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Induced mutation of curcuma hybrid cv. sweetmemory through tissue culture by ethyl methanesulphonate (EMS)

Yoosumran, V.[1,2], Saetiew, K.[1*], Ruamrungsri, S.[3], Akarapisarn, A.[4] and Teerarak, M.[1]

[1]School of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand; [2]Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand; [3]Departments of Plant and Soil Science, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand; [4]Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.

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Abstract The ethyl methanesulphonate (EMS) is induced mutation in Curcuma hybrid cv. sweetmemory. The shoot size 1 cm were cultured on Murashige and Skoog medium (MS) supplemented with 2 mg/l BA for plantlet induction. After 8 weeks of culture, it was observed that the shoots soaked in 0% EMS at 60 and 120 minutes survived 100%. In additions it was found the highest percentage of shoot emergence, number of shoots, and plant height. While plants treated with EMS solution had decreased survival and growth rates. Thereafter, existing plantlets were potted, and growth was observed. It turned out that the plants none receiving EMS solution (control) had the survival rate and growth rate were better than those of the plants receiving EMS solution. Results showed that control plants receiving 0% EMS at 60 minutes gave highest content of chlorophyll a as 4.06 μg/cm^2, chlorophyll b of 2.08 μg/cm^2 and carotenoid of 3.09 μg/cm^2 but there was none statistical difference with the plants that received EMS solution. The control plants had larger stomata sizes than those that received the EMS solution. However, there was no difference in the number of chloroplasts among treatments. The living plantlets from all treatments were tested Random amplified polymorphic DNA (RAPD) marker and genomic DNA was extracted from fresh leaves. A dendrogram was constructed polymorphic bands using the NTSYSpc program (version 2.10p) showing 2 clusters which separated with similarity coefficients ranging from 0.74-1.00.

Keywords: EMS, Mutation, Curcuma hybrid and RAPD

Introduction

Krachiew (*Curcuma* spp.), belongs to the Zingiberaceae and is classified under the subgenus Eucurcuma. Distinctive features of the Krachiew group include white or yellow true mouth petals and inflorescences that arise directly

[*]**Corresponding Author:** Saetiew, K.; **Email:** kanjana.sa@kmitl.ac.th

from the rhizome. These inflorescences are characterized by large, cup-shaped blooms with broad coma bracts and a short peduncle. The species has a basic of 21 chromosome number. It grows and flowers well during the rainy season and goes dormancy by collapsing and dying, leaving the bulbs underground in winter to wait for growth in the next season. Originating in Indochina, Myanmar, and Thailand, Krachiew is widespread throughout Thailand, particularly in the northern and northeastern regions where species diversity is highest (Bunya-atichart *et al*., 2004). These ornamental plants have gained popularity both domestically and internationally, with annual rhizome exports valued at approximately 20-30 million baht (Anonymous, 2011) main export markets include the United States, Japan, the Netherlands, Germany, and Australia (Thongwai and Kunopakarn, 2007).

In vitro culture combined with induced mutations can accelerate breeding programs by generating variability through selection, particularly for flower color and shape. This approach is not only facilitating rapid plant multiplication but also enhances diversity in Krachiew, thereby easier and expediting the breeding process. Ethyl methanesulphonate (EMS) a chemical mutagen from the alkylating group, has been widely used in plant research due to its ability to induce high gene mutation frequencies while causing relatively few chromosomal aberrations. Commonly applied to seeds, EMS has more recently been utilized to treat *in vitro* explants in various species (Saxena *et al*., 1990; Duron, 1992) and can be used with good results in areas where irradiation is not available. However, these mutagens are not widely used because they penetrate plant tissues such as shoots and stems and are popularly used in plant breeding because they cause genetic mutations resulting in morphological changes (Van Harten, 1998). Currently, induced mutants are crucial in plant molecular genetics, serving as a base tool for studying developmental genetics and advancing breeding programs. Their identification and utilization significantly enhance molecular research. William *et al.* (1990) developed DNA fingerprinting using RAPD markers. RAPD markers are commonly employed for evaluating genetic diversity and relationships within and between populations in various plant species. The method offers advantages such as speed, low cost, simplicity, and the requirement of only small DNA samples without prior sequence knowledge. In the Zingiberaceae family, RAPD markers have been utilized to assess genetic diversity, identify cultivars, and study the phylogenetic relationships of ginger. (Theerakulpisut *et al*., 2005; Kizhakkayil and Sasikumar, 2010; Das *et al*., 2017). The use of RAPD molecular markers to investigate the diversity of Zingiberaceae plants has been reported by Vanijajiva (2012) studied Low genetic variation of *Boesenbergia tenuispicata*, a species endangered and endemic to Thailand, using RAPD markers it was found that out of the 19 primers

tested, 8 primers were able to differentiate of *Boesenbergia tenuispicata*. Hence, the objectives were to induce mutation in *Curcuma* hybrid cv. sweetmemory using EMS to solution to obtain morphologically distinct Curcuma hybrid cv. sweetmemory from unmodified plants and using the RAPD technique efficient methods for analyzing the mutated generations by assessing the degree polymorphism of Curcuma hybrid cv. sweetmemory.

