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Identification of Genetic Diversity among Mutant Taro (*Colocasia esculenta* L. *cv* WANGI) Using Agro-Morphological Trait and Simple Sequence Repeats (SSR) Molecular Markers

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Genetic diversity Taro

ABSTRACT

Taro (*Colocasia esculenta*) is one of the traditional crops with enormous sources of dietary fiber, carbohydrates, vitamins, and minerals contents. Mutation breeding using gamma radiation is one of the most preferred approaches used to induce mutation in taro studies. Molecular markers are widely used to detect such induced mutation and genetic diversity in plants. Therefore, the present study was carried out to evaluate genetic diversity among irradiated taro genotypes in comparison with standard taro variety by using simple sequence repeats (SSR). A total of 200 of M_1V_4 taro genotypes were used in this study derived from segregating population of chronic-gamma irradiated taro *cv* Wangi with different ranges of gamma dose. The agro-morphological results revealed that genotype exposure in T6 (120.12 Gy) has the highest plant height (54.53 cm), leaf length (32.24 cm), and leaf width (24.87 cm). Corm's weight was decreased significantly with an increased dose of treatment. All mutants recorded a lower number of corm weight as compared with the control genotype. Out of 10 SSR primers tested, 9 primers have successfully amplified 43 amplicons. The polymorphism information content (PIC) values of SSR markers ranged from 0.20 to 0.80. Cluster analysis classified taro into 3 subgroups mutant and parent genotypes. The results clearly showed that SSR markers are important tools to distinguish mutant genotypes

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and confirmed their usefulness for phylogenetic studies. Finally, the present investigation indicated that genotypes exposed by T6 (120.12 Gy) are promising high-yielding genotypes that can be recommended as new cultivars and possessed an attractive phenotype appropriate for ornamental use.

1 Introduction

Colocasia esculenta L. Schott locally known as taro is a major source of energy, and minerals including calcium, iron, fiber, and vitamins that are good for human health. According to Zulkhairi et al. (2020), taro ranked fifth worldwide among the tuber crops and starchy roots after cassava, yam, potato, and sweet potato, and is categorized as an underutilized crop. With the rise in consumer population and patterns in search for healthy food, the demand for taro production has been increasing, and there has been a great deal of research aiming to develop new taro varieties (Koffi et al. 2021).

The naturally existing genetic variability in taro is not sufficient to achieve the desired improvement. Genetic variability in this crop has been exhausted due to natural selection and hence, conventional breeding methods are not very fruitful due to labor-intensive and time-consuming (Miyasaka et al. 2019; Legesse and Bekele 2021). Additionally, taro is affected by at least 10 major diseases and pests in different parts of the world including taro leaf blight (TLB) caused by the fungus-like oomycete *Phytophthora colocasiae*, Raciborski which can reduce corm yield by up to 50% (Mishra et al., 2019; Mbi et al. 2021). Therefore, mutation breeding has been an alternative way of achieving new taro cultivars.

Mutations can occur spontaneously or be generated by mutagens, which are divided into two categories: physical and chemical mutagens (Ma et al., 2021; Kazama et al., 2008). The effect of chemical mutagens on plant materials is generally considered milder, however, chemical mutagens are generally carcinogenic and have uncertain penetration of the target plant (Seetohul et al., 2007; Oladosu et al. 2015). On the other hand, physical mutagen such as ionizing radiation takes less time for irradiation and creates more damage to DNA strands, therefore considerably helping to improve plant breeding.

Previous researchers have used mutation breeding programs to enhance the genetic properties of *C. esculenta* var. *esculenta* for resistance against taro leaf blight (TLB) as shown by Sahoo et al. (2015). These findings indicated that γ-rays suppressed the growth of Phytophthora spores in the leaf tissues of taro cultivars namely Telia and Satasankha under in vitro conditions. Fadli et al. (2018) used white taro cultivars (*Xhanthosoma sagittifolium* L) to study the effect of cobalt-60 gamma-ray irradiation. Findings suggested that among various doses of gamma rays tested (30; 60; 90; and 120 grays), leaf color variation only occurred at irradiation rates of 30 and 90 gray while plant heights occur at all levels of irradiation. In addition, these researchers identified the Lethal dose (LD50) at 60 grays. In addition, Matsumaya et al. (2020) performed mutational breeding by heavy-ion beam irradiation of multiple shoots of the 'Chiba maru' cultivar. These researchers reported that by using 2–10 Gy neon and carbon ion beams, they achieved a plant survival rate of more than 90 % and used 94 surviving plants for genomic screening. Unfortunately, till now, there is no reported research on the taro mutation breeding program in Malaysia. Therefore, this study is an attempt to use the chronic gamma irradiation approach to improve taro *cv* Wangi in Malaysia.

