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Induced mutation in ornamental gingers (Zingiberaceae) using chemical mutagens viz. Colchicine, Acridine and Ethylmethanesulphonate

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Abstract Induced mutations have great potential and serve as a complimentary approach in genetic improvement of crops. Mutation studies in Zingiberaceae members has proven to be an ideal tool for generating material to be used in investigating genotypic as well as phenotypic variation. Now gingers have high ornamental demand and it has high potential in cut flower arrangements as well as in landscaping. Mutagenesis may induce some useful variation in them. An experiment was conducted using chemical mutagents for induced mutation in selected ornamental ginger species. The chemical mutagens used for the present study was ethyl methanesulphonate (EMS), acridine and colchicine. Experiments were conducted in different dosages of mutagents and washed with running water after regular intervals. The mutated and controlled plants are planted at the Calicut University Botanical Garden and the variations observed at maturity and data were recorded.

Key words

Chemical mutagents, Mutation, Ornamental gingers, Zingiberaceae

Mutation breeding has been widely used for the improvement of plant characters in various crops. It is a powerful and effective tool used by the plant breeders especially for autogamous crops having narrow genetic base (Micke, 1988). Mutation studies in Zingiberaceae members has proven to be an ideal tool for generating material to be used in investigating genotypic as well as phenotypic variation (Adamee *et al*., 2007). Ornamental gingers encompass a diverse and versatile group of plants coming under the family Zingiberaceae with 53 genera and over 1377 species (Kong *et al*., 2010). They are mainly distributed in tropics and subtropics with the centre of distribution in the Indo-Malayan region, but extending through tropical Africa to Central and South America (Tomlinson, 1969; Kress *et al*., 2002; Kong *et al*., 2010). Recently they are gaining increased recognition in the flowering pot plant, landscape and cut flower markets especially some genera such as *Alpinia* (Shell Ginger), *Curcuma* (Hidden Ginger), *Globba* (Dancing Ladies), *Hedychium* (Butterfly Ginger), *Kaempferia* (Peacock Ginger) and *Zingiber* (Shampoo Ginger) (Prabhu *et al*., 2010a). The climate and soil of our country is suitable for the cultivation of these plants, Indian horticultural field has to exploit its ornamental value. Now the gingers are slowly becoming popular in the gardens in India (Prabhu *et al*., 2014a).

In any mutation breeding programme, selection of an effective and efficient mutagen is very essential to produce high frequency of desirable mutation. Many chemical mutagens have been employed for obtaining useful mutants in various crop species (Singh and Singh, 2001). However the various workers emphasizes that artificial induction of mutation by colchicine (Col), ethylmethanesulphonate (EMS) and acridine (Acr) provides tool to overcome the limitations of variability in plants especially carnation induces specific improvement without disturbing their better attributes (Mensah Obadoni, 2007; Islam, 2010; Roychowdhury and Tah, 2011). It might be considered that, these chemical induced growth abnormalities were mainly due to cell death and suppression of mitosis at different exposures. Ornamental plants appear to be ideal systems for mutation breeding as many characters of economic interest, i.e. flower traits or the growth habit are easily monitored after mutagenic treatment (Schum, 2003). Mutation techniques are mainly employed for improving crop cultivars, enhancing biodiversity and increasing farmer's income. Usually mutant cultivars are higher yielding, disease-resistance and with better nutrition. Mutations can be beneficially utilized for tailoring better varieties of crop plants. With these objective three wild potential ornamental gingers were selected and detailed improvement studies are carried out.

Materials and Methods

Materials Selected

Three wild ginger species viz. *Boesenbergia siphonantha* (Baker) M. Sabu *et al.*, *Curcuma inodora* Blatter J., and *Larsenianthus careyanus (*Benth.) W. J. Kress & Mood. of the family Zingiberaceae, have been selected for the present study based on their ornamental potential (Plate 1).

Plate 01. Materials selected. A. & B. *Boesenbergia siphonantha* (Baker) M. Sabu *et al.* - Habit and flower; **C. & D.** *Curcuma inodora* Blatter J. - Habit and inflorescence; **E.&F.** *Larsenianthus careyanus* (Benth.) W.J. Kress & Mood-Habit and inflorescence.

