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# Identification of Mutant Heliconia Using DNA Fingerprinting Approach (*Heliconia Nickerensis*)

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**Abstract:** Ornamental breeding focused on commercial traits such as flower color, longer shelf life, fragrance modification, and plant and flower architectures. Using gamma radiation, the induced mutation technique has been widely used for varietal development in ornamental plants. Induced mutation in ornamental plants helps to increase genetic variability by modifying genetic constituents through deletion (removal of DNA segment), doubling or rearrangement of the chromosomes. This genetic variability can be distinguished by DNA fingerprinting. Molecular markers have been derived to visualize DNA sequence polymorphism. One of the molecular markers that are widely utilized in DNA fingerprinting is the Simple Sequence Repeat (SSR) marker. In this study, rhizomes of *Heliconia nickerensis* were mutated by exposing them to gamma rays at different levels of dosage: 55 Gy, 65 Gy and 75 Gy. The mutants were then screened using 10 SSR markers to detect polymorphism at the genetic level. 0 Gy and *Heliconia stricta* were also included in this experiment to act as the control and outgroup samples. Out of 10 SSR markers, only 8 SSR markers can amplify the collected samples. A dendrogram was constructed to understand the effective doses of radiation on *Heliconia* mutation and effect of radiation which resulted in the DNA alterations.

**Key words:** dendrogram, gamma radiation, mutation, polymorphism, Simple Sequence Repeats (SSR) marker

## 1. Introduction

*Heliconia* species are widely distributed in tropical forests. *Heliconia* belongs to the *Heliconiaceae* family which comprises only a single genus. *Heliconia* consist of 250 to 300 species in the world. The largest number of species was found in Colombia [1]. These species usually propagate through rhizomes and seeds. The production of *Heliconia* has been increased due to the high market demand. To meet this demand, introducing a new plant variety can be an alternative way to enhance the production of *Heliconia*. The induced mutation technique in ornamental breeding was found to be the most efficient and effective technique in producing new plant varieties [2].

Many plant crops which have high market value in the food, ornamental, pharmaceutical, cosmetic and other industries are undergoing mutation induction. Using mutation radiation, ornamental plant crops have produced many new varieties such as achimenes, chrysanthemum, carnation, roses, cape primrose, lily, dahlia, bougainvillea and azalea [3]. The effect of mutation has given rise to a new variety of ornamental plant in different characteristic phenotypes such as flower color, shape and size [4].

The induction of mutagenesis can occur through radiation and chemical mutagens. In the present research, there are 90% mutant varieties obtained by the induction of gamma rays and 22% with x-rays [5]. Gamma ray radiation treatment uses electromagnetic radiation with high energy bombardment. They assist

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in rupturing the hydrogen bond between the base pair which breaks the DNA strand of the sequence.

This DNA alteration can be detectable using molecular markers. Much research has been undertaken, on microsatellites for example, using molecular markers to validate their mutants [6, 7] plus RAPD [8] and AFLP [9]. In studies of *Heliconia*, there are several molecular markers that have been used for breeding programs and diversity studies such as isoenzyme and RAPD markers [10], AFLP [11, 12] and Simple Sequence Repeat (SSR) markers [13].

SSR markers have been used for *Heliconia acuminata* species to study the effects of forest fragmentation on the population of the plant species [13]. According to the research there are 10 genomic SSR markers that have been designed and the effectiveness of the markers was evaluated. All SSR markers were proven useful for population studies of *H. acuminata* species. Thus, in the present study, these markers have been used to identify mutants of *Heliconia nickerensis* in order to understand the effect of radiation on the DNA alteration of *Heliconia*.

## 2. Materials and Methods

### *2.1 Plant Material*

In this study, 192 samples consisting of 2 species, *H. nickerensis* and *Heliconia stricta*, from the Malaysian Agricultural Research and Development Institute (MARDI) were used. The rhizomes of *H. nickerensis* accessions were irradiated using acute gamma ray with four dosages ranging from 0 (control), 55, 65 and 75 Gy from a Caesium-137 source (BioBeam GM8000) at the Malaysian Nuclear Agency [14, 15]. *H. stricta* were included in this study to act as outgroup to the species *H. nickerensis* under study.

### *2.2 DNA Extraction*

Fresh sample leaves were extracted using the conventional DNA extraction protocol. Early before extraction, the samples were kept at a temperature of -80°C for one day. The samples were disrupted and homogenized in TissueLyser II. Then, the supernatants were separated from the raw samples using the Beckman Coulter automated liquid handler (BiomekRNXp). The DNA samples were quantified using Epoch BioTek and Floroskan Ascent FL. The DNA stock samples were screened using 0.8% agarose gel. A sufficient amount of DNA was later processed using the PCR amplification protocol.