The identification of crop diversity is important in plant breeding programs. This is because the identification and characterization of crops are the initial steps in every introduction and improvement program where it can provide valuable information for their introduction and genetic improvement. Previously, identification based on morphological characteristics has been used for many cultivars. However, morphological identification is very subjective because some of the morphological structures are susceptible to the effect of environmental factors and significant variations have been observed to vary in different regions. Therefore, the existing DNA marker technology is the utmost way for assessing genetic relatedness and variability among the crops (Cretazzo et al. 2022). The uses of molecular markers have been employed to determine the diversity of various crops (Rasco et al. 2016) where the simple sequence repeats (SSR) tend to be among the most polymorphic genetic marker types and have been used in the plant genetic identification process (Korir et al. 2013) compared to other markers such as amplified fragment length polymorphism (AFLP) and restriction fragment length polymorphism (RFLP). This is because SSRs do not necessitate high concentrations or quality of DNA and show great repeatability across laboratories and are genetically codominant. Thus, it is suitable for gathering information on polymorphisms to differentiate across genotypes and identify the representative materials for the genetic diversity collection (Romero et al. 2019). Recently, SSR markers were extensively used to study phylogenetic relationships and differences among taro cultivars in Malaysia and India, respectively (Khatemenla et al. 2019; Shahril et al. 2020). In the present study, genetic differences of mutant lines derived from chronic gamma irradiation *cv* Wangi were also determined using SSR markers.

2 Materials and Methods

2.1 Plant Material and Breeding scheme

The Malaysian Agricultural Research and Development Institute (MARDI) provided a local taro cultivar (*C. esculenta* L. cv Wangi). Constant and healthy suckers of taro Wangi variety (a total of 200) were irradiated using chronic gamma-ray (radiation source: cesium-137) at Nuclear Malaysia Gamma Greenhouse facility for 35 days control group was placed in a shelter house nearby. The shoot growth from the irradiated sucker (M_1V_1) was planted on the field on day 35 after irradiation. The irradiated sucker growth from the M_1V_1 plant was planted at the MARDI greenhouse to develop M_1V_2 populations. Growth data were collected in M_1V_2 generations and chimeric structures were observed and eliminated. Selected individuals with M_1V_2 mutated sucker were propagated for further observation and uniformity in M_1V_3 generation. Then, the potential individuals M_1V_3 mutated sucker with good growth traits were further propagated and planted until being harvested in the field to assess the growth, the agronomy, and also phenotypic traits. The selection process was repeated to generate the M_1V_4 mutant generation. For this study, a total of seven advanced mutant taros from the different treatments of chronic gamma irradiation as shown in Table 1 with one control parent (non-mutant cv Wangi) were selected. Monitoring was done in every week with recommended management practice followed by Hasan et al. (2020).

2.2 Agro-morphological analysis

The agro-morphological characteristics of taro were observed after 6 to 8 months before planting in the field. According to International Plant Genetic Resources Institute (2000), nine quantitative traits such as plant height, leaf length, leaf width, number of leaves, number of suckers, number of stolons, corm weight, corm diameter, and corm width traits were measured in the M_1V_4 generations.

2.3 Extraction of genomic DNA

The healthy young leaves were harvested from each treatment of M_1V_4 generation taro genotypes for extraction of genomic DNA. The DNA extraction was executed following the CTAB protocol given by Khumaida et al. (2017) with slight modification. The young leaf tissue was cut into small pieces and put into 1.5ml centrifugation tubes with 800µL of CTAB and ground by using a tissue lyser. Then, the sample was incubated in a water bath in 65°C for 1 hour. The equal volumes of chloroform:isoamyl alcohol (24:1) was added into the centrifuge tubes before being centrifuged at 14000 rpm for 15 minutes. After that, the supernatant was transferred into fresh tubes and 200µL of cold isopropanol was added. The pelleting of nucleic acids was obtained by centrifuging at 14000 rpm for 5 minutes. The supernatant was removed and the pellet was washed with 70% of ethanol and centrifuged at 13000 rpm for 4 minutes. The ethanol was discarded and dried at room temperature to obtain the pellet. The obtained DNA was suspended into 100µL of TE buffer and stored at 4°C until it is ready to be used. The quantification of extracted DNA was tested by using a NanoDrop Spectrophotometer (Thermo Scientific NanoDrop™ 1000 spectrophotometer) and 2% of high-resolution agarose gel electrophoresis.

2.4 Polymerase Chain Reaction (PCR) based SSR Marker

Ten SSR primers previously evaluated by Khatemenla et al. (2019) were used as shown in Table 2. PCR amplification was conducted on a thermal cycler in a final volume of 25.0µL containing 1.0µL template DNA, 5.0µL buffer, 0.5µL dNTPs, 3.0µL $MgCl_2$, 1.0µL each of forward and reverse primers, 0.2µL Taq polymerase, and 13.3µL molecular water. The SSR profiles were denaturated for five minutes at 94°C, then went through 35 amplification cycles (denaturation at 94°C for 15 seconds, annealing between 47°C and 51°C for 15 seconds, extension at 72°C for 15 seconds, and final extension step at 72°C for 7 minutes). A total of 5µL of amplified PCR product was separated through gel electrophoresis on 3% agarose gel stained with FloroSafe DNA Stain and 100 bp DNA ladder as a reference before the product was visualized using Molecular Imager® (GelDoc™ XR, Bio-Rad).

