Journal of Academia Vol. 10, Issue 2 (2022) 81 – 86

SCREENING OF LOW OXALATE CONTENT IN M1V4 MUTANT LINE OF TARO (*Colocasia esculenta*) CV. WANGI

Nur Alia Nadhirah Zainal[1], Siti Nursyazwani Maadon[1], Siti Nor Atika Baharin[2], Nor'Aishah Hasan[1]

[1]*School of Biology, Faculty of Applied Sciences*
[2]*School of Chemistry and Environment, Faculty of Applied Sciences*
Universiti Teknologi MARA (UiTM), Cawangan Negeri Sembilan, Kampus Kuala Pilah, 72000 Kuala Pilah, Negeri Sembilan, Malaysia

Corresponding author: nursyazwani87@uitm.edu.my

Abstract

Raw taro causes unpleasant sensations such as prickliness or itchiness, hence it needs to be cooked before consumption. It is caused by calcium oxalate that formed as needle-shaped crystals in parts of taro, *Colocasia esculenta*. The purpose of this study is to determine the effects of chronic gamma irradiation in local taro cultivar, Wangi to its oxalate content. The effect of gamma irradiation in mutant line of taro, M1V4 to reduce the oxalate content was discussed. Using volumetric analysis, total oxalate content is significantly reduced in the treatment groups as compared to the control group. Treatment groups 1 (0.66 Gy) and 2 (0.33 Gy) that has been exposed to the highest dosages of chronic gamma irradiation reported the lowest oxalate contents among the samples. The results analyzed with one-way ANOVA and least significant difference (LSD) test supported that a higher dosage of gamma irradiation affected the oxalate content in the corms adversely. Although chronic gamma irradiation is found to be capable of reducing oxalate content in taro corms, precise and critical methods should be employed to evaluate for low oxalate content in taro mutant lines.

Keywords: Total oxalate, corm, taro, *Colocasia esculenta*, permanganometric titration

Article History:- Received: 12 September 2022; Revised: 2 October 2022; Accepted: 9 October 2022; Published: 31 October 2022
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Introduction

Taro is one of the main crops cultivated in Asia. *Colocasia esculenta*, commonly known as taro, is propagated in a widespread manner across tropical areas with high rainfall. Locally known in Malaysia as 'keladi', it is a root crop that is regarded as staple in Asia Pacific regions such as the Philippines, Papua New Guinea, Palau, and Fiji (Rashmi, 2018). Taro is favoured and cultivated mainly for its edible parts which are the corms, roots, and leaves. Besides, it can also be grown under variable climate conditions, and can be found in flooded area, swamps, irrigated terraces, and even dry lands (Mabhaudhi, 2014). Due to the flexibility of growing taro, it can also be found in domestic homes and small fields. Taro is easily manipulated to be made into palatable food, as its fleshy corms can be baked, boiled, mashed, and fried. The leaves are usually blanched or simmered but it can be added to soups or stews. The versatile consumption makes it easy to be manipulated into any palatable dishes.

In Malaysia, taro is not only a source of food, but it is also integral to the culture as herbal remedies and traditions item. The cultivation of taro is not fully optimized as some cultivars are grown only for its petioles and leaves (Onwueme, 1999). Malaysia might be producing its own taro, but the total oxalate content of local taro cultivar, especially of variant wangi is high with an average of 178.3 ± 5.1 mg/100 g DW, as compared to other variants such as minyak, cina, hijau, tapak badak, and hitam (Zulkhairi, et al., 2020). Kaushal et al., (2015) have highlighted that taro is also one of the most consumed crops in the tropical countries including Malaysia. However, the production is not parallel to the demands it received. Through mutation breeding, the treatment of taro of cultivar wangi with gamma irradiation has produced a mutant line, M1V4, that can yield taro with potentially lower oxalate content. Diet with high oxalate content cause adverse health effect particularly because the oxalate will cause reduced mineral absorption. The oxalate will bind with the minerals inside body and form kidney stones (Mitchell et al., 2019). Through this study, the yield of M1V4 mutant line of taro wangi can be compared to its original strain. Evaluation of M1V4 mutant line quality which has lower oxalate content compared to the taro in the control group as the mutant line have been treated with gamma radiation can be conducted. With these findings,the production of taro will be able to attract the interest of locals towards this acclimatized crop as it can produce yields that can reduce the preparation time prior to consumption. Subsequently, with the discovery of the new mutant line, it can be incorporated in staple diet as quality of taro as food is increased alongside reduced adverse health effect.

