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Changes in Flower Development, Chlorophyll Mutation and Alteration in Plant Morphology of *Curcuma alismatifolia* by Gamma Irradiation

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Abstract: Problem statement: *Curcuma alismatifolia* Gagnep., commonly known as 'Thai tulip' or pathumma is a member of the family Zingiberaceae. This herbaceous perennial which can be grown from rhizomes have a great potential for use as a cut flower, flowering potted plant and as a garden plant for tropical landscaping. Unlike other bulbous floral crops, research on *C. alismatifolia* using gamma irradiation has been a least subject of investigation. **Approach:** Presently, the use of induced mutation in plant improvement had become a proven way that can generate new sources of genetic variations in creating new varieties. This additional tool is important in plant improvement, which was a valuable approach to plant breeding in the world of ornamental industry. A study on the effects of gamma irradiation on mutagenesis of *Curcuma alismatifolia* was conducted to determine the optimal dose for radiosensitivity test (LD_{50}) of the plants and also to determine the effects of induced mutation on the species. This study was carried out at the Malaysian Nuclear Agency (Nuclear Malaysia), Bangi. Ten levels (dose rate) of gamma irradiation were used in this study. Data collection for days of shoot emergence, plants height, number of leaves and shoots, days to bloom, height of flowering stalks, inflorescence size and the days to anthesis (post-production longevity) were taken. **Results:** Results obtained showed that the highest survival rate was 67% obtained from the non-irradiated rhizomes (0 Gy). Fifty percent when were treated with 10 Gy and 63% survival rate when treated at 20 Gy. Mean survival rate fell sharply from 63% at 20 Gy to 7% at 30 Gy. This decreasing trend was followed by 2% survival at 40 Gy. Results indicated that the radiosensitivity test (LD_{50}) for the *Curcuma alismatifolia* was approximately at 25 Gy. **Conclusion:** Gamma irradiation had exerted various effects on growth of *C. alismatifolia,* including the survival rate of rhizomes, extension of days to shoot emergence, plant height, leaves and shoots number as well as modifications in plant morphology and flower development.

Key words: *Cucurma alismatifolia*, Gamma irradiation, mutagenesis, flower development, chlorophyll mutation

INTRODUCTION

Commonly known as Thai tulip or 'pathumma', a member of the family Zingiberaceae[1], *C. alismatifolia* has a great potential for use as cut flower, flowering pot plants and as a garden plant for tropical landscaping in various regions. They are herbaceous perennials, often with a dormancy period. The underground parts consist of short fleshy rhizomes, which were the principal organ for N storage and the storage roots were the major organs for carbohydrate storage[1]. Under natural conditions, sprouting starts in May and the dormant period starts in October [2]. Its showy inflorescence on a long peduncle comprised a compound spike of colorful spiral main bracts, which subtends a group of two to seven true flowers[1]. The prominent elliptical coma bracts are generally purplish pink with brownish green tips. Unlike other well-established bulbous floral crops, studies on *C. alismatifolia* for induced mutation have not been fully investigated. The objectives of this study were to investigate the optimum dose for radiosensitivity test of *Curcuma alismatifolia* and to determine the effects of various doses of gamma irradiation on *C. alismatifolia*.

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MATERIALS AND METHODS

The experiment was conducted at the Malaysian Nuclear Agency (Nuclear Malaysia), Bangi. Rhizomes of *Curcuma alismatifolia* var. Pink were irradiated with 10 different doses namely 0 (control), 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 Gy[3]. 30 rhizomes were treated for sensitivity testing for each dose and 30 non-irradiated rhizomes were used as control. Irradiation of the rhizomes was conducted in Block 41, MINTec-Sinagama; Agrotechnology and Biosciences Division, Taman Technology MINT, Bangi. The source of gamma rays is ^{60}Co (J.L Shepherd M-109) at 1. 858 Gy sec^{-1}, of the dose rate. The rhizomes were wrapped in newspapers and the Fricke's solution was attached together in order to measure the absorbed doses of gamma rays during irradiation. Immediately after irradiation, the rhizomes were planted in sand bed and watered once a day by mist at Ladang Flora Genetika, MINT[4]. No fertilizers were applied while the plants were growing. Radio-sensitivity test of the plant was determined by measuring the survival rate [lethal dosage (LD_{50})] of the plant 40 days after irradiation [3]. The experimental design was a Completely Randomized Design (CRD) and a significant treatment effect was determined by using the Analysis Of Variance (ANOVA). Results from the radio-sensitivity test and the observation on the highest frequency of chlorophyll mutants were used for the next stage in order to expose the rhizomes with the single dose treatment. Usually, the study can be continued until fourth generation (M_1V_4) to see the stability of the induced traits before selecting as mutated plants. However, according to Brunner[3]: Selection of mutants usually begins in the second generation or later generation for the Vegetatively Propagated Plants (VVP) because it will be much easier to recognize mutants as they segregate out. Weekly data were taken for the days for shoot emergence, plant height, number of leaves and shoots, days to bloom, height of flowering stalks, inflorescence size and the days to anthesis (post-production longevity). Morphological changes of the plants were also screened for variations in shape and chlorophyll changes on their leaves as well as modifications in flower development including changes in color and form.