Materials and methods

Plant materials

The young inflorescence *Curcuma* hybrid cv. sweetmemory washed with running tap water for 30 min followed by surface sterilization with 20% Clorox (1.2% sodium hypochlorite) adding 2 drops of tween-20 for 20 min. and 5% Clorox adding 2 drops of tween-20 for 5 min. After that the explants were 3 times rinsed with sterilize distilled water for 5 min. The young flower approximately 1 cm in length were cultured on MS medium (Murashige and Skoog 1962) supplemented with 0.5 mg/l NAA and 0.5 mg/l BA to induce the new shoot. The explants were cultured under cool fluorescent lamps at light intensity 40 $\mu Molm^{-2}$ with a 16 h/day light at temperature 25±2°C. The young flower was transferred to the fresh medium after 30 days for shoot induction. Shoot was used explants.

EMS induced mutation

The shoot regenerated form young inflorescence was used as explants. Explants were immersed in 0, 0.5 and 1.0% EMS for 60 and 120 minutes and shake on the 80 rpms shaker. The shoots were wiped water on sterile paper. They were transferred to the MS medium supplemented with 2 mg/l BA they were observed for their growth after 30 days recovery period, after which surviving new shoots were counted. Experimental design used in this research was 3×2 factorial in completely randomized design, 6 treatments, 3 replications and 10 explants per replication. Determination of percentage of survival, shoot regeneration, shoot number and stem hight.

Morphological observation

Survival plants were grown in 6 inchs plastic pots using soil: coconut pieces: chaff (1:1:1) and maintained in greenhouse for 3 months. The plants were observed weekly for mutation characteristics. Plant parameters are including

survival rate, plant height, length of flower, flower size and bulb size measured the circumference, and bulb number.

Determinations of Chlorophyll a, chlorophyll b and carotenoids

Chlorophyll a (chl a), chlorophyll b (chl b) and carotenoid content of the leaves were determined as described by adaptive method from Lichhtenthaler (1987). The freshly weighed 0.2 g of leaf was crushed and extracted with 80% acetone, 5 mL volume, covered in aluminum foil, and kept in the darkness for 3 hr prior to filtering. Filter paper No. 93, 125 mm size, was used for filtration. The filtered extracts were then centrifuged for 10 min at 10,000 rpm. The resulting absorbance values were then recorded at wavelengths of 470 nm, 647 nm, and 663 nm. The evaluations were conducted by calculating the value.

chlorophyll a (µg/ml) = 12.25(A663) - 2.79(A647),
chlorophyll b (µg/ml) = 21.50(A647) - 5.10(A663), and
carotenoids (µg/ml) = [1000(A470) - 1.82(chl a) – 85.02(chl b)]/198
chlorophyll a, b content (µg/cm^2) = (chlorophyll value × 5 ml)/leaf area
carotenoids content (µg/cm^2) = (chlorophyll value × 5 ml)/leaf area

The results were expressed as chlorophyll and carotenoid contents in leaf of *Curcuma* hybrid cv. sweetmemory (µg/cm^2).

Measurement of stomata and chloroplast number

The stomata size and number of chloroplasts were studied by removing the upper epidermis surface with a razor blade. The lower epidermis was mounted on glass slides and a light microscopy “Olympus CX31” with a digital camera “DP20” was used to photograph size of stomata (width and length) and number of chloroplasts with magnification of 40x.

RAPD analysis

DNA was extracted from one gram of fresh leaves ground in liquid nitrogen. Genomic DNA was extracted by the method recommended by CTAB/Chloroform-Isoamyl Alcohol DNA Extraction Protocol (Doyle and Doyle 1987). The quality and quantity of the DNA was assessed on a 2% agarose gel. A total of 18 primers synthesized at Humanizing Genomics macrogen were used for the study (Table 1). Amplication reactions were in volumes 25 µl containing of genomic DNA 2 µl, ONE PCR (Tag Mix Red) 12.5 µl, Primer 10 mM 1 µl and dH_2O 9.5 µl. Amplifications were performed for an initial denaturation at 94ºC for 4 min, 45 cycles of 1 min 30 sec denaturation at 94ºC, 1 min at a specific annealing temperature for each primer and 2 min extension at

72ºC and a final extension of 5 min at 72ºC and then at 4ºC until storage. Along with the PCR amplified products, 100 bp DNA ladder as standard marker was subjected to electrophoresis in 2% agarose gel in 0.5X TBE buffer. Bring the twenty-nine samples were used for the study. Scoring was carried out only for the clear and unambiguous bands. The scores '1'and '0' were given respectively for the presence and absence of bands. RAPD bands within a genotype is scored as missing if they were poorly resolved on the gel or if the template DNA did not amplify well. Genetic distance was calculated on the basis of Jaccard's coefficient method. A dendrogram was constructed using the TREE procedure by the Numerical Taxonomy and Multivariate Analysis System (NTSYS) based on Jaccard's similarity coefficient using Unweighted Pair Group with Arithmetic Mean method (UPGMA).