### *2.3 SSR Genotyping Using ABI 3730XL DNA Analyzer*

Ten SSR primers were designed and used for PCR amplification which was performed in a final volume of 10 µl containing 10X PCR buffer, 50 mM $MgCl_2$, 10 mM dNTP, 10 µM forward and reverse primer, 5 µM M13 primer, 20 mg/ml BSA, and 5 U/µl Taq. The amplicons were screened using 2% agarose gel and later processed for SSR genotyping using the ABI 3730XL DNA Analyzer.

### *2.4 Data Scoring and Cluster Analysis*

The data genotype was analyzed using GeneMapper software 5. The data were scored and tabulated using Microsoft Excel. The file data were further analyzed for the allelic frequency using PowerMarker V3.25 [16]. The genetic distance was calculated based on the genetic distance method of Nei [17]. A dendrogram was constructed using the UPGMA method in the NTSYSpc 2.21s software.

## 3. Results and Discussion

### *3.1 DNA QC*

The DNA concentration obtained from the *Heliconia* samples was around 110-680 ng/µl. DNA screening using gel electrophoresis showed that the extracted DNA samples were intact and sufficient for the genotyping process.

### *3.2 SSR Genotyping and Data Scoring*

Out of 10 SSR markers tested only 8 produced scorable peaks for further analysis (Table 1).

**Table 1 The 8 SSR markers used for cluster analysis.**

| SSR marker | Primer sequence 5' | Repeat motif | Annealing temperature (°C) | Allele Size (bp) |
|---|---|---|---|---|
| Hac-A103 | F: GCATTGGCTTCCTTTCTC<br>R: CTTGCTTGGTTCCTGTTG | $T_9$…$(CA)_{13}(GA)$ | 54 | 257-276 |
| Hac-A12 | F: CATCGTCTTTGCTGTAATCTTC<br>R: GTCGTAATGCTTCTTGTGATTG | $(CT)_4(GT)_{13}$ | 62 | 180-195 |
| Hac-A5 | F: TGGTCAAATCACCTTTTCAAC<br>R: GGACACCCACTCAGTCAAA | $(AT)_6(GT)_{14}$ | 58 | 178-198 |
| Hac-B117 | F: TTGCGACAGTTAAAATGAGTG<br>R: ACATACCCACTGCACGAGT | $T(TTG)_7$-TGG-$(TTG)_2$ | 58 | 215-230 |
| Hac-B6 | F: ACCAAGACCACCTCCACTC<br>R: AGGAACGAACGGCAGATAAG | $(CAA)_7$ | 59 | 274-291 |
| Hac-C114 | F: ACCTCCAAAAGGAGTAAAGCTA<br>R: AAGGTAAGGGACTGTCCTACA | $(AGA)_9$ | 62 | 254-260 |
| Hac-C7 | F: GAAGCCTCCATCATCTCTT<br>R: GGCAGAAACTGAGTGGTG | $(CTT)_7$ | 56 | 213-215 |
| Hac-D1 | F: GCGAAGAAGATGAAGAGC<br>R: CCCGACAGAAGCCCTAA | $(ATG)_9$ | 54 | 184-199 |

*3.3 Cluster Analysis*

A dendrogram was constructed using the UPGMA method in the NTSYSpc 2.21s software (Fig. 1). Cluster analysis showed that the samples were clustered into two main groups: Group A and Group B. Group A can be divided into three sub clusters: Group AI, Group AII, and Group AIII. The group details are listed in Table 2.

Group AI classified as a mixture of samples irradiated by 0, 55, 65 and 75 Gy. The samples irradiated by 55, 65 and 75 Gy that were included in this group showed allele similarity with 0 Gy samples

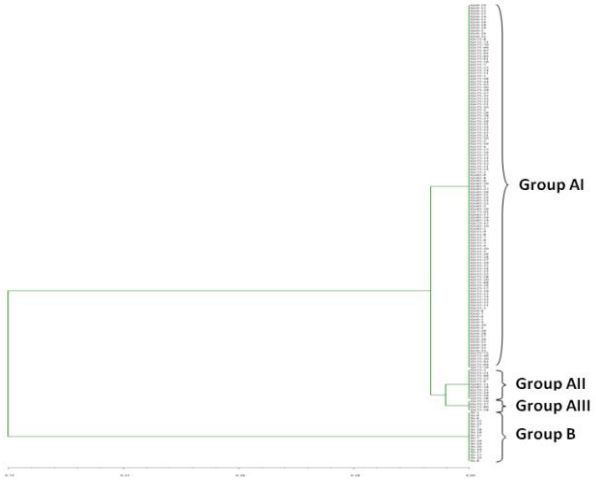


**Fig. 1 *Heliconia* mutant dendrogram generated from 8 SSR markers based on the genetic distance calculation of Nei [13] by using the UPGMA method.**