Boesenbergia siphonantha (Baker) M. Sabu & al.: 'Island Purple Ginger' is an important potential ornamental plant endemic to Andaman Island seen at an altitude of 5-45 m (Sabu *et al.* 2004). It produces very beautiful purple coloured flowers and shining broad leaves, which is suitable for ornamental ground cover or hedge plants and also as pot plants in gardens (Prabhu *et al.,* 2010b, 2012&2013). They will be dormant during the months of November to May and produces new sprouts by the end of May. It completes vegetative maturity within three months after sprouting and produces beautiful flowers during June to October.

Curcuma inodora Blatter J.: *C. inodora* is a small, 30-60 cm tall plant endemic to Peninsular India and collected from Sanquiline of Goa and Malwan of Maharashtra with an altitude of 20-200 m MSL. .*C. inodora* is an important ginger with high ornamental value and commonly known as 'Hidden or Surprise gingers' (Prabhu *et al.,* 2014a). The inflorescence comes directly from the underground rhizomes, even before the emergence of leafy shoot. The plant can be grown as a cut flower crop and as a ground cover plant and propagated by division of rhizomes. The variation in the flower colour and bract colour gives an additional value to its use as an ornamental cut flower plant (Thomas *et al.,* 2010).

Larsenianthus careyanus (Benth.) W. J. Kress & Mood.: The plant is naturally growing in the evergreen forest of North-East India especially in Assam, Meghalaya, Arunachal Pradesh, Manipur and neibouring country Bangladesh. The plant is growing in an altitude ranging from 150-1000 m above MSL. The plant takes almost 6-10 months to flower. Flowers are soft pink in colour, with the shape of the insect 'praying mantis' and so termed as 'praying mantis ginger' (Nissar *et al.,* 2008). It is now used as a cut flower plant. The inflorescence shows a good vase life of 5-10 days. Plants flower during July to January and set seeds (Prabhu *et al.,* 2014b).

Methodology

The present study was conducted at the Calicut University Botanical Garden (CUBG), Department of Botany, University of Calicut, Kerala, India. The parts used for experiment are stem cuttings of *H. careyana* and rhizomes of *C. inodora* and *B. siphonantha*. 1% EMS solutions in distilled water were prepared and immersed the budding portion of the materials in the solution. The duration is set for 12 hours. After 12 hours the plants were thoroughly washed in tap water and planted. Control plants were dipped in water. Colchicine is usually used for inducing polyploidy in plants. Different concentration of colchicine solution is prepared like 100 ppm, 250 ppm and 500 ppm and the materials were dipped in solution for 24 hours and then washed in tap water for 15 minutes. It was then planted. The materials were treated with 0.5 %, 1% and 2% acridine solutions for 10 minutes and are then washed under tap water for 10 minutes and planted in sand.

Result and Discussions

For the improvement of traits in selected ornamental gingers, two to three different concentrations of aqueous solutions of colchicine, acridine and EMS were used. The rhizomes of *B. siphonantha* and *C. inodora,* stem cuttings of *L. careyanus* were treated with 1% (Ch1, A1, E1), 2% (Ch2, A2, E2) and 4% (E3) colchicine, acridine and EMS respectively. There were 3 replicates which included 5 rhizomes/stem cuttings per treatment. The entire surfaces of the stem cuttings/rhizome were dipped in 250 flasks coatings different concentrations of colchicine/acridine/EMS solutions separately. The materials were kept for 12 hrs. in solution. After treatment all the rhizomes/stem cuttings were washed in distilled water. The materials were planted using potting mixture contains soil, sand and cowdung in 1:1:1 ratio. Separate Control was also used for each treatment. The character evaluated were plant height, leaf number, leaf length, lamina length, lamina breadth, petiole length, length of inflorescence, number of bracts, no. of flower per inflo., flower length, length of calyx, length of corolla tube length of petals, breadth of petals, labellum length, labellum breadth, staminodes length, staminodes breadth, stamen length, anther length of epigynous glands and length of ovary.

***Boesenbergia siphonantha*:** It was found that rhizome of *B. siphonantha* treated with 2% colchicine was not responded. Not a single sprout was emerged out from the rhizome treated with 2% colchicine after 2 months of planting. Rhizomes treated with 1% colchicine responded by producing sprouts after 1 week of planting. The changes were observed in leaf size, number and plant height. The character variation due to colchicine treatment is shown in table 1. The characters such as plant height, leaf number, leaf length and lamina length are reduced in size and number. The height of treated plant shortened to 9.4 cm as compared to control plant with 16. 5 cm. Leaf number became 3, and length of the leaf reduced 5.8 cm in contrast with leaf number of 4 and leaf length of 11.8 cm in control. No flowering was observed in the treated plants with 1% colchicine (Table 1).