RESULTS

Radiosensitivity test of *Curcuma alismatifolia* was determined by measuring the LD_{50} of the plants. A graph was plotted in order to determine the optimal doses for mutagenesis. The decreasing trend of survival rate was clearly shown in Fig. 1.

The highest mean of survival rate was 67% obtained from the non-irradiated rhizomes. At 10 Gy, the mean survival rate was 50% and at 20 Gy, the survival rate was 63%. Mean survival rate dropped sharply from 63% at 20 Gy to 7% at 30 Gy. This decreasing trend was followed by 2% survival at 40 Gy. Results indicated that the radio-sensitivity test (LD_{50}) for the *Curcuma alismatifolia* was approximately at 25 Gy. The use of gamma irradiation however extended the days for the shoot emergence from 27 days for the untreated rhizomes (control) to 40 days for the irradiated rhizomes. Plant height decreased with increasing dosage of gamma rays (Fig. 2). The tallest plant was recorded for the untreated rhizomes (0 Gy) at 79 cm, followed by 10 Gy at 74 cm and 20 Gy at 56 cm. Six weeks after irradiation, all rhizomes were killed when subjected to the treatments of 30-100 Gy.

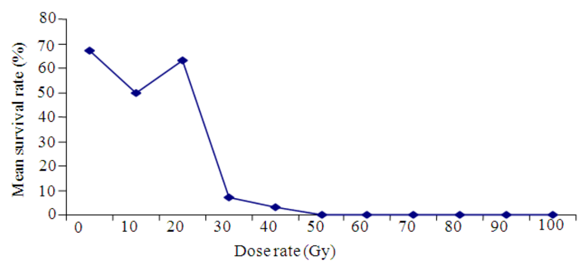

Fig. 1: Dose determination for radiosensitivity test (LD_{50}) of *Curcuma alismatifolia*

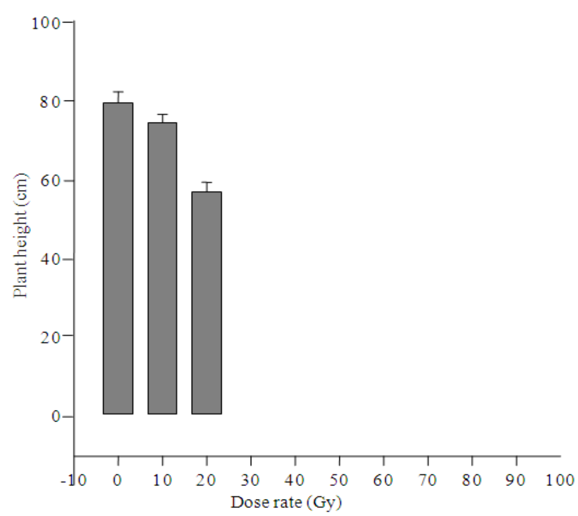

Fig. 2: Effects of gamma irradiation on plant height of *Curcuma alismatifolia*

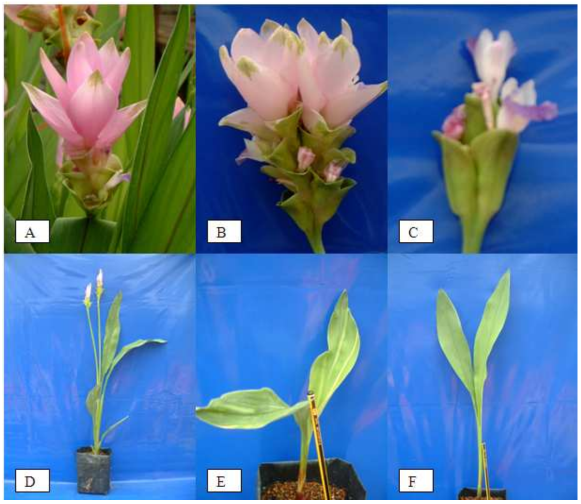

Fig. 3: Effects of gamma irradiation on flower development of *Curcuma alismatifolia* and the chlorophyll changes on their leaves; (A): *Curcuma alismatifolia* var. Pink; (B): Double inflorescence within one stalk; (C): The inflorescence without bracts; (D): Double stalk per plants; (E): Chlorophyll mutants (mericlinal chimera) observed in mutated plants; (F): Plants with untreated rhizomes (control)