Table 1. List of random primers used in RAPD analysis in this study

Primer number	Nucleotide sequence 5′ to 3′	Primer number	Nucleotide sequence 5′ to 3′
OPA-02	TGCCGAGCTG	OPB-01	GTTTCGCTCC
OPA-03	AGTCAGCCAC	OPB-14	TCCGCTCTGG
OPA-04	AATCGGGCTG	OPC-01	TTCGAGCCAT
OPA-10	GTGATCGCAG	OPC-05	GATGACCGCC
0PA-18	AGGTGACCGT	OPD-02	GGACCCAACC
OPAM-01	TCACGTACGG	OPD-03	GTCGCCGTCA
OPAM-03	CTTCCCTGTG	OPD-08	GTGTGCCCCA
OPAM-12	TCTCACCGTC	OPD-18	GAGAGCCAAC
OPAM-18	ACGGGACTCT	OPK-05	TCTGTCGAGG

Statistic analysis

All data were analized using ANOVA and Duncan's multiple range tests at $p \leq 0.05$ by SAS programme.

Results

Effect of EMS on survival rate and shoot size in tissue culture

After culture in 8 weeks, it was found that the shoot from explants soaked in sterile water without EMS had the highest survival 100% whereas highest percentages of shoot formation 83.33% and the highest number of shoots per

plant 2.00 shoots, while the the shoot soaked in concentration of 1% of EMS in 120 minutes had the lowest percentage of shoot formation 43.33% and the lowest number of shoots per plant 1.06. The stems were different size, the plant height tended to increase every week. The shoots that were soked in sterile water with out EMS (control) had the highest average plant height of 5.70 cm, while the concentration of 1% EMS solution in 120 minutes had the minimum plant height 4.33 cm (Table 2, Figure 1).

Table 2. Effect of EMS concentration and duration of soak on survival rate, shoot regeneration, number of shoot and plant height after culture 8 weeks in vitro

EMS (%) (A)	Duration (minutes) (B)	Survival rate (%)	Shoot regeneration (%)	No. of shoot	Plant height (cm)
0	60	100.00±0.00a	83.33±5.77a	2.00±0.00a	5.70±0.12a
	120	100.00±0.00a	70.00±5.77ab	1.96±0.05a	5.35±0.13b
0.5	60	96.67±5.77ab	66.67±0.00ab	1.36±0.11b	5.10±0.17c
	120	86.67±5.77bc	56.33±5.77ab	1.33±0.15b	4.67±0.12d
1.0	60	80.00±10.00cd	46.67±5.77b	1.13±0.05c	4.59±0.03d
	120	73.33±5.77d	43.33±5.77b	1.06±0.15c	4.33±0.19e
F-test A		**	*	**	**
F-test B		ns	ns	ns	**
F-test A*B		**	**	**	**
CV%		6.45	28.47	7.13	2.80

ns = non significant difference *Significant different at P≤ 0.05 **Significant different at P≤0.01
Means within column followed by the same later are not significant different as determined by Duncan's multiple range test

Effect of EMS on number of survival rate, and growth rate in greenhouse

After transferring the plants with roots to the soil pots and culture them for 8 weeks. The result showed the highest survival rate of plant at 0% EMS 60 minutes (control) had 70% and found had the height of stem 31.35 cm, length of flower 16.15 cm, circumference of flower 14.86 cm, circumference of bulb 6.72 cm and number of bulb 12.77 bulb which is the most effective concentration (Table 3, Figure 2A, 2B and 2C). While the concentration of 1% EMS solution in 120 minutes it was found the survival rate 55.00% and had the height of stem 30.14 cm, length of flower 15.06 cm, circumference of flower 13.94 cm, circumference of bulb 5.72 cm and number of bulb 10.50 bulb which is the least effective concentration. (Table 3, Figure 2D, 2E and 2F) However, every treatment has the characteristics of stems, leaves and flowers that are not different. By *Curcuma* hybrid cv. sweetmemory distinctive features flowers are yellow true mouth petals, and the inflorescence flowering is caused by the

rhizome directly, inflorescences from the tops of artificial stems, some plant had a long flower peduncle, some had a short flower and the inflorescence is large.