B. siphonantha treated 2% acridine was not sprouted. Rhizomes treated with 1% acridine responded by producing sprouts after 1 week of planting. No flowering was observed in treated plants. The changes were observed in leaf size, leaf number and plant height. The height of the treated plant shortened to 9.2 cm as compared to control plants with 16. 5 cm. Leaf number became 3, and length of the leaf reduced to 5.6 cm in contrast with leaf number of 4 and leaf length of 11.8 cm in control plants. *B. siphonantha* treated with 1%, 2% and 4% EMS are not sprouted (Table 1 & Plate 02).

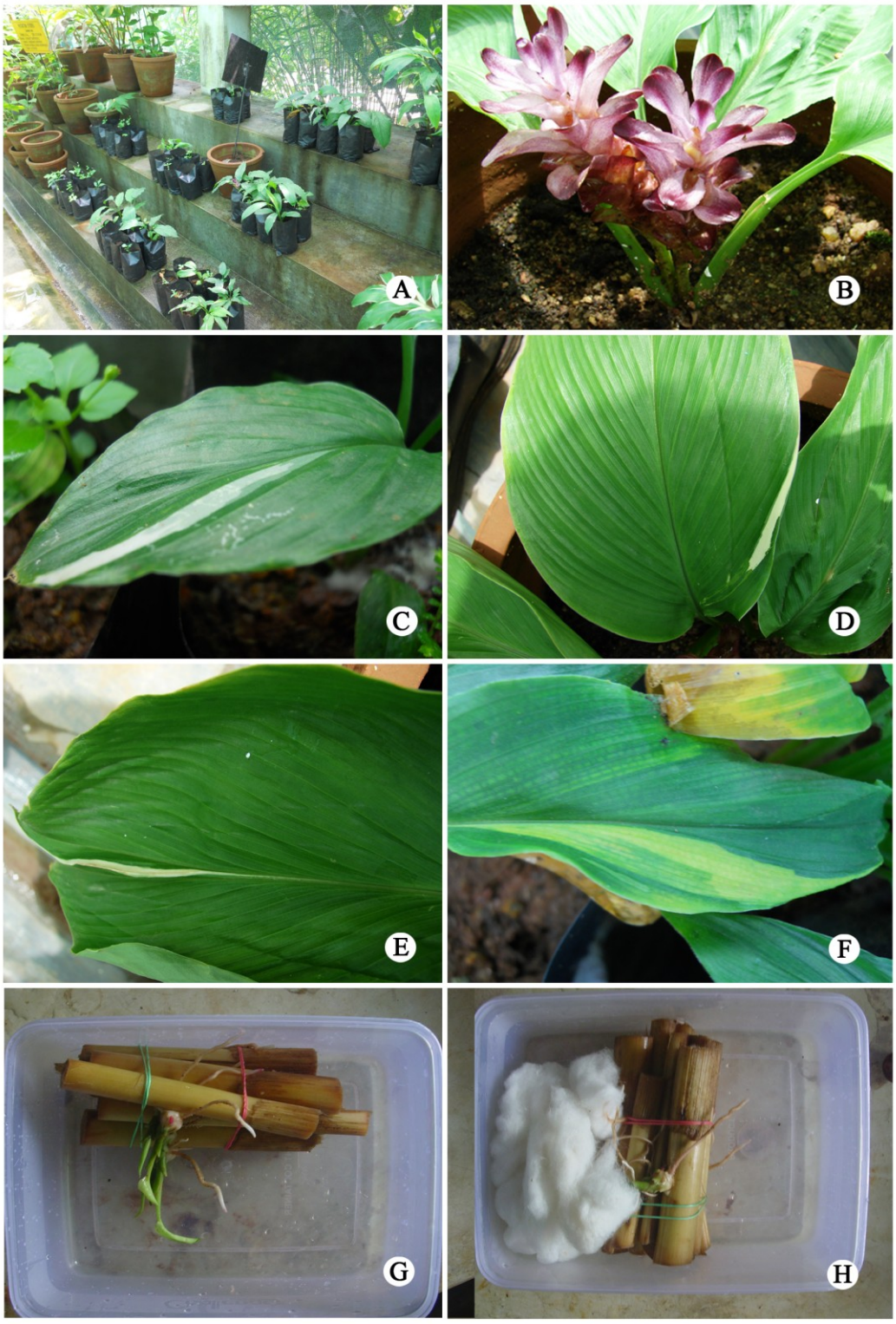

Plate 02. Mutation studies. A. Experimental plants; *Curcuma inodora* **B.** Double inflorescence from a single plant using 1% EMS; **C.-E.** White patches on leaves using 1% EMS and 2% Colchicine; **F.** White patches on leaf of *Boesenbergia siphonantha* using 1% Acridine; **G.&H.** Acridine treatment on the bulbils of *Larsenianthus careyanus.*

