

EFFECT OF EMS AND COLCHICINE ON MORPHOLOGY AND YIELD CHARACTERS IN OKRA (*Abelmoschus esculentus* (L) moench) VARIETIES OF TCR 2086 AND MAHYCO HYBRID IN M₂ GENERATION

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ABSTRACT

Mutation breeding in crop plants is an effective approach in improvement of crop having narrow genetic base such as Bendi or Okra (*Abelmoschus esculentus* (L)moench). The main objective of the present study is to determine the effect of different concentration of ethyl methane sulphonate (EMS) and colchicine. In Mahyco hybrid, colchicine (0.4, 0.6 and 0.8 percent concentrations) and EMS (0.8 percent) and in TCR 2086 variety (Col 0.4, 0.6, 0.8 percent) EMS (0.8, 1.0, 1.2 percent). The observations were recorded in M₂ generations of different morphology and yield characters such as plant height, number of branches per plant, number nodes per plant, fruit length, fruit girth, number of fruits per plant, number of seeds per fruit, fruit weight and fruit yield per plant. In M₂ preplanning Mahyco hybrids and TCR 2086. Significant increase in colchicine treatment which compound to EMS for most of yield and its component traits.

Keywords: EMS, Colchicine, Okra, Mutation

INTRODUCTION

Bhendi (*Abelmoschus esculentus* L.) is a warm season annual herbaceous vegetable crop which can be found in nearly every market in Africa. It is grown primarily for its young immature green fruits and fresh leaves used in salads, soups and stews. The crop which is generally self-pollinated (Martin, 1983) belongs to the family Malvaceae (Mallow) and has its origin from West Africa (Joshi *et al.*, 1974). The Bhendi provides important source of vitamins and minerals (Lamont, 1999). Grubbenet *al.* (1977) have also reported significant levels of carbohydrate,

potassium and magnesium. The seeds of Bhendi are reported to contain between 15 and 26% protein and over 14% edible oil content (NARP, 1993). The crop is the fourth most popular vegetable in Ghana after tomatoes.

The World Bhendi production was estimated at 4.8 million tons (Gulsenet *al.*, 2007). In Ghana, Bhendi is found in its fresh state in almost all markets in Ghana during the rainy season and in a dehydrated form during the dry season. Particularly in northern Ghana due to its strong commercial value. Bhendi has vital importance as food diet among the inhabitants of cities and villages. The fresh fruits of *Abelmoschus esculentus* (L.) are a common component of Indian diet. In addition the plant has been used medicinally in treatment of several disorders. Anticancer, antimicrobial and hypoglycemic activities of plant are reported. The anti-ulcer activity of fresh fruits is recently reported. This is a coarse, erect branched more or less hairy, annual herb 0.6 to 1.5 meters in height. The only published work so far on the potential application of this mucilage's as a binder in tablet formulation was on sodium salicylates a highly water soluble drug that is no longer in therapeutic usage. The present work is an attempt to the effect of chemical mutagens induces any desirable variations in Bhendi which could be used in crop improvement programme.

MATERIALS AND METHODS

The present investigation was carried out at the Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalainagar during 2013 to 2014. The materials used and the methods followed are described below.

MATERIALS

Biological material

Table 1. Source and special characters of the parents

S.No	Parents	Source	Special Character
1.	TCR 2086	Trichur, Kerala	Medium tall plants. Dark green fruits
4.	Mahyco hybrid	Maharashtra Hybrid Company, Maharashtra	Tall plans. Dark green and long fruits

Mutagens employed

Chemical mutagens like ethyl methane sulphonate (EMS) and colchicine were used at various concentrations to induce mutagenesis.

Ethyl methane sulphonate (EMS) ($\text{CH}_2\text{SO}_2\text{OC}_2\text{H}_5$)

The chemical was obtained from Koch-light Laboratory, Colnbrook -Bucks -England, having a dosimetry 1 half-life period of 30 hours with a molecular weight of 124.16 and density of 1.20.

Colchicine ($\text{C}_{22}\text{H}_{25}\text{NO}_6$)

The chemical was purchased from HI-MEDIA Laboratories, Mumbai, having a molecular weight of 399.45 with a melting point of $147^\circ - 150^\circ\text{C}$.