**Table 2 Groups details of *Heliconia* mutant dendrogram.**

| Group | No of samples | | | | |
|---|---|---|---|---|---|
| | 0 Gy | 55 Gy | 65 Gy | 75 Gy | St |
| AI | 27 | 26 | 18 | 58 | - |
| AII | - | 2 | 2 | 7 | - |
| AIII | - | - | - | 4 | - |
| B | - | - | - | - | 18 |
| Total | 27 | 28 | 20 | 69 | 18 |

*0, 55, 65 and 75Gy (*H. nickerensis* accessions were irradiated using acute gamma ray with four dosages: 0 (control), 55, 65 and 75 Gy); St (*H. stricta*)

(Table 3). Since there are no allele differences, these samples were considered to be non-mutated at the locus markers used in this study, even though they were exposed to the highest ray strength of 75 Gy. This might be that the SSR markers used have a conserved flanking region, thus mutation would be difficult to occur in this locus in order that the plant can protect itself from damage [11, 18].

Group AII classified as a mixture of samples irradiated by 55, 65 and 75 Gy. Slight allele differences were detected in the samples of this group. Samples amplified by the Hac-B117 marker showed changes in alleles: 220/230 instead of 230/230 when compared to samples of Group AI (Table 3). The allele changes may be due to the effect of gamma rays that ruptured hydrogen bonds between the base pairs and breaks in

**Table 3 Summary of alleles for each sample in a cluster group.**

| SSR Markers | **Group AI** (0, 55, 65, 75 Gy) | **Group AII** (55, 65, 75 Gy) | **Group AIII** (75 Gy) | **Group B** (St) |
|---|---|---|---|---|
| **Hac-A103** | 257/259 | 257/259 | 257/259 | 257/276 |
| **Hac-A12** | 186/195 | 186/195 | 186/195 | 180/184 |
| **Hac-A5** | 178/185 | 178/185 | 178/185 | 178/198 |
| **Hac-B117** | 230/230 | 220/230 | 220/220 | 215/230 |
| **Hac-B6** | 274/287 | 274/287 | 274/287 | 291/291 |
| **Hac-C114** | 254/260 | 254/260 | 254/260 | 257/257 |
| **Hac-C7** | 213/213 | 213/213 | 213/213 | 215/215 |
| **Hac-D1** | 184/199 | 184/199 | 184/199 | 189/199 |

one of the DNA strands [19]. Thus, we considered this as a slight mutation occurring in these samples.

Group AIII has only samples irradiated by 75 Gy. There are no any samples from 0, 55 and 65 Gy included in this group. Samples amplified by the Hac-B117 marker also showed changes in alleles: 220/220 instead of 230/230 (Group A1) and 220/230 (Group AII). Thus, 75 Gy may modify the genetic constituents of these samples through ruptured hydrogen bonds between the base pairs and breaks in both DNA strands [19]. The number of samples that mutated is very low even though 75 Gy could be modifying the DNA, and out of the 69 samples analyzed, only 4 samples were included in this group. Therefore, this dosage level still needs to be considered regarding whether or not it is efficient in inducing mutation in *Heliconia*.

Group B has only St samples. All SSR markers showed different alleles compared to 0 Gy samples. These samples were genetically different and proved that St samples can act as an outgroup to the species under study: *H. nickerensis*.

Regarding the overall observation, out of 8 SSR marker used in cluster analysis, only one marker, Hac-B117, can detect allele changes while the rest of the markers showed no changes. There is a possibility that the mutation rate may low in *H. nickerensis*, suggesting the use of a wide range of radiation levels [20] or adding more abundant SSR markers that would be distributed throughout the genome in order to detect mutations in more loci [18].

## 4. Conclusion

Irradiation with 75 Gy could modify DNA strands in *Heliconia*, however the probability for samples to be mutated in this study was very low. A wide range of effective radiation levels need to be studied in order to ensure the mutation is successful. An additional number of SSR markers used would also be necessary to detect as many mutations as possible in many loci throughout the whole genome.

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