Table 1

Comparison between mutated and control plants of *B. siphonantha*

Sl. No	Characters (cm)	Colchicine		Acridine		Ethylmethanesulphonate (EMS)			Control
		Ch1	Ch2	A1	A2	E1	E2	E3	C1
1	Plant height	9.4	NG	9.2	NG	NG	NG	NG	16.5
2	Leaf number	3	NG	3	NG	NG	NG	NG	4
3	Leaf length	5.8	NG	5.6	NG	NG	NG	NG	11.8
4	Lamina length	2.1	NG	2.0	NG	NG	NG	NG	10.6
5	Lamina breadth	2.2	NF	NF	NF	NG	NG	NG	4.3
6	Petiole length	1.8	NF	NF	NF	NG	NG	NG	2.1
7	Length of inflo.	6.1	NF	NF	NF	NG	NG	NG	6.8
8	Number of bracts	4	NF	NF	NF	NG	NG	NG	4
9	No. of flower per inflo.	NF	NF	NF	NF	NG	NG	NG	5
10	Flower length	NF	NF	NF	NF	NG	NG	NG	10.2
11	Length of calyx	NF	NF	NF	NF	NG	NG	NG	0.8
12	Length of Corolla tube	NF	NF	NF	NF	NG	NG	NG	9.6
13	Length of petals	NF	NF	NF	NF	NG	NG	NG	1.7
14	Breadth of petals	NF	NF	NF	NF	NG	NG	NG	0.5
15	Labellum length	NF	NF	NF	NF	NG	NG	NG	2.4
16	Labellum breadth	NF	NF	NF	NF	NG	NG	NG	1.5
17	Staminode length	NF	NF	NF	NF	NG	NG	NG	0.8
18	Staminode breadth	NF	NF	NF	NF	NG	NG	NG	0.4
19	Stamen length	NF	NF	NF	NF	NG	NG	NG	0.7
20	Anther length	NF	NF	NF	NF	NG	NG	NG	0.4
21	Length of epigynous gland	NF	NF	NF	NF	NG	NG	NG	0.5
22	Length of ovary	NF	NF	NF	NF	NG	NG	NG	0.4

***Curcuma inodora*:** The rhizomes of *C. inodora* treated in 2% colchicine were not sprouted. Rhizomes treated with 1% colchicine responded by producing sprouts after 1 week of planting. The changes were observed in leaf size, leaf number and plant height. The character variation due to colchicine treatment is shown in table 2. The characters viz. plant height, leaf number, leaf length and lamina length are reduced in size and number. The height of the treated plant shortened to 15.8 cm as compared to control plants with 19. 5 cm. Leaf number became 3, and length of the leaf reduced 4.5 cm in contrast with leaf number of 4 and leaf length of 5.2 cm in control plants. Flowering was also initiated in plants treated 1% colchicine. Size of the flower has been slightly increased (5.5 cm) compared to control (5.2 cm) (Table 2).

The rhizomes of *Curcuma inodora* treated in 2% acridine were not sprouted. Rhizomes treated with 1% acridine responded by producing sprouts after 1 week of planting. The changes were observed in leaf size and number and plant height. The character variation due to Acridine treatment is shown in table 2. The height of the treated plant is increased to 26.5 cm as compared to control plants with 19. 5 cm. Number of leaves remains the same in both treated plants and control. The leaf length of leaf increased to 22.5 cm and is 14.5 cm in control plants. Flowering was also initiated in plants treated 1% acridine. Size of the flower has been slightly increased (5.4 cm) compared to control (5.2 cm). Details regarding the size of floral organs are shown in table 2 (Plate 02).