Methods

Plant materials

Samples of mutated taro *Colocasia esculenta* cv. wangi of mutant line M1V4 is obtained from Malaysian Agricultural Research and Development Institute (MARDI) Serdang, in which the taros were treated with chronic gamma irradiation from radiation source Caesium-137, at Malaysian Nuclear Agency (Suhaimi et. al, 2020). The doses of each treatment depended on its position, or rings, from the radiation source (**Error! Reference source not found.**). There were 6 different rings used as noted in Table 1 below. Chronic gamma irradiation dose for each ring depends on the distance between the ring to the source (Caesium-137). The taro suckers were exposed to chronic gamma irradiation for 35 days, and received a varying degree of accumulated dose (Gy). Radiation doses were measured with a dosimeter.

Table 1. The rings and its corresponding doses (Gy/day) (Hasan et al., 2020)

Treatment	Rings	Chronic Gamma Irradiation Dose (Gy/hr)	Accumulated dose (Gy)
Control	-	0	0
1	2	0.66	268.28
2	3	0.33	120.12
3	4	0.17	68.07
4	5	0.11	44.05
5	6	0.07	28.03
6	10	0.03	12.01

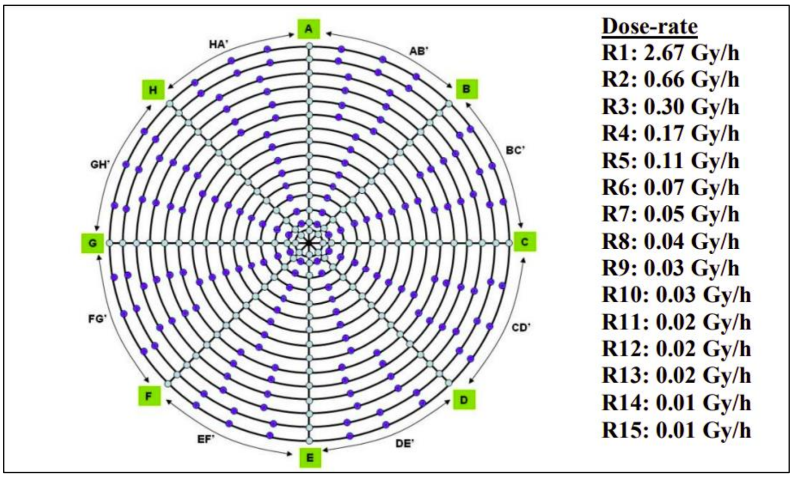

Figure 1. Dose mapping inside the Gamma Greenhouse of Malaysian Nuclear Agency, Bangi (Ahmad et. al., 2018)

Oxalate content

The oxalate content was extracted by the method employed in research by Nurilmala and Mardiana (2019), which is a three-step process that included maceration of taro corms, oxalate precipitation and permanganometric titration. The taro corms from each treatment were dried and made into powder. 2 g of sample powder were macerated in 20 mL of 0.15% citric acid for 6 hours at room temperature, A platform shaker was used to ensure that the powder is thoroughly soaked in the acid. After 6 hours, the solution was filtered and acidized with 10% tungstophosphoric acid. Precipitation will occur after 5 mL of tungstophosphoric acid was added. The mixture was centrifuged at 1500 rpm for 5 minutes. Precipitate and supernatant obtained were dissolved with 2M H_2SO_4 and heated until the solution reached 70ºC. The solution was titrated against 0.01M $KMnO_4$ to determine the calcium oxalate content. This method is known as permanganometric titration. Titration was made in triplicate and the calcium content amount was determined from the amount of $KMnO_4$ consumed to complete the titration.