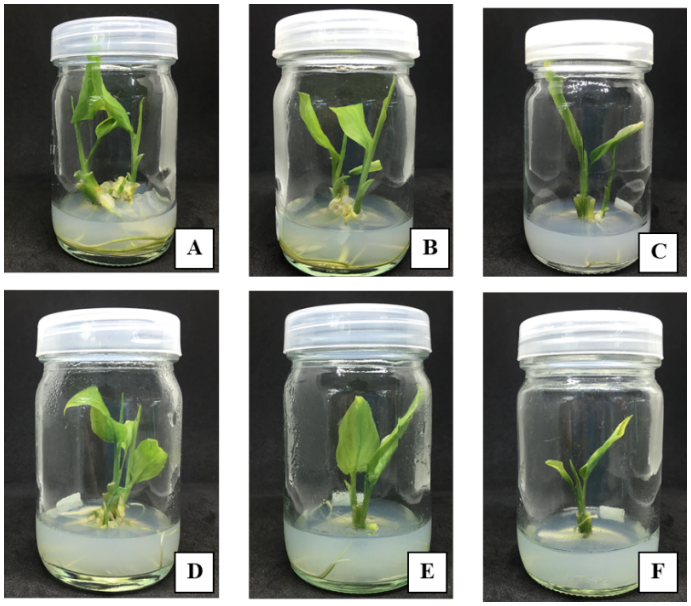

Figure 1. *Curcuma* hybrid cv. sweetmemory after treating with various concentration of EMS and cultured on MS medium for 8 weeks (A) control 0% EMS, 60 min (B) 0.5% EMS, 60 min (C) 1.0% EMS, 60 min (D) control 0% EMS, 120 min (E) 0.5% EMS, 120 min (F) 1% EMS, 120 min

Effect of EMS on total chlorophyll (a+b), carotenoid content, stomata size and chloroplast number

The leaves of *Curcuma* hybrid cv. sweetmemory at 8 weeks were taken to determine the amount of chlorophyll a, b and carotenoid. The result showed every treatment had no difference in total chlorophyll (a+b) and carotenoids values. But at 0% EMS 60 minutes (control) had the highest total chlorophyll (a+b) 5.96 µg/cm^2, and carotenoids 3.09 µg/cm^2. After taking the leaves of *Curcuma* hybrid cv. sweetmemory under a microscope it was found at the control had the most stomata size, width 25.51 µm, length 36.82 µm and chloroplast number 30.56 grain (Figure 3A). At 1% EMS 120 minutes had the minimum stomata size, width 20.72 µm, length 32.24 µm and chloroplast number 30.10 grain (Figure 3B, Table 4).

Table 3. Effect of EMS concentration and duration of soak on number of survival rate, plant height, length of flower, circumference of flower, circumference of bulb and number of bulbs after transplant 8 weeks

EMS (%) (A)	Duration (minutes) (B)	Survival rate (%)	Plant height (cm)	Length of flower (cm)	Circumference of flower (cm)	Circumference of bulb (cm)	Number of bulbs
0	60	70.00±5.00a	31.35±5.00	16.15±0.50a	14.86±0.47	6.72±0.19	12.77±0.51a
	120	68.33±5.77ab	31.10±5.77	16.10±0.69ab	14.68±0.60	6.31±0.19	11.00±0.88b
0.5	60	63.33±7.63abc	30.58±7.63	15.47±0.24abc	14.77±0.47	6.14±0.31	10.98±0.49b
	120	58.33±5.77bc	30.46±5.77	15.30±0.40abc	14.54±1.24	5.90±0.73	10.96±0.28b
1.0	60	58.33±2.88bc	30.20±2.88	15.17±0.06bc	14.12±0.54	5.76±0.47	10.95±0.28b
	120	55.00±5.00c	30.14±5.00	15.06±0.62c	13.94±0.34	5.72±0.49	10.50±0.55b
F-test A		**	ns	*	ns	*	**
F-test B		ns	ns	ns	ns	ns	**
F-test A*B		**	ns	**	ns	ns	**
CV %		8.88	2.40	3.05	4.70	7.27	4.83

ns = non significant difference *Significant different at P≤ 0.05 **Significant different at P≤0.01 Means within column followed by the same later are not significant different as determined by Duncan's multiple range test

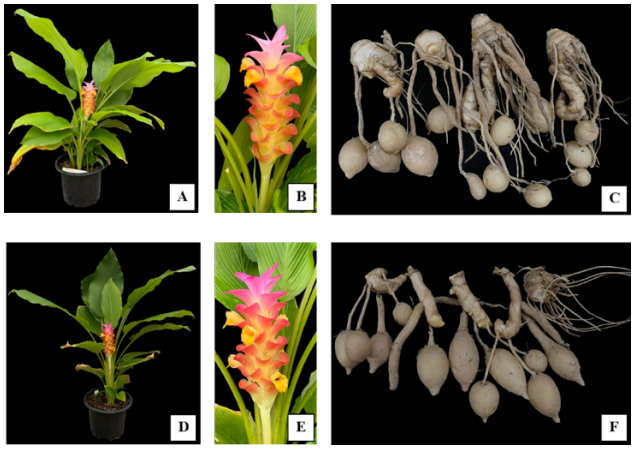

Figure 2. *Curcuma* hybrid cv. sweetmemory after transplant 8 weeks (A), (B) and (C) treated with 0% EMS 60 minutes produced highest parameters of growth and (D), (E) and (F) treated with 1% EMS 120 minutes produced least parameters of growth

