. in Gamma Green House

Treatment	Ring	Survival Rate (%)	Plant Height (cm)	Petiole Height (cm)	Leaves Width (cm)	Leaves Number (n)
Days of exposure to gamma radiation (35^{th})						
Accumulated dose (Gy)						
12.01	10	100^{a}	40.60^{c}	34.21^{cd}	12.30^{b}	3.04^{bc}
16.02	8	100^{a}	40.26^{c}	30.67^{d}	15.24^{a}	2.48^{cd}
28.03	6	100^{a}	47.43^{bc}	36.53^{bc}	15.01^{a}	3.56^{ab}
44.05	5	100^{a}	52.63^{ab}	40.06^{b}	15.06^{a}	3.72^{a}
68.07	4	100^{a}	53.32^{a}	41.17^{b}	14.78^{a}	3.04^{bc}
120.12	3	100^{a}	49.86^{ab}	40.64^{b}	14.40^{a}	3.12^{abc}
268.28	2	88^{c}	47.24^{c}	40.74^{b}	10.44^{b}	3.05^{cd}
Control	0	96^{b}	58.67^{a}	48.86^{a}	15.04^{a}	2.31^{d}
Mean			48.75	39.11	14.03	3.04
SD			6.30	5.44	1.74	0.48
CV			12.92	13.91	12.40	15.79

Mean within a column followed by the same letter are not significantly different ($p < 0.05$)

As observed in this study, higher doses of gamma irradiation have adverse effect on the physiology of the irradiated plants. According to Ritongga and Wulansari (2010), this phenomenon is defined as the damage in chromosomes resulted from the exposure of the irradiated plants at cellular level to physical mutagen. They stated that the higher the dose of the physical mutagen, the lower the plant height, due to the damage that the physical mutagen inflicted to the chromosomes, resulting the upset of the plant growth.

Genetic mutation is the heritable changes to an individual genetic composition. These changes usually producing new traits that are passed on from parent to offspring and eventually will leads to evolution. Naturally, mutation is caused by errors in replication of DNA. However, these changes also can be induced by the exposure of radiations (Spencer-Lopes et al., 2018). In this study, it was observed that as doses of the radiation increase, the frequency of leaf mutation was also increased (Figure 2). At cumulative dose of 268.28 Gy (Ring 2), all the irradiated plants showed changes in their leaf's appearance. At the lowest dose rate and accumulated dose, 12.01 Gy or Ring 10, the irradiated plants have the lowest number of mutated leaves. The similar pattern was also observed in studies conducted by Fadli et al. (2018).

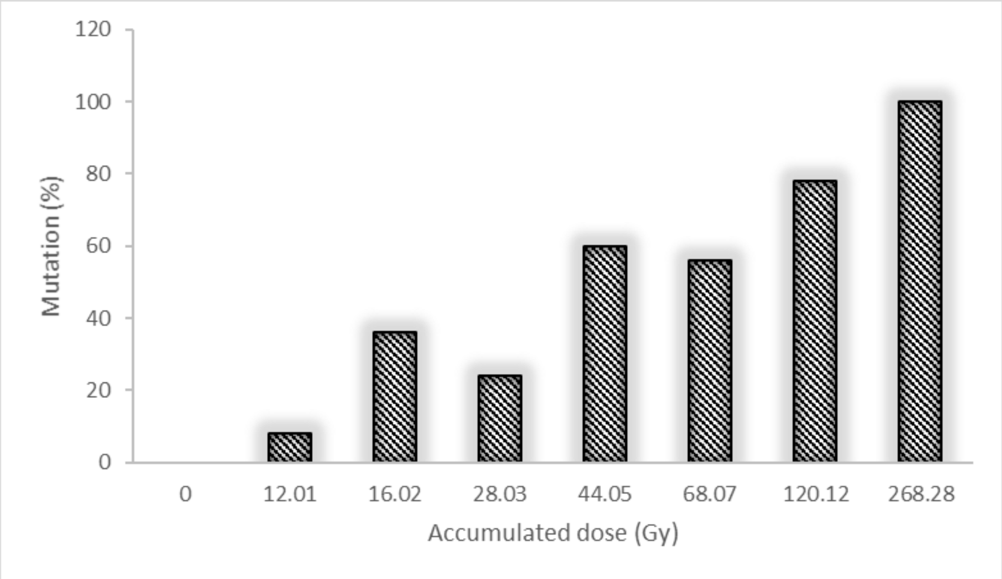

Figure 2: The frequency of leaf mutation occurred in irradiated plants

Most changes observed on leaves of irradiated *C. esculenta* L. were wrinkle and variegation. Variegation of leaves is a phenomenon in which there is different coloured zones appearance in the leaves, and sometimes also occurs on the stem (Wang et al., 2004). Variegation occurs if there is a lack of chloroplast on the leaves or if chloroplast DNA is damaged and cannot formed chlorophyll (Fadli et al., 2018). As the irradiated plants being exposed to the physical mutagen, the ionizing energy will cause damage to the chloroplasts in the plant cells of the irradiated plant leaves, unabling the chloroplasts to produce chlorophyll. This results in the variegation of leaves. Therefore,increasing the dosage of irradiation will also increase the frequency of variegated leaf formation (Figure 3).

Figure 3: Different forms of *C. esculenta* leaves after irradiation. From left to right: wrinkled (268.28 Gy, Ring 2), variegated (120.12 Gy, Ring 3) and normal leaves (0 Gy, control)

CONCLUSIONS

In this study, the LR25 values for *C. esculenta L. 'Wangi'* using chronic gamma irradiation were 245.90 Gy. Increasing dosage of gamma irradiation was found to greatly affect the growth parameters. The frequency of mutated leaf formation increases as the irradiation dose increase. This finding is very useful to serve as the basic information for taro breeders to develop new varieties by using chronic gamma irradiation approach.

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