Table 1 Taro Mutant and Dosage Rate of Chronic Gamma Irradiation

Treatment	Ring	Chronic gamma doses (Gy)	Accumulated dose (Gy)
T0	-	0	0
T1	10	0.03 Gy/h	12.01
T2	8	0.04 Gy/h	16.02
T3	6	0.07 Gy/h	28.03
T4	5	0.11 Gy/h	44.05
T5	4	0.17 Gy/h	68.07
T6	3	0.30 Gy/h	120.12
T7	2	0.66 Gy/h	268.28

Ring 10, 10 m from the radioactive source to 0.67 Gy/day; Ring 2, 2 m from the radioactive source

Table 2 The nucleotide sequences (5' to 3') of the primers used for SSR analysis

Primer name	Forward primers	Reverse primers	Temperature (°C)
Ce1 A06	5'-GCT TGT CGG ATC TAT TGT- 3'	5'-GGA ATC AGT AGC CAC ATC-3'	51
Ce1 B03	5'- TTG CTT GGT GTG AAT G-3'	5'- CTA GCT GTG TAT GCA GTG T-3'	51
Ce1 C03	5'- TGT TGG GAA AGA GGG-3'	5'- GGG GAA TAA CCA GAG AA-3'	51
Ce1 C06	5'- CCA GAA GAG ACG TTA CAG A-3'	5'- ACG ACT TTG GAC GGA-3'	47
Ce1 F04	5'- AGG GAA TAC AAT GGC TC-3'	5'- ACG AGG GAA GAG TGT AAA-3'	47
Ce1 H12	5'- TAG TTA GCG TGC CTT TC-3'	5'- CAA CAA CTT AAT GCT TCA C- 3'	51
Uq73-164	5'- ATG CCA ATG GAG GAT GGC AG-3'	5'- CGT CTA GCT TAG GAC AAC ATG-3'	47
Uq84-207	5'- AGG ACA AAA TAG CAT CAG CAC-3'	5'- CCC ATT GGA GAG ATA GAG AGA- 3'	51
Uq97- 256	5'- GTA ATC TAT TCA ACC CCC CTT-3'	5'- TCA ACC TTC TCC ATC AGT CC-3'	49
Uq201- 302	5'- CTA AGG AGA GGA GAT CCG AAC-3'	5'- CAA GAC GAT GCT GAA CCA- 3'	49

2.5 Data analysis

Data were analyzed with one-way ANOVA using the statistical program (SPSS version 20). Means separation was carried by the least significant difference (LSD) and Tukey's multiple range tests with significance determined at $P \leq 0.05$. The bands obtained in DNA samples amplified were scored '0' for absence and '1' for presence to create a binary data matrix. The polymorphism information content (PIC) of each marker was calculated using the following formula:

$$PIC = 1-\Sigma x2i$$

The xi is the frequency of i^{th} allele for each SSR locus. The similarity index was calculated by using Jaccard's coefficient to identify the genetic similarity. The genetic similarity (GS) matrix between treatments based on molecular data was computed using Wang et al. (2021) coefficient where the similarity matrix was used to produce an agglomerative hierarchical clustering by employing Unweighted Pair Group Method with Arithmetic Averages (UPGMA) with average linkage then graphically converted into a dendrogram by using NTSYSpc version 2.1 software.

3 Results and Discussions

Physical mutagenesis by gamma irradiation can be used to induce mutations and to create genetic variation with a desirable mutant. Similar to other countries, taro is also considered as a neglected crop and cultivated by a small farmer in Malaysia as a carbohydrate source. However, with the increasing demand for a healthy lifestyle, it is a great potential to promote taro as an alternative carbohydrate source (Okpul et al. 2005). With 43 local varieties available in Malaysia, breeders are actively finding an alternative way to improve the production of taro with high yield, early maturity, and ability to tolerate the local climatic environment (Zulkhairi et al. 2020). However, there is limited information on the genetic variability of taro. Taro *cv* Wangi is a popular Malaysian local genotype for its large central corm. However, it contains high oxalate contents (Abdullah et al. 2021). To the best of our knowledge, this is the first study in Malaysia that has stated the use of the mutation induction approach in taro that can be applied to improve the genetic variation of taro.

3.1 Agro-morphological Traits

The morphological traits of each taro genotype were observed in each replicate of each treatment. The average of agronomic data such as plant height, leaf length, leaf width, number of leaves, number of suckers, number of stolons, corm weight, corm length, and corm diameter was recorded as shown in Table 3.

On average, plants exposed by T6 (102.12 Gy) were the tallest among all materials tested, with a mean height of 54.53 cm. On the other hand, the shortest plant was observed in T1 (12.01 Gy) among all mutants with a 34.98 cm mean height. The leaf length and the leaf width displayed a similar trend. Plant height is the popular metric for assessing the biological impacts of physical mutagenesis. Findings in this study demonstrated that the higher gamma irradiation doses resulted in slower height growth. Previous research by Fadli et al. (2018) on Indonesian taro *cv* white suggested that plant stunted or decreased in plant height was due to the impact of high doses in gamma irradiation that triggered its physiological disorder or chromosomal damage.

For leaf traits, the longest length and the widest width leaf were observed in mutants' plants in T4 and T6, while the shortest was recorded in plants exposure to T1 (12.01 Gy) and T2 (16.02 Gy) (Table 3). The plant in T6 (120.12 Gy) was observed to have a greater number of leaves than the plant exposed by T2 (16.02 Gy) which showed a fewer number of leaves. Similarly, Gharib (2021) showed that gamma rays influenced the number of leaves, and had the greatest effect at a 10 Gy dose, but demonstrated no effect on shoots. In addition, a study from Nurilmala and Mardiana, (2018) on the effect of gamma rays in stevia stated that the low dose exposure