Table 4. Effect of different duration of EMS treatment on total chlorophyll Carotenoid, stomata size and number of chloroplasts for 8 weeks

EMS (%) (A)	Duration (minutes) (B)	Total Chlorophyll a+b(µg/cm²)	Carotenoid (µg/cm²)	Stomata size		Number of chloroplasts
				Width (µM)	Length (µM)	
0	60	5.96±0.32	3.09±0.02	25.51±0.80a	36.82±1.35a	30.56±0.22
	120	5.90±0.04	2.98±0.05	24.04±0.57b	35.92±0.87ab	30.40±0.20
0.5	60	5.76±1.01	3.11±0.49	22.38±0.60cd	34.42±0.51bc	30.43±0.34
	120	5.76±0.35	3.05±0.19	22.96±1.14bc	34.80±0.73bc	30.26±0.15
1.0	60	5.39±0.25	2.87±0.17	21.41±0.33de	33.27±0.89cd	30.20±0.21
	120	5.23±0.63	3.04±0.52	20.72±0.41e	32.24±0.15d	30.10±0.05
F-test A		ns	ns	**	**	ns
F-test B		ns	ns	**	**	ns
F-test A*B		ns	ns	**	**	ns
CV%		9.48	10.32	3.07	2.42	0.71

ns = non significant difference **Significant different at P≤0.01
Means within column followed by the same later are not significant different as determined by Duncan's multiple range test

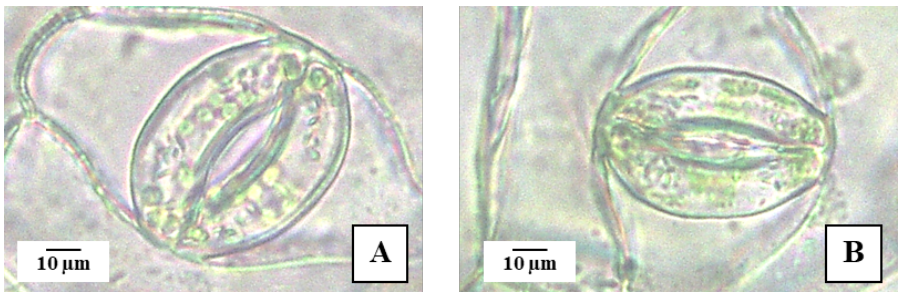

Figure 3. Size and Chloroplast number of stomata from the segment explants of *Curcuma* hybrid cv. sweetmemory with EMS: (A) 0% EMS 60 minutes and (B) 1% EMS 120 minutes

Genetic variation of Curcuma hybrid cv. sweetmemory exposed to EMS solution at different concentrations and durations using RAPD technique

The genetic variance analysis of 29 samples of *Curcuma* hybrid cv. sweetmemory exposed to EMS solution at different concentrations and durations from the treatments in which they were immersed in EMS solution at concentrations of 0, 0.5 and 1% for 60 and 120 min, the DNA extracted from all samples was tested for genetic variance using 18 RAPD primers (Table 1). It was found that there were 5 primers that could differentiate the DNA bands: OPA-10, OPA-18, OPAM-01, OPAM-03 and OPD-02 which gave 6, 5, 3, 5 and 7 clear DNA bands respectively (Table 5), with sizes ranging from 1,500-3,000 base pairs. It gave a total of 97 DNA bands and a total of 11 DNA bands with polymorphism, which is 11.34%.

Table 5. The number of DNA bands from DNA fingerprints of Sweetmemory cultivar treated with EMS solution at different concentrations and durations by RAPD technique with 5 primers and the number of DNA bands giving polymorphism from each primer

Primer	Number of DNA bands	Number of different DNA bands	% polymorphic bands	PICs
OPA10	6	4	66.67	0.12
OPA18	5	2	40.00	0.06
OPAM01	3	1	33.33	0.16
OPAM03	5	1	20.00	0.19
OPD02	7	3	42.85	0.12
Total	26	11	202.85	0.65
Average	5.2	2.2	40.57	0.13

Number of DNA bands giving polymorphism and PICs values from each primer pair

The genetic variation study of *Curcuma* hybrid cv. sweetmemory treated with EMS solution at different concentrations and durations by RAPD technique found a total of 5 primers that gave different values and Polymorphic Information Contents (PICs). Each primer had the following PICs: OPA-10 had a PICs value of 0.12, OPA-18 had a PICs value of 0.06, OPAM-01 had a PICs value of 0.16, OPAM-03 had a PICs value of 0.19, and OPD-02 had a PICs value of 0.12, giving an overall average value of 0.13 (Table 5).