Rhizomes treated with 1% (E1), 2% (E2) and 3% (E3) EMS responded by producing sprouts after 1 week of planting flowering after one month. The changes were observed in leaf size, number and plant height. The character variation due to EMS treatment is shown in table 2. The height of the treated plant is increased to 27.5 cm as compared to control plants with 19. 5 cm. Number of leaves remains the same in both treated plants and control. The leaf length of leaf increased to 22.5 cm and is 14.5 cm in control plants. One interesting change observed in 1% treatment was the initiation of two inflorescence from a single plant at a time. The reproductive characters not show any major variations. Compared to E2 and E3, E1 show better results both in qualitative and quantitative characters. Size of the flower has been slightly increased (5.4 cm) compared to control (5.2 cm). (Table 2 & Plate 02).

Table 2

Comparison between mutated and control plants of *C. inodora*

Sl. No	Characters (cm)	Colchicine		Acridine		Ethylmethanesulphonate (EMS)			Control
		Ch1	Ch2	A1	A2	E1	E2	E3	C1
1	Plant height	15.8	NG	26.5	NG	27.5	19.5	20.7	19.5
2	Leaf number	3	NG	4	NG	5	4	4	4
3	Leaf length	12.5	NG	22.5	NG	22.7	18.5	16.2	14.5
4	Leaf breadth	4.5	NG	5.4	NG	6.4	6.2	5.4	5.2
5	Number of comma bracts	3	NF	5	NF	7	5	4	5
6	Number of fertile bracts	5	NF	16	NF	23	11	9	7
7	Number of flower/ inflorescence	6	NF	12	NF	19	9	8	5
8	Length of flower	5.5	NF	5.4	NF	5.3	4.9	5.3	5.2
9	Length of sepal	1.18	NF	1.2	NF	1.2	1.1	1.2	1.15
10	Breadth of sepal	0.98	NF	0.1	NF	0.1	0.1	0.98	0.1
11	Length of petals	1.42	NF	1.4	NF	1.6	1.54	1.55	1.55
12	Breadth of petals	1.32	NF	1.3	NF	1.30	1.32	1.3	1.31
13	Labellum length	2.0	NF	2.1	NF	2.05	2.0	1.95	2.0
14	Labellum breadth	1.60	NF	1.62	NF	1.6	1.6	1.58	1.55
15	Staminode length	1.55	NF	1.54	NF	1.54	1.52	1.54	1.52
16	Staminode breadth	0.75	NF	0.76	NF	0.78	0.75	0.76	0.75
17	Length of Anther	0.42	NF	0.4	NF	0.40	0.41	0.4	0.4
18	Length of epigynous gland	0.40	NF	0.4	NF	0.42	0.4	0.4	0.42
19	Length of Ovary	0.23	NF	0.25	NF	0.28	0.3	0.28	0.26
20	Diameter of ovary	0.21	NF	0.2	NF	0.21	0.2	0.2	0.22

***Larsenianthus careyanus*:** The stem cutting of *Larsenianthus careyanus* treated in 2% colchicine was not sprouted. Stem cuttings treated with 1% colchicine responded by producing sprouts after 2 week of planting. The character variation due to colchicine treatment is shown in table 3. The characters (Plant height, Leaf number, Leaf length, Lamina length is increased in size. The height of the treated plant increased to 25.5 cm as compared to control plants with 18. 5 cm. Length of the leaf increased to 17.5 cm in contrast with 13.5 cm in control plants. Flowering was also initiated in plants treated 1% colchicine. Size of the flower has been slightly increased (6.1 cm) compared to control (5.8 cm) (Table 3)

The stem cutting of *L. careyanus* treated in 2% acridine was not sprouted. Stem cuttings treated with 1% acridine responded by producing sprouts after 2 week of planting. The major change observed in *L. careyanus* using 1% acridine was the variegation on leaves. A white coloured lines on the leaves add more beauty to the plants in the horticulture field. The material selected for the experiment was bulbils, ie., a vegetative part of the plant. Hence the data of more than 7 generation is essential to confirm the status of experimental plant as a mutant variety. Presently data of 5 generation already recorded and the plants under observation. The results showed that, still the plant shows the variegation (Plate 3). The character variation due to acridine treatment is shown in table 3. The height of the treated plant increased to 25 cm as compared to control plants with 18. 5 cm. Length of the leaf increased to 17.2 cm in contrast with 13.5 cm in control plants (Plate 03).