Table 3 Morphological traits of taro mutants in M_1V_4 generations

Mutant plants	Plant height (cm)	Leaf length (cm)	Leaf width (cm)	Number of leaf (n)	Number of sucker (n)	Number of stolon (n)	Corm weight (kg)	Corm length (cm)	Corm diameter (cm)
Control (parent)	43.67	24.76	18.14*	4.64	0.84*	0.36	1.62*	8.60	15.08
T1	34.98*	18.02*	12.38*	4.68	1.00	0.36	0.27	10.12	15.92
T2	37.93*	18.98*	13.37*	3.96*	0.96	0.40*	0.31	8.36	14.72
T3	36.39*	19.21*	12.53*	4.36*	0.72*	0.16	0.19*	6.16	9.44*
T4	46.48	25.83	17.75*	5.08	1.52*	0.40*	0.37	9.88	16.84*
T5	44.66	23.02	16.60*	5.16	0.92	0.32*	0.27*	7.88	15.24
T6	54.63*	32.24*	24.87*	6.36*	1.36*	0.60	0.34	9.80	17.52*
T7	47.48	25.52	17.40*	4.88	1.12	0.36	0.17*	5.88	9.72*
LSD (0.05)	1.24	2.10	3.07	1.26	1.54	1.58	0.99	1.08	1.34

Mean separation within columns by LSD and Turkey's multiple range tests at 5% level

Table 4 Summary statistics of SSR polymorphisms in this study

Primer	No Amplified Products	No of Polymorphic Products	Polymorphic Loci (%)	Polymorphic information content value (PIC)
Cel A06	1	1	100	0.61
Cel B03	2	1	50	0.80
Cel C03	9	2	22	0.65
Cel C06	4	4	100	0.59
Cel F04	7	4	57	0.72
Cel H12	3	3	100	0.44
Uq 73- 164	1	0	0	0
Uq 84- 207	2	2	100	0.44
Uq 97- 256	7	6	85	0.20
Uq 201-302	7	7	100	0.80
Total	43	30	714	5.22
Average	4.3	3.0	71.4%	0.52

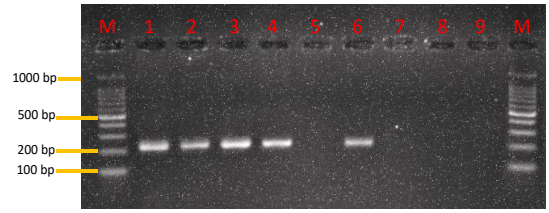
of gamma rays can enhance the growth and the development of the leaves. Furthermore, the highest number of suckers were recorded in plant exposure in T4 and T6. In contrast, plants in T3 recorded the lowest number of suckers and stolon. Corm weight varied significantly among the various mutants. The parent genotypes showed the highest corm weight when compared with other mutants. In contrast, plant exposure in T7 (268.28 Gy) and T3 (28.03 Gy) showed a minimum corm weight of 0.17 and 0.19 kg, respectively. Among mutants, plants of T6 (120.12 Gy) and T4 (44.05 Gy) recorded the highest weight of corm. This was aligned with a study by Lee et al. (2020) where he reported the highest corm weight by using 30 Gy of gamma irradiation. The highest corm length was recorded in the plant of T1 (12.01 Gy) and the lowest was recorded in plant exposure by T7 (268.28 Gy). The widest corm was measured in plants exposed by T6 (120.12 Gy) while the narrowest corm was measured in the plant of T3 (28.03 Gy). The mutation technique has been successfully employed in taro to isolate good mutants with desired economic plant characters (Sianipar and Maarisit 2015). Previous studies indicated that irradiation of mutants can increase carbohydrate and low calcium oxalate contents (Manzila et al. 2020). Morphological characteristics application to evaluate the genetic diversity was reported to have certain disadvantages as the expression of the phenotypes is influenced by the growth stages and various environmental factors (Ahn et al. 2018).

Therefore, the use of DNA markers is encouraged as it is simple, rapid, and reproducible by experiment regardless of environmental conditions, accurate monitoring of seed purity, and determination of cultivars, and it also can be performed at a low cost (Cretazzo et al. 2022).

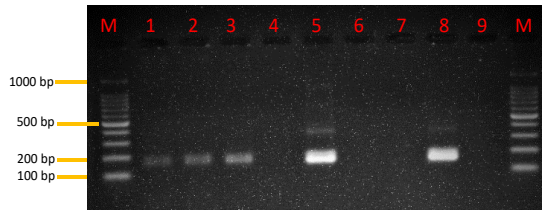
3.2 Microsatellite polymorphism and genetic variability analysis among genotypes

A total of 10 SSR loci were used for the estimation of genetic diversity among 8 mutants and parent taro *cv* Wangi parental lines. The level of polymorphism among these 8 genotypes of taro was

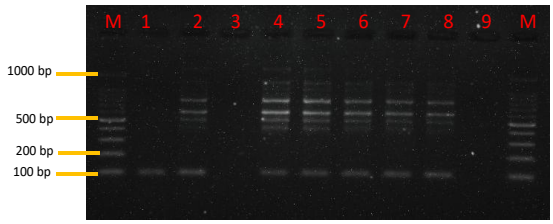
evaluated by calculating allele number and PIC values for each of the 10 SSR loci. The SSR primer pairs used for genetic diversity analysis, the number of alleles for each SSR locus, and the PIC values are presented in Table 4.