Data analysis

The data were recorded and the variation between the means of each treatment were observed with analysis of variance (ANOVA) using SPSS version 20. The data collected were presented as means ± standard error (SE).

Result and Discussion

The calcium oxalate content range obtained from the study conducted has large difference compared to the total of oxalate content in taro in another research by Zulkhairi et al. (2020). T6 has the highest amount of total oxalate among the irradiated taro, at 11424.81 ± 505.17 mg/100g, in which it was treated to Caesium-137 at 0.03 Gy/day. The lowest value of total oxalate in corms was recorded at 70.5 ± 20.1 mg/100g. From Table 2, it is observed that chronic gamma irradiation affected the oxalate content in taro as the oxalate content differs from the control group.

Table 2. Mean values (± SE) for oxalate content in mg/100g in taro corms that have been treated with different dosage of chronic gamma irradiation

Treatment	Mean oxalate content (mg/100g)
Control	12375.75 ± 329.43 ^{a,b}
1	6092.78 ± 344.54 ^{c}
2	7374.96 ± 937.45 ^{c}
3	9431.77 ± 309.08 ^{d,e}
4	10221.03 ± 494.00 ^{d,f,g}
5	10267.17 ± 97.99 ^{e,f,h}
6	11424.81 ± 505.17 ^{b,g,h}

Statistically significant at α=0.05; based on LSD test and 95% confidence, means that do not share the same letter are significantly different.

As visualized in the bar chart, Figure 2 the total oxalate content in the treatment groups is generally lower than in the control group. Treatment group 1 which has received the highest accumulated dose of irradiation has the lowest oxalate content at 7374.96 ± 937.45 mg/100g while treatment group 6 with lowest accumulated dose at 12.01 Gy has been analyzed to contain a closer amount to the control group, at 11424.81 ± 505.17 mg/100g. However, the values obtained through volumetric analysis in this study were higher than the concentration reported previously, in which the oxalate content of taro ranges from 24% to 88.9% of total oxalate content (Hang et al., 2011). The total oxalate content in a study by Kristl et al. (2021) ranges from 133.7–229.7 mg 100 g^{-1} DW. In comparison, cultivars in Madeira reported oxalate content ranging from 153.2–353.4 mg 100 g^{-1} DW (Gouveia et al., 2018).

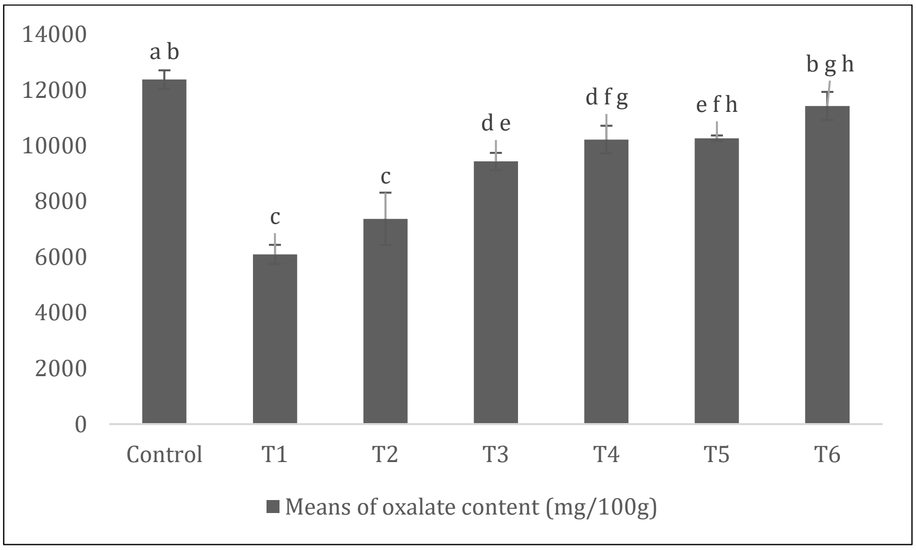

Figure 2. Bar chart for oxalate content in different treatments of irradiated corms of taro. Means that do not share the same letters are significantly different by LSD test ($p<0.05$).