When the stem cuttings treated with 1 % EMS, the height of the plant increased more than twice to 42.5 cm, increased to 26.2 cm when treated with 2% and 24.5 cm in plants treated with 4% EMS (Control size is 18.5 cm). In all the treatments viz. 1%, 2% and 3 % EMS, the size of the leaf increased like 24.5 cm, 16.5 cm and 15.5 cm respectively as the control is 13.5 cm. The size of the flower produced by the control plant is 5.9 cm, whereas the sizes of flowers produced by treated plants are 6.2 cm (1%), 6.1 cm (2%) 5.95 cm (4%). Details regarding the vegetative and floral attributes are given in the table 3.

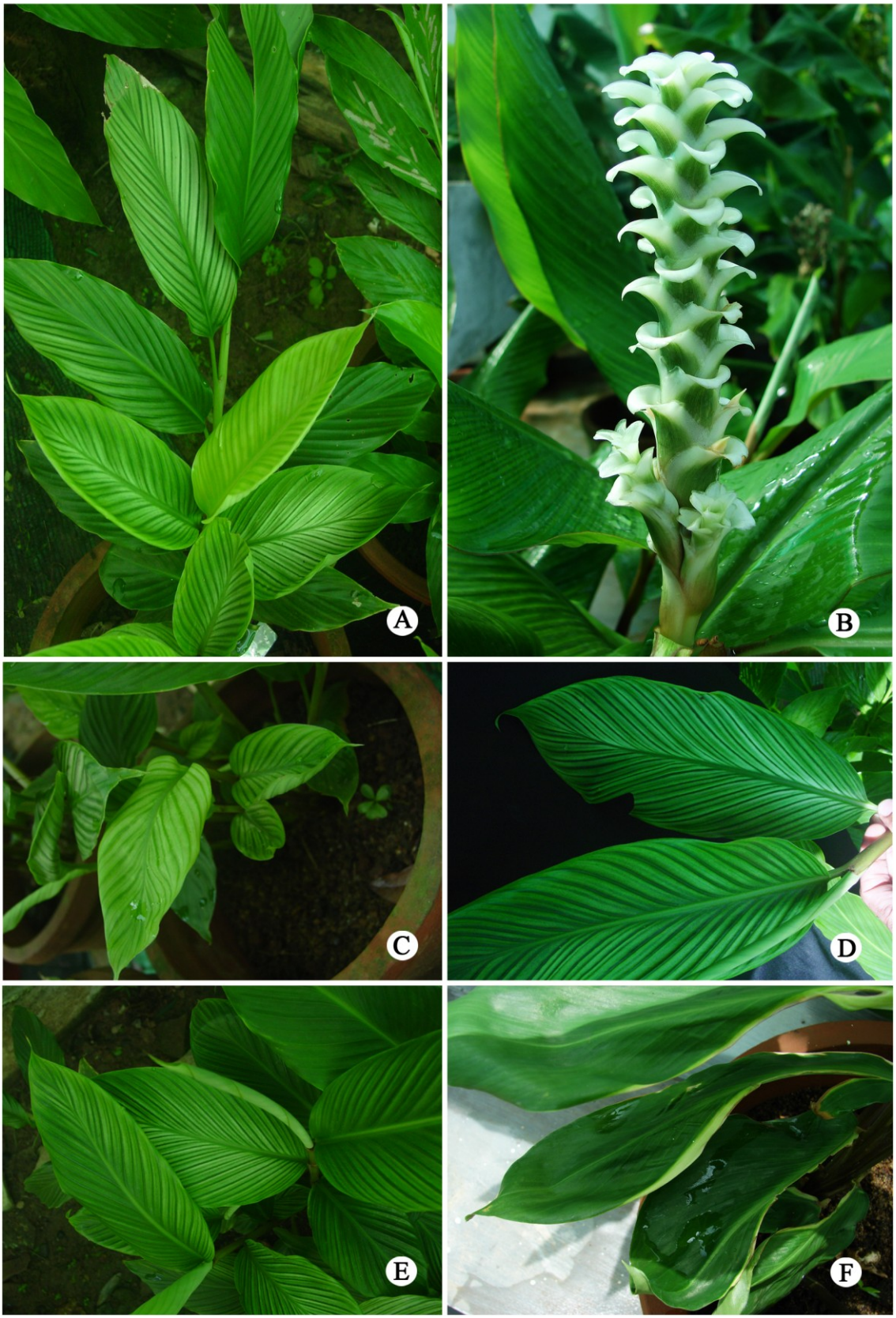

Plate 03. Mutation studies in *Larsenianthus careyanus* (Benth.) W.J. Kress & Mood. **A. & E.** Induced variegated leaves by using 1% Acridine mutagent; **F.** Unsusual white patches on leaves using 2% EMS.