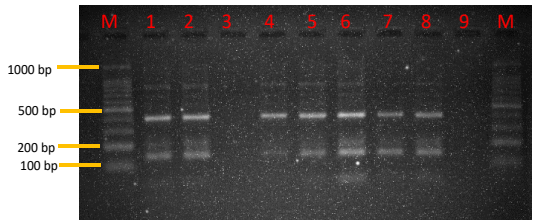
a) SSR primer Cel A06



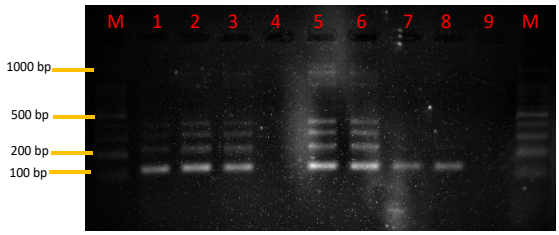
b) SSR primer Cel B03



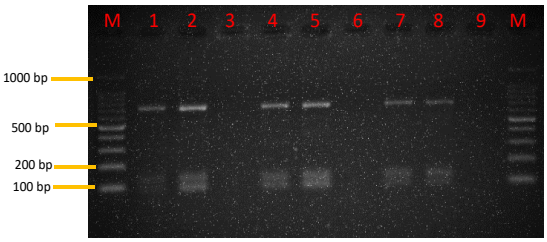
c) SSR primer Cel C03



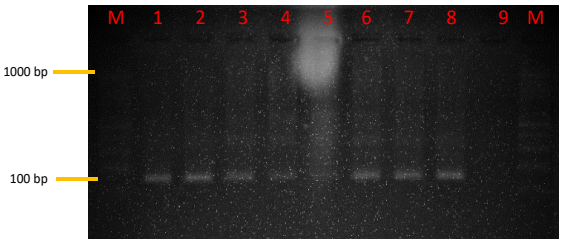
d) SSR primer Cel C06



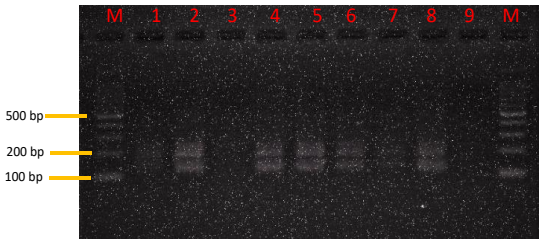
e) SSR primer Cel F04



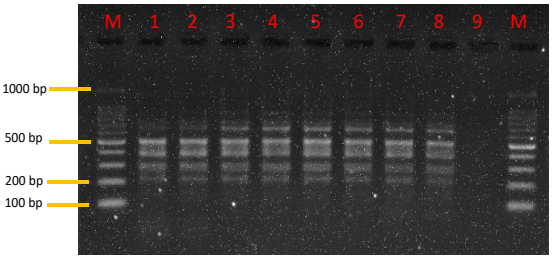
f) SSR primer Cel H12



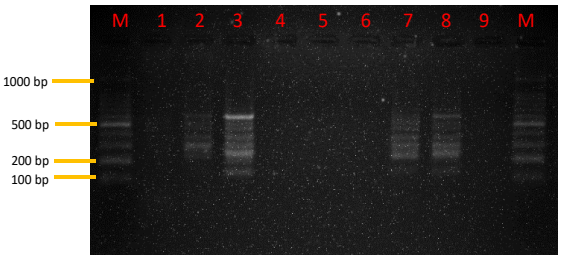
g) SSR primer Uq73- 164



h) SSR primer Uq84- 207



i) SSR primer Uq97- 256



j) SSR primer Uq201-302

Figure 1 Banding pattern of mutant genotype and parent revealed by 10 SSR primers; Lane 1: Parent (taro); Lane 2-8: mutant genotype; Lane 9: Negative control, M: 100bp DNA Ladder

The data from the banding pattern of taro mutant genotypes have been analyzed using 10 polymorphic SSR markers yielding a total of forty–three (43) scorable amplicons were detected with an average of 4.3 alleles per locus. In this study, the total of DNA bands obtained were 30 polymorphic bands out of 43 bands with an overall polymorphism percentage was 71.4 percent. Primer Cel C03 produced the maximum number of amplified fragments with 9 bands meanwhile primer Uq 73- 164 and primer Cel A06 produced the minimum number of amplified fragments (Table 4; Figure 1).

Nine out of ten primers showed polymorphic changes. However, the analysis using primer Uq 73- 164 showed a monomorphic band (Table 4) (Figure 1). Based on the previous study by Oladosu et al. (2015) in mutant rice, monomorphic bands are consistent bands that cannot be used for genetic diversity research but polymorphic bands produce differences that can be used to observe the systematic relationship between the populations that is important to identify genetic changes. In addition, a previous study by Singh et al. (2012) stated that some of the mutants can produce different bands from the control even if the mutants were irradiated by the same dose of gamma rays. Meanwhile, the same size of DNA bands between mutants showed amplified DNA sequence homology while specific bands in one mutant proved the unique genetic variety making the DNA bands profile depend on the degree by which irradiation affects the cells.

Some of the SSR primers were very informative with polymorphic information content (PIC) ranging from 0.20 to 0.80 with an average of 0.52 while one primer which is Uq 73- 164 primer showed no PIC value since it is monomorphic in Table 4. These results were comparable to the PIC values of some mutant crops from previous studies by Manzila et al. (2020) on induced chili pepper mutants (0.53), Shahril et al. (2020) on induced rice mutants (0.51), and gamma-irradiated cowpea mutants (0.51) by Olasupo et al. (2018). The most informative SSR primers were Cel B03 and Uq 201-302 which have the same value (PIC = 0.80). Similarly, Khatemenla et al. (2019) worked on taro in Northeast India and reported Uq 201-302 with (PIC = 0.82) as the most informative marker. SSR markers with a PIC value of 0.5 or higher were considered effective in selecting the polymorphism rate and have potential in the evaluation of genetic variance (Sianipar et al. 2015). Meanwhile, the least informative primer was Uq 97- 256 with (PIC = 0.20) since the PIC value was closer to one considered to be more informative (Khatemenla et al. 2019).