Clustering of Curcuma hybrid cv. sweetmemory treated with EMS solution at different concentrations and durations using NTSYS 2.10p program

The cluster analysis, using the similarity matrix representing Jaccard's coefficient to group the data by UPGMA method with NTSYS 2.10m program. The genetic similarity value was 0.83, and the genetic relationship of *Curcuma* hybrid cv. sweetmemory strain could be classified into 2 groups from 29 samples (Figure 4) and the cophenetic correlation coefficient (r) was 0.74. By group 1 showed short peduncle with a large cylindrical spike, dark pink coma bracts of more than 1/3 of inflorescence, dark purple flower bracts, obtuse to rounded bract apex and yellow flower Group 2 showed long peduncles with a slender cylindrical spike, pink coma bracts of more than 1/3 of inflorescence, light purple flower bracts, obtuse to rounded bract apex and yellow flower (Figure 4).

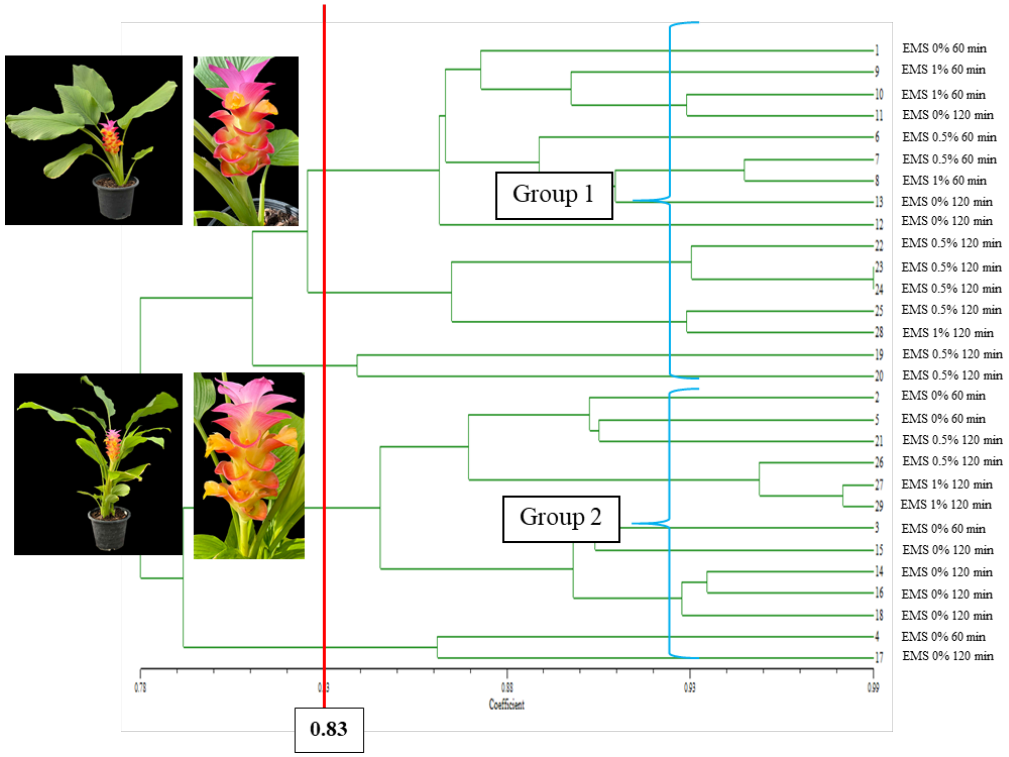

Figure 4. Phylogenetic tree of *Curcuma* hybrid cv. sweetmemory treated with EMS solution at different concentrations and durations was analyzed using NTSYS 2.10m program based on data from RAPD technique

Discussion

In this experiment, it was found that plants not exposed to EMS solution had a higher survival percentage than plants exposed to EMS solution and had the highest percentage of shoot formation and the highest number of shoots per section because EMS solutions are the most commonly used chemicals for inducing mutations and are effective against a wide range of plant species. In addition, the experiment also showed that soaking seedlings under shaking conditions allowed the EMS solution to be absorbed more thoroughly causes tissue damage. However, the success of mutagenicity depends on several factors, such as the nature of the plant tissue (tissue type, tissue size, and stage of tissue development), or the potency of the mutagen (pH and concentration). Including the soaking time is also very important in determining the success of the mutagenicity (Khan *et al*., 2009; Gruszka *et al*., 2012). In addition using a high concentration of mutagen will result in a high mutation rate but a reduced survival rate of plant tissues. On the contrary, using a low concentration of mutagen will result in a low mutation rate but a higher survival rate of plant tissues. Therefore, using an appropriate concentration of mutagen is essential because it will provide an appropriate amount of plant tissues that survive and the chance of finding the desired mutants (Wu and Mooney, 2002). Existing plantlets were potted, and growth was observed. It turned out that the plants not receiving EMS gave 70% survival by Allum *et al*. (2007) the reported that the ability to transport mutagens into plant tissues and genetic characteristics of plants were factors effecting the percentage of survival. The infestation of insects, pests and unfavorable weather conditions have resulted in a low survival percentage for plants. The analysis of total chlorophyll (a+b), and carotenoids revealed no significant difference in the quantities of chlorophyll and carotenoids, as both pigments are essential for light energy absorption in the photosynthetic process. Warner and Edwards (1989) reported the chlorophyll content in plant leaf tissue serves as an indicator of the capacity for photosynthesis and responsiveness to environmental and they enhance the change of light energy into chemical energy. This conversion facilitates the synthesis of nutrients and enhances the growth of plants. Measurement of the width, length and number of chloroplasts of stomata revealed that the width and length of stomata were different. Plants that did not receive EMS solution had larger stomata than plants that received EMS solution. However, the number of chloroplasts was not different in each treatment. Because EMS solution caused mutations at the gene level, which showed different morphological and physiological characteristics from plants that did not receive EMS solution.