Table 3

Comparison between mutated and control plants of *L. careyanus*

Sl. No	Characters (cm)	Colchicine		Acridine		Ethylmethanesulphonate (EMS)			Control
		Ch1	Ch2	A1	A2	E1	E2	E3	C1
1	Plant height	25.5	NG	25	NG	42.5	26.2	24.5	18.5
2	Leaf number	7	NG	6	NG	9	6	5	7
3	Leaf length	17.5	NG	17.2	NG	24.5	16.5	15.5	13.5
4	Leaf breadth	5.6	NG	5.2	NG	5.8	6.1	5.4	4.5
5	Length of Inflorescence	8.2	NF	NF	NF	16.5	11.2	8.5	7.2
6	Length of spike	4.4	NF	NF	NF	13	8.1	5.2	4
7	Number of comma bracts	3	NF	NF	NF	5	4	4	5
8	Number of fertile bracts	9	NF	NF	NF	24	12	9	10
9	No. of flower/ inflorescence	7	NF	NF	NF	21	13	11	12
10	Length of flower	6.1	NF	NF	NF	6.2	6.1	5.95	5.9
11	Length of sepal	1.3	NF	NF	NF	1.4	1.3	1.3	1.3
12	Breadth of sepal	0.54	NF	NF	NF	0.58	0.60	0.55	0.58
13	Length of petals	1.3	NF	NF	NF	1.35	1.25	1.23	1.3
14	Breadth of petals	0.28	NF	NF	NF	0.35	0.3	0.25	0.25
15	Labellum length	1.7	NF	NF	NF	1.9	1.8	1.8	1.85
16	Labellum breadth	0.7	NF	NF	NF	0.75	0.70	0.75	0.72
17	Staminode length	0.3	NF	NF	NF	3.2	0.3	0.27	0.30
18	Staminode breadth	0.28	NF	NF	NF	0.28	0.26	0.3	0.28
19	Length of Anther	0.48	NF	NF	NF	0.55	0.5	0.45	0.5
20	Length of stamen	2.4	NF	NF	NF	2.5	2.46	2.6	2.3
21	Length of epigynous gland	0.4	NF	NF	NF	0.48	0.38	0.5	0.42
22	Length of Ovary	0.42	NF	NF	NF	0.48	0.42	0.45	0.45

Conventional mutation techniques have often been used to improve yield, quality, disease and pest resistance in crops, or to increase the attractiveness of flowers and ornamental plants (Maluszynski *et al*., 1995). Many studies on this aspect were already completed in different crops such as *Musa acuminata* (Saradhuldhat and Silayoi, 2001), *Curcuma longa* (Rao, 1999), *Gymostachyum* spp. (Khaing *et al.,* 2007), *Zingiber Officinale (*Rashid *et al*., 2013*)*, *Phaseolus vulgaris* (Borkar and Moore, 2010), *Vigna mungo* (Bhosale and Hallale, 2011), *Trigonella* (Biswas and Datta, 1988), *Gymnostachyum* spp. (Khaing *et al*., 2007) and *Kaempferia galanga* (Kak and Kaul, 1982&1988).

Conclusion

Genetic variation is necessary for crop improvement programmes. Induced mutations are highly effective to genetic variations. The result showed that muatagenic treatments produced variability in plants. *Boesenbergia* treated with colchicine and acridine showed decreased in overall size of leaves. *Curcuma inodora* treated with cochine become shorter in size but its size increased when treated with acridine. *Larsenianthus careyanus* treated with both acridine and colchicine showed increased in number of leaves, size in vegetative and floral attributes. An improved variety of *L. careyanus* has been produced through chemical mutagenesis. From the mutagents used viz., colchicine, acridine, EMS and among this acridine treated plants showed better changes than others. 1% acridine treated plants in *L. careyanus* produced beautiful white variegation on leaves. All the experimental plants are under observation. Data were regularly observed and recorded. The result revealed that the efficient concentration of acridine or colchicine for inducing mutation in *Boesenbergia, Curcuma inodora* and *Larsenianthus careyanus* is 1%. The useful mutant isolated through present study need to be tested on a wider scale to establish any changes in chromosome or allele frequency and also to assess its performance in later generations.

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