3.3 Genetic relationship between mutants and control *cv* wangi

The DNA bands of the obtained taro mutants had been converted into a binary number where “1” indicated the presence of a band and “0” indicated the absence of a band. The binary data of the SSR profile can be used to produce a genetic similarity matrix using NTSYSpc software and Jaccard’s coefficient of similarity. The genetic similarity based on SSR markers ranged from 0.22 to 0.83 (Table 5). Genetic similarity analysis has been done previously by Singh et al. (2012) in mung bean mutants which ranged from 0.78 to 1 based on SSR molecular markers. The highest genetic similarity was observed between mutant genotypes exposure in T6 (120.12 Gy) and T7 (268.28 Gy) (0.83) followed by T1 (12.01 Gy) and T7 (268.28 Gy) (0.72). The highest genetic similarity was found between control with mutant genotypes exposure in T1 (12.01 Gy) (0.65) and T4 (44.05 Gy) (0.63) while the lowest similarity was reported in mutant genotypes T2 and T3 (0.22). The genetic similarity between mutant genotype and control was observed between control and plant in T2 (16.02 Gy) (0.38). An earlier study on rodent tuber mutants by Sianipar et al. (2015) reported a significant effect of gamma irradiation on plant mutant genotype, the smaller genetic similarity with more differences were observed in the mutant genotype. This can be observed between control and mutant genotypes exposure of T2 (16.02 Gy), T3 (28.03 Gy), T6 (120.12 Gy), and T7 (268.28 Gy) that have smaller genetic similarities in this study. These results also indicated that the control and taro mutant lines were genetically different.

Table 5 Similarity matrix based on Jaccard’s Coefficient of Similarity of taro mutants

	Control	T1	T2	T3	T4	T5	T6	T7
Control	1.00							
T1	0.65	1.00						
T2	0.38	0.53	1.00					
T3	0.53	0.50	0.22	1.00				
T4	0.63	0.59	0.29	0.61	1.00			
T5	0.60	0.56	0.38	0.58	0.68	1.00		
T6	0.40	0.58	0.41	0.56	0.53	0.48	1.00	
T7	0.46	0.72	0.47	0.57	0.57	0.50	0.83	1.00

Note: “T= treatment”, “1 to 7= number of treatments”

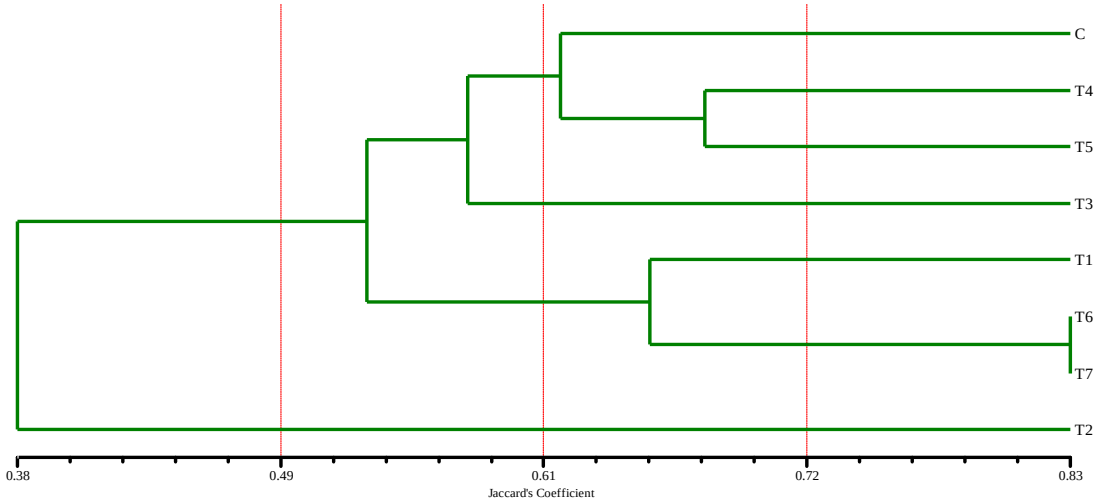


Figure 2 Dendrogram of taro mutant lines and the control based on SSR marker analysis constructed with the UPGMA method

The analysis was carried out by using quantitative similarity with Jaccard's similarity coefficient while clustering using Sequential Agglomerative Hierarchical Nested (SAHN) analysis to generate a dendrogram of mutant taro and control based on SSR markers analysis by using UPGMA cluster (Figure 2). The analysis method revealed that the dendrogram with a similarity coefficient of 0.38 to 0.83. At a similarity coefficient of 0.49, three clusters were formed cluster I consisted of 3 mutant genotypes and control (parent) samples while cluster II consisted of 3 mutant samples, and cluster III consisted of only one mutant sample. Cluster I was divided into two sub-clusters at the coefficient of similarity of 0.61 consisted sub-cluster A with 3 samples which were 2 mutants that have been irradiated by T4 (44.05 Gy) and T5 (68.07 Gy) together with a control sample. Sub-cluster B consisted of only one sample that was irradiated with T3 (28.03 Gy) gamma-ray. Whereas, cluster II consisted 3 mutant samples which were exposed to T1 (12.01 Gy), T6 (120.12 Gy), and T7 (268.28 Gy), while cluster III consisted of only one sample which was in T2 (16.02 Gy) that showed to be divergent and clustered on its own main cluster.