This can be due to the reasons that the study is conducted on a varietal difference, as this study focuses on variety wangi. Cultivar wangi has the third highest oxalate content compared to other varieties (Zulkhairi et al., 2020). Besides that, the huge difference may be impacted by the nutrient availability factor. *C. esculenta* is a root crop that grows its corms underground. As such, the abiotic factors interacting with the plant such as temperature, soil type and climate could have affected the results as well. These abiotic factors are also known as agro-ecological differences. The physiological basis for interactions among different plant nutrients are dynamic as it is affected by the plant itself and its mechanism of regulating the plant growth. Higher dosage of gamma irradiation could also be inflicted on the taro sucker before propagating it to further lower the oxalate content. Personal error in the analysis could not be avoided as volumetric analysis through titration requires observation of endpoint through the change of color in titrate, which might be biased as the color change occurs subtly and for a short while.

The total oxalate contents in the irradiated groups are generally lower than in the control groups, as shown in Figure 2. From the result, it can be inferred that exposure to higher dosages of chronic gamma irradiation has affected the oxalate content in the corms of *C. esculenta.* Even though Siddhuraju et al., (2002) has found that gamma irradiation can significantly reduce the antinutritional factors in plant materials such as soybean, the opposite occurs in this study. Similarly, total yield of this mutant line needs to be compared. In this study, gamma irradiation amplifies the oxalate content instead of reducing it. According to Ahuja et al. (2014), radiolysis occurs more often when gamma radiation is absorbed by plant material, among other interaction with atoms and minerals inside the cells. Water content in plants, as supported by Marcu et al. (2013), becomes the main target of the ionizing radiation, leading to increase of free radicals that could damage or modify the physiological or biochemical processes of the organism in study. The moisture content after exposure to gamma ray might alter the pathway of calcium oxalate formation. Synthesis of ascorbic acid utilized in formation of oxalate (Nakata, 2003) may have been modified as well. As supported by Singh and Datta (2010), they stated that low gamma irradiation improved plant nutrition but not the quality of grains related to micronutrients. The antinutritional value and nutritional value alike need to be investigated together for a clear correlation to be made.

Conclusion

Based on this experiment, the study managed to determine the oxalate content of M1V4 mutant line in corms of taro, *C. esculenta* and compared it to the control group. It can be concluded that higher dosage and exposure to chronic gamma irradiation have an adverse effect on the oxalate content in taro by reducing it. However, the total oxalate content for the mutant line M1V4 acquired through this study is comparatively higher than the reported ranges from various taro cultivated in other countries. Although screening of low oxalate content cannot be determined through volumetric analysis, it can serve as a preliminary screening before proceeding with other methods that are costly or proceeding with the selection of suitable breeds to be propagated for further analysis or use. Further studies on the determination of oxalate content in taro should be conducted using UV-Vis spectrophotometer and HPLC to determine the oxalate content in the corms and possibly other parts of taro such as the petioles and leaves. Studies on the nutritional content of local taro could also be conducted to ascertain the values of carbohydrates, proteins and fats obtained from its consumption.

Acknowledgement

Authors are thankful to Malaysia Nuclear Agency and Malaysian Agricultural Research and Development Institute (MARDI) for their supportive encouragement and guidance for the smooth completion of the work.

Author Contribution

NAN Zainal – collecting data, data processing, and analysis, manuscript writing; SN Maadon, SNA Baharin, NA Hasan – experimental design, conceptualization, supervision, manuscript writing, review, and editing.

Conflict of Interest

The authors declare no competing interests

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