From the analysis of genetic variance of 29 samples exposed to different concentrations of *Curcuma* hybrid cv. sweetmemory EMS solution and time by RAPD technique using 18 primers. It was found that RAPD markers can be used to detect genetic variance of *Curcuma* hybrid cv. sweetmemory, with 5 primers giving differences in DNA bands by looking at the rate of polymorphism, the genetic relationships of *Curcuma* hybrid cv. sweetmemory can be classified into 2 groups by group 1 comprises plants immersed in a 75% EMS solution, whereas group 2 consists primarily of control plants. Because the guanine molecule has ethyl groups, the EMS solution causes base pairs to switch places. This changes the ionization properties of the molecule and causes unusual base pairing. Due to the presence of ethyl groups at various places within the purine bases, the EMS solution induces the loss of purine bases from DNA strands. This cleavage of the bonds linking sugars and bases results in the loss of purine bases and the formation of gaps. Subsequently, during DNA repair processes, mistakes may arise at bases distinct from the original, resulting in transition and transversion mutations. Cutting phosphate groups and sugars may also cause single-stranded and double-stranded DNA to separate, which can cause DNA segments to be lost and, eventually, mutations. As a result, the plants exposed to the EMS solution display unique morphological characteristics in contrast to those that did not receive the EMS solution treatment (Iaea *et al.,* 1977). Which is consistent with the research Boonsrangsom (2020) studied the diversity of 'Wan Chak Motluk' (*Curcuma comosa* Roxb.) in Thailand using the RAPD technique it was found that dendrogram constructed from unweighted pair group method with arithmetic average (UPGMA) showed that all samples could be classified into two major clusters; the 'Wan Chak Motluk' samples (I) and the other *Curcuma* sp. (II). Moreover, the 'Wan Chak Motluk' cluster could be divided into six sub-clusters. According to the report of Yu *et al*. (2012) PICs are highly efficient when PICs > 0.5 PICs are moderately effective when 0.25 < PICs < 0.5 and PICs have low efficiency when PICs < 0. 2. The study found that all 18 primers that differentiated DNA bands gave low efficiency PICs by the experimental PICs value was 0.13. However, a small number of primers were used in this study, which may not be suitable primers for differentiation of *Curcuma* hybrid cv. sweetmemory. The information obtained from the appearance of DNA bands is therefore not sufficient for classification and grouping of *Curcuma* hybrid cv. sweetmemory according to morphological characteristics. Therefore, other types of primers should be tested in future DNA fingerprinting studies. The RAPD technique has effectively distinguished closely related species (Zhang *et al.,* 2001; Theanphong *et al.,* 2016). The assessment of genetic similarities using RAPD markers offers a more dependable method for determining phylogenetic relationships and identifying variations compared to only evaluating external

characteristics (Vanijajiva *et al.,* 2005; Palai and Rout, 2007). This study utilized RAPD markers as a straightforward and rapid method to assess the genetic diversity and relationships among Curcuma species. Nonetheless, RAPDs serve as a predominant marker but have been documented to exhibit several shortcomings, including inadequate repeatability and low fidelity (Williams *et al.,* 1990). To further investigate genetic diversity and species identification, more genetic markers and/or more dependable and efficient markers, like sequence-related amplified polymorphism (SRAP) and simple sequence repeat (SSR) markers, may be added in the future (Robarts and Wolfe, 2014; Vieira *et al.,* 2016).

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References

Allum, J. F., Bringloe, D. H. and Roberts, A. V. (2007). Chromosome doubling in *Rosa rugosa* Thunb. Hybrid by exposure of *in vitro* nodes to oryzalin: the effects of node length, oryzalin concentration and exposure time. Plant Cell, 26:1977-1984.

Anonymous. (2011). Pathumma. http://previously.doae.go.th/plant/patumma.htm. Accessed: 29 June, 2011.

Boonsrangsom, T. (2020). Genetic diversity of 'Wan Chak Motluk' (*Curcuma comosa* Roxb.) in Thailand using morphological characteristics and random amplification of polymorphic DNA (RAPD) markers. South African Journal of Botany, 130:224-230.

Bunya-atichart, K., Ketsa, S. and Van Door, W.G. (2004). Postharvest physiology of *Curcuma alismatifolia* flowers. Postharvest Biology and Technology Biharean Biologist 10: 134-136.