Conclusion

In the present study, taro mutant genotypes showed a wide range of agronomic characteristics as the effect of chronic gamma irradiation. The present study elucidated morphological and genetic characteristics of parent genotype and seven selected mutants to determine the best genotype. Plant exposure by T7 (268.28 Gy) showed as the promising mutant with the highest plant height (54.53 cm), leaf length (32.24 cm), and leaf width (24.87 cm). The markers generated by SSR primers allowed detection of genetic polymorphism and genetic fingerprinting among the mutants of taro tested. In doing so, they have shown to be useful for the construction of a germplasm collection and providing additional information that could form the basis for the rational design of breeding programs. The present characterization strategies will help to exploit different mutant lines for developing new taro genotypes with desired traits.

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References

Abdullah, S., Fauzi, N.Y.M., Khalid, A.K., Osman, M., & Mohamad, A. (2021). Effect of Gamma Rays on Seed Germination, Survival Rate and Morphology of *Stevia rebaudiana* Hybrid. *Malaysian Journal Fundemantal Applied Science*, *17*(5), 543-549.

Anh, V.L., Inoue, Y., Asuke, S., Vy, T.T.P., Anh, N.T., & Wang, S. (2018). Rmg8 and Rmg7, wheat genes for resistance to the wheat blast fungus, recognize the same avirulence gene AVR-Rmg8. *Molecular Plant Pathology*, *19*, 1252–1256.

Cretazzo, E., Moreno Sanz, P., Lorenzi, S., Benítez, M.L., Velasco, L., & Emanuelli, F. (2022). Genetic Characterization by SSR Markers of a Comprehensive Wine Grape Collection Conserved at Rancho de la Merced (Andalusia, Spain). *Plants*, *11*, 1088.

Fadli, N., Syarif, Z., Satria, B., & Akhir, N. (2018). The effect of gamma cobalt-60 ray irradiation on cultivar growth in taro white

(*Xhanthosoma sagittifolium* L.). *International Journal Environmental Biotechnology Agricultural*, *3*(6), 268284.

Gharib, A.H. (2021). Inducing Genetic Variation in Taro Using Gamma Irradiation. *Egyptian Journal Basic Applied Science*, *36*(3), 73-86.

Hasan, N.A., Suhaimi, L., Ahmad, F., Mohamed Bahari, U., Harun, A.R., & Rafii, M.Y (2020). Effects of Chronic Gamma Irradiation on the Growth of Local Taro Variety (*Colocasia esculenta L.* WANGI). *Jurnal Sains Nuklear Malaysia*, *32*(2), 23 – 30.

International Plant Genetic Resources Institute (IPGRI) (2000). IPGRI Annual Report (1999). Rome, Italy: IPGRI (pp. 40)

Kazama, Y., Saito, H., Yamamoto, Y.Y., Hayashi, Y., et al. (2008).LET-dependent effects of heavy-ion beam irradiation in *Arabidopsis thaliana*. *Plant Biotechnology*, *25*, 113– 117.

Khatemenla, Alam, S., Barooah, M., Phookan, D.B., et al. (2019) SSR Marker-Based Molecular Characterization of Some Upland Taro (Colocasia esculenta L. Schott) Cultivars of North-East India. International Journal of Current Microbiology and Applied Sciences, 8(06), 2310-2320.

Khumaida, N., Ardie, S.W., & Astuti, M.S. (2017). Characterization of Irradiation Induced Mutants of Cassava (*Manihot esculenta* Crantz) Generated from Jame-jame and Adira-4 Genotypes at M_1V_2 Generation. *KnE life science*, 22-28. DOI https://doi.org/10.18502/kls.v2i6.1016.

Koffi, J., Koffi, K., Bonny, S., & Bi, A. (2021) Genetic Diversity of Taro Landraces from Côte d'Ivoire Based on Qualitative Traits of Leaves. *Agricultural Sciences*, *12*, 1433-1446.

Korir, N.K., Han, J., Shangguan, L., Wang, C., et al. (2013). Plant variety and cultivar identification: advances and prospects. *Critical Review Biotechnology*, *33*(2), 111-125.

Lee, H.Y., Moon, S., Ro, H.S., Chung, J.W., & Ryu, H. (2020). Analysis of Genetic Diversity and Population Structure of Wild Strains and Cultivars Using Genomic SSR Markers in *Lentinula edodes*. *Mycobiology*, *48*(2), 115–121.

Legesse, T., & Bekele, T. (2021). Evaluation of improved taro (*Colocasia esculenta* (L.) Schott) genotypes on growth and yield performance in North-Bench woreda of Bench-Sheko zone, South-Western Ethiopia. *Heliyon*, *7*(12), e08630.

Ma, L., Kong, F., Sun, K., Wang, T., & Guo. T. (2021) From Classical Radiation to Modern Radiation: Past, Present, and Future of Radiation Mutation Breeding. *Frontier Public Health*, *9*,768071.

Manzila, I., Priyatno, T.P., Nugroho, K., Terryana, R.T., & Hidayat, S.H. (2020). Molecular and morphological characterization of EMS-induced chili pepper mutants resistant to Chili veinal mottle virus. *Biodiversity Journal*, *21*(4), 1448-1457.