The highest number of leaves was obtained from the untreated rhizomes with 7 leaves and for treatment at 10 Gy, there were 6 leaves. Irradiation at 20 and 30 Gy produced only 5 leaves. There were only 2 shoots recorded for both control and treatments at 10 Gy. While there was only 1 shoot for 20 and 30 Gy treatments. Induced mutation also created new genetic variations within crop varieties. It can enhance the mutation frequency compared with spontaneous mutation in nature. The alterations in plant morphology and the changes in color of leaves and flowers were observed after the irradiation. The changes include differences in flower development, color intensity, double toner on bracts, double inflorescence within one stalks, double stalk per plants, the inflorescence without bracts and as well as chlorophyll mutation on leaves (Fig. 3). The plants are currently at the M_1V_2 generation for the sensitivity testing stage and M_1V_1 for the single dose treatment.

DISCUSSION

Radio-sensitivity test is a perquisite step before the mutagenic treatment is started[5]. The main purpose of this test is to investigate the most effective dosage of irradiation to be used and also to estimate the frequency and mutation spectrum using gamma irradiation. The half reducing fatality by the lethal exposure to plants, the extended level of damage using gamma irradiation and the lethal exposure rate to plants can be used as an indicator for radio-sensitivity test.

Generally, for mutagenesis either with chemical or physical mutagens, the dose or concentration that reduces the growth 50% of the control is considered suitable for mutation induction. The choice of the dose to be applied for the highest mutant rescue is then left to the breeder's experience with the specific plant material, its genetics and its physiology. The suitable dose should be about 20% higher and about 20% lower from that found by laboratory tests to be the midpoint of the estimated desirable dose range[6] while Heinze and Schmidt[7] advised to operate at a dose giving LD50 (±10%), or a dose resulting in 20% survival of the treated material.

High doses of gamma irradiation lowered the formation of new shoots in *C. alismatifolia*. Generally, if the doses are too high, too many plants will be killed because mutagens can have direct negative effects on plant tissue and many mutations can be lethal. This is due to the fact that primary injuries are retardation or inhibition of cell division, cell death induction of mitotic activity effects of growth rate or growth habit and changes in plant morphology. However, if the dose is too low, there will not be enough mutation generated because of low mutation frequency and results in small-mutated sector[4].

Various effects on the plants will be observed in the different generations after mutation induction. M1 generations are heterogeneous where different plants will carry different mutations. It also exhibits non-heritable direct effects on mutagens such as sterility, chimeric, heterozygous at mutated sector and reduced vigor[4]. Genetic effects or mutations are changes of genetic material and they may be transferred from M1 to the following generations[8].

Induced mutation also creates new genetic variations within crop varieties. It can enhance the mutation frequency compared with spontaneous mutation in nature. Variation observed at *C. alismatifolia* were not solely due to genetic mutation, instead it could be due to somatic mutation. Somatic mutation occurs when the mutant cell continues to divide; the individual will contain a patch of tissue with genotype different from the cells of the rest of the body[9]. These include karyotypic changes, point mutations, somatic crossing-over and sister chromatic exchange, somatic gene rearrangement, changes in organelle DNA, DNA amplification, insertion or excision of transposable elements and segregation of pre-existing chimeral tissue[10]. Most of the somatic mutation cannot be inherited and such variations will disappear in the next generation[5].

Plant height is quantitative trait which is predominantly controlled by polygene. Each gene contributes small effects, which is called genetic addictive effect. The efficiency of selecting the desired mutant is control by single genes[6,10]. In some cases if the mutations are not stable, it will undergo recombination process during meiosis. It is known that, multi-cellular organism have the ability to recover form sub lethal doses of ionizing radiation. Even within a cell, non-damaged molecule may be able to take over metabolic processes and exert a gradual recovery to normal levels.

CONCLUSION

The use of gamma irradiation has been shown to affect the survival rate of *C. alismatifolia*. Gamma irradiation at a dose of 30 Gy has resulted in a sharp decrease of the survival rate of *C. alismatifolia*. The radiosentitivity test of *C .alismatifolia* has indicated that the LD_{50} of this crop was approximately 25 Gy. Besides, various effects of induced mutations on *C. alismatifolia* have also been demonstrated. Studies have shown that gamma irradiation has extended the days of shoot emergence, affecting the plant height, leaves and shoots number, as well as modifications in plant morphology, flower development and chlorophyll mutation on leaves resulting in chimeras. In the future, it is hope that gamma irradiation can develop a new variety of this crop with improved commercial properties suitable for Malaysian landscape.

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