Das, A., Gaur, M., Barik, D. P. and Subudhi, E. (2017). Genetic diversity analysis of 60 ginger germplasm core accessions using ISSR and SSR markers. Plant Biosystems, 151:822-832.

Doyle, J. J. and Doyle J. L. (1987). Isolation of plant DNA from fresh tissue. Focus, 12:13-15.

Duron, M. (1992). Induced mutations through EMS treatment after adventitious bud formation on shoot internodes of *Weigela* cv. Bristol ruby. ACTA Horticulturae, 320:113-118.

Gruszka, D. Szarejko, I. and Maluszynski, M. (2012). Plant Mutation Breeding and Biotechnology. CABI International Wallingford, 159-166.

Iaea. (1977). Manual on mutation breeding. Technical reports species no. 119. 2nd ed. Joint FAO/IAEA division of Atomic Energy in Food and Agriculture. Vienna Austria, 298 p.

Khan, S., Al-Qurainy, F. and Anwar, F. (2009). Sodium azide: A chemical mutagen for enhancement of agronomic traits of crop plants. Environment and We International Journal of Science and Technology, 4:1-21.

Kizhakkayil, J. and Sasikumar, B. (2010). Genetic diversity analysis of ginger (*Zingiber officinale* Rosc.) germplasm based on RAPD and ISSR markers. Scientia Horticulturae, 125:73-76.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid grow and bioassay with tobacco tissue culture. Plant Physiology, 15:473-497.

Palai, S. K. and Rout, G. R. (2007). Identification and genetic variation among eight varieties of ginger by using random amplified polymorphic DNA markers. Plant Biotechnology, 24:417-420.

Robarts, D. W. H. and Wolfe, A. D. (2014). Sequence-related amplified polymorphism (SRAP) markers: A potential resource for studies in plant molecular biology. Applications in Plant Sciences, 2.

Saxena, P. K., Williams, D. and King, J. (1990). The selection of chlorsulfuron-resistant cell lines of independent origin from an embryogenic cell suspension culture of *Brassica napus* L. Plant Science, 69:231-237.

Theanphong, O., Thanakijcharoenpath, W., Palanuvej, C., Ruangrungsi, N. and Rungsihirunrat, K. (2016). RAPD marker for determination of phylogenetic relationships of 15 Curcuma species from Thailand. Bulletin of Health, Science and Technology, 14:45-56.

Theerakulpisut, P., Chantaranothai, P., Thong-a-ram, D., Khampila, J., Triboun, P. and Mahakam, W. (2005). Genetic variation of the genus Zingiber (Zingiberaceae) in Thailand detected by RAPD markers. In: Proceedings of the Thirty-first Congress on Science and Technology of Thailand, 18-20 October 2005. Technopolis, Suranaree University of Technology, 67 p.

Thongwai, N. and Kunopakarn, J. (2007). Growth inhibition of *Ralstonia solanacearum* PT1J by antagonistic bacteria isolated from soils in the northern part of Thailand. Chiang Mai Journal of Science, 34:345-354.

Van Harten, A. M. (1998). Mutation Breeding, Theory and Practical Applications, Cambridge University Press, United Kingdom.

Vanijajiva, O. (2012). Low genetic variation of *Boesenbergia tenuispicata*, a species endangered and endemic to Thailand, using RAPD markers. Thai Journal of Genetics, 5:67-78.

Vanijajiva, O., Sirirugsa, P. and Suvachittanont, W. (2005). Confirmation of relationships among *boesenbergia* (Zingiberaceae) and related genera by RAPD. Biochemical Systematics and Ecology, 33:159-170.

Vieira, M. L. C., Santini, L., Diniz, A. L. and Munhoz, C. F. (2016). Microsatellite markers: what they mean and why they are so useful. Genetics and Molecular Biology, 39:312-328.

Warner, D. A. and Edwards, G. E. (1989). Effects of polyploidy on photosynthetic rates, photosynthetic enzymes, contents of DNA, chlorophyll and sizes and numbers of photosynthetic cells in C4 dicot *Artiplex confertifolia*. Plant Physiology, 91:1143-1151.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A. and Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research, 18:6531-6535.

Wu, J. H. and Mooney, P. (2002). Autotetraploid tangor plant regeneration from in vitro Citrus somatic embryogenic callus treated with colchicine. Plant Cell, Tissue and Organ Culture, 70:99-104.

Yu, J. Z., Fang, D. D., Kohel, R. J., Ulloa, M., Hinze, L. L., Percy, R. G. and Jones, D. C. (2012). Development of a core set of SSR markers for the characterization of *Gossypium* germplasm. Euphytica, 187:203-213.

Zhang, K. Y. B., Leung, H. W., Yeung, H. W. and Wong, R. N. S. (2001). Differentiation of *lycium barbarum* from its related lycium species using random amplified polymorphic DNA. Planta Medica, 67:379-381.

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