Matsuyama, T., Watanabe, M., Murota, Y., Nakata, N., et al. (2020). Efficient mutation induction using heavy-ion beam irradiation and simple genomic screening with random primers in taro (*Colocasia esculenta* L. Schott). *Scientia Horticulturae*, *272*,109568.

Mbi, T.K., Godswill, N.F., Brice, T.L., & Emmanuel, Y. (2021). Field management of Taro (*Colocasia esculenta* (L.) Schott) leaf blight via fungicidal spray of foliage. *Journal Cameroon Academy Science*, *16*(3), 197-208.

Mishra, D., Bhoi, L., Dash, M., Tripathy, S.K., et al. (2019). Mutagenic effectiveness and efficiency of EMS and gamma rays on rice bean (*Vignaumbellate* (Thunb) Ohwi and Ohashi): An underutilized legume crop. *International Journal of Conservation Science*, *7*(3),2060-2064.

Miyasaka, S.C., Renee, M.B., Michael, B., Kantar, M.H., Thomas, W.R., & Paudel, M.S. (2019). Genetic Diversity in Taro (*Colocasia esculenta*). In: Nandwani, D. (eds) *Genetic Diversity in Horticultural Plants, Environmental Development Sustainability*, vol 22, (pp 191–215), Springer, Cham.

Nurilmala, F., & Mardiana, D. (2018). Nutrients and Anti-nutrients Content Analysis of Bogor Taro Mutant Clone (*Colocasia esculenta*). *IOP Conference Series: Earth and Environmental Science*, *334*,012070.

Okpul, T., Mace, E.S., Godwin, I.D., Singh, D., & Wagih, M.E. (2005). Evaluation of variability among breeding lines and cultivars of taro (*Colocasia esculenta*) in Papua New Guinea using ISSR fingerprinting and agro-morphological characterization. *Plant Genetic Resources Newsletter*, *143*, 8-16.

Oladosu, Y., Rafii, M.Y., Abdullah, N., Malek, M.A., et al. (2015). Genetic variability and diversity of mutant rice revealed by quantitative traits and molecular markers. *Agrociencia Uruguay*, *49*(3), 249-266.

Olasupo, F.O., Ilori, C.O., Stanley, E.A., Owoeye, T.E., & Igwe, D.O. (2018). Genetic Analysis of Selected Mutants of Cowpea (*Vigna unguiculata* [L.] Walp) Using Simple Sequence Repeat and rcbL Markers. *American Journal Plant Science*, *9*(13), 2728.

Rasco, J.L.S., Mendoza, M.R.R., & Abustan, M.A.M. (2016). Molecular Characterization of Taro [*Colocasia esculenta* (L.) Schott] Using SSR Markers. *Philippine Journal Crops Sciences*, *41*(3),65-73.

Romero, M., Mujica, A., Pineda, E., Ccamapaza, Y., & Zavalla, N. (2019). Genetic identity based on simple sequence repeat (SSR) markers for Quinoa (*Chenopodium quinoa* Willd.). *Ciencies Investigacion Agraria*, *46*(2), 166-168.

Sahoo, B.B., Kole, P.C., & Sahoo, M.R. (2015). Effects of γ Irradiation on Leaf Blight Disease of Some Taro (*Colocasia esculenta* (L.) Schott) Genotypes. *International Journal Biology Resources*, *6*(1),007-014.

Seetohul, S., Puchooa, D., & Ranghoo-Sanmukhiya, V.M. (2007). Genetic Improvement of Taro (*Colocasia esculenta var esculenta*) through in-vitro mutagenesis. *University Mauritius Research Journal*, *13A*, 79-89.

Shahril, A.R., Azman, N., Kamaruzaman, R., Amri, S.Y., et al. (2020). Genetic diversity of released Malaysian rice varieties based on single nucleotide polymorphism markers. *Czech Journal Genetics Plant Breeding*, *56*, 62-70.

Sianipar, N.F., Laurent, D., Purnamaningsih, R., & Darwati, I. (2015). Genetic variation of the first generation of rodent tuber (*Typhonium flagelliforme* Lodd.) mutants based on RAPD molecular markers. *HAYATI Journal Bioscience*, *22*(2), 98-104.

Sianipara, N.F., Maarisit, W.A. (2015). Detection of Gamma-Irradiated Mutant of Rodent Tuber (*TyphoniumflagelliformeLodd*.) *In Vitro* Culture by RAPD Molecular Marker. *Procedia Chemistry*, *14*, 285 – 294

Singh, S.K., Lavanya, G.R., Bhat, K.V., Babu, G.S., et al. (2012). Microsatellite markers revealed genetic diversity in mungbean mutant lines. *Indian Journal Hill Farming*, *25*(1), 38-43

Wang, S., Ge, S., Colijn, C., Biller, P., Wang, L., & Elliott, L. T. (2021). Estimating Genetic Similarity Matrices Using Phylogenies. *Journal of computational biology: a journal of computational molecular cell biology*, *28*(6), 587–600.

Zulkhairi, A.M., Razali, M., Umikalsum, M.B., Norfaizal, G.M., Athirah, A.A., & Aisyah, M.S. (2020). Determination of Oxalates in Corms of Selected Taro (*Colocasia esculenta*) Varieties in Malaysia Using Ultra High-Performance Liquid Chromatography. *Asian Journal Chemistry Sciences*, *7*(3), 28-37.