Determination of LD50 value

Ethyl methane sulphonate

Two sets containing 200 well filled healthy seeds were selected for treatment to determine the LD50 value. Seeds were presoaked in water for 12 hours followed by EMS at 0.20,0.40,0.60,0.80,1.00,1.20,1.40,1.60,1.80 and 2.00 per cent concentrations. The seeds after soaking in EMS were thoroughly washed under tap water for 8 to 10 times. The treated seeds were then subjected to germination test. Based on reduction of germination to 50 per cent. LD₅₀ value was determined. Three concentrations of EMS around LD₅₀ were used for further studies.

Colchicine

Two sets containing 200 well filled healthy seeds were selected for treatments. The seeds were presoaked in water 12 hour followed by soaking in colchicine at 0.20, 0.40, 0.60, 0.80, 1.00, 1.20, 1.40, 1.60, 1.80 and 2.00 per cent concentrations. The seeds after soaking in colchicine were thoroughly washed under tap water for 8 to 10 times. The treated seeds were then subjected to germination test. Based on reduction of germination to 50 per cent LD₅₀ value was determined. Three concentrations of colchicine around LD₅₀ were used for further studies.

Mutagen treatment

Ethyl methane sulphonate

The required concentrations of EMS were prepared in distilled water. The volume of solution is about three times the volume of seeds. The seeds were presoaked in distilled water for 12 hours. The excess moisture in theseeds was removed by pressing it in folds of filter paper. Then the seeds

were soaked in for 12 hours at room temperature ($26\pm 2^{\circ}\text{C}$) with intermittent shaking. After the treatment, the seeds were thoroughly washed with tap water for 8 to 10 times before sowing in the main field.

Colchicine

The seeds were soaked in distilled water for 12 hours. Then the excess moisture in seeds was removed by pressing in folds of filter paper. The seeds were then soaked in colchicine prepared in distilled water for 12 hours at room temperature ($26\pm^{\circ}\text{C}$) with intermittent shaking. After the treatment, the seeds were thoroughly washed in tap water for 8 to 10 times before sowing in the main field.

Control

Seeds soaked in distilled water for 24 hours were used as control.

GENERATION STUDIES

Study of M₂ generation

The seeds harvested from M₁ generation were bulked from individual treatments and were used for raising M₂ generation (Panda and Subudhi, 1994). But few treatments such as 0.8 per cent, 1.0 per cent and 1.2 per cent EMS in Arka Anamika variety. The M₂ generation was grown with three replications in randomized block design and 25 plants were maintained for each treatment per replication. Biometric observations were recorded and individual plant data and mean values were used for statistical analysis. Observations recorded for Plant height, Number of branches per plant, Number of nodes per plant, Fruit length, Fruit girth, Number of fruits per plant, Number of seeds per fruit, Fruit weight and Fruit yield per plant.

RESULTS AND DISCUSSION

EMS

The present investigation revealed that as the concentration of EMS increased there was a decrease in germination percentage. Fifty per cent of reduction in seed germination was observed at 1.0 per cent concentration. Hence the LD₅₀ value for EMS was found to be 1.0 per cent to induce mutagenesis Mahyco Hybrid and TCR 2086 varieties. (Table 2).

Colchicine

It was observed that the reduction in germination increased with the increase in concentration. In colchicine treatment 50 per cent reduction in seed germination was observed at 0.6 per cent concentration for Mahyco Hybrid and TCR 2086 varieties.(Table 2).

Wider range of plant height in Mahyco hybrid was found in 0.4per cent colchicine (99-124 cm). Minimum plant height in Mahyco hybrid was found in 0.8 per cent EMS (114.11 cm), while maximum plant height in Mahyco hybrid was found in 0.4 per cent colchicine (118.6 cm).

Higher range of plant height in TCR 2086 variety was found in 1.2per cent EMS (100-125 cm). Minimum plant height in TCR 2086 variety was recorded in 0.8 per cent EMS (105.11 cm), while maximum plant height in TCR 2086 variety was recorded in 0.6 per cent colchicine (113.5 cm).

Wide range of number of branches was found in Mahyco hybrid in 0.6 per cent colchicine (1-5 branches). Maximum number of branches in Mahyco hybrid was found in 0.6 per cent colchicine (1.20 branches) and minimum number of branches in Mahyco hybrid was found in 0.8 per cent colchicine (1.04 branches), while control had 1.55 branches.

Higher range of number of branches in TCR 2086 variety was found in 0.4 per cent colchicine (1-5 branches). Maximum number of branches in TCR 2086 variety in 1.2 per cent EMS (1.45 branches) and minimum number of branches was found in TCR 2086 variety in 1.0 per cent EMS(1.25 branches) while control had 1.55 branches.

Wide range of number of nodes in Mahyco hybrid was found in 0.6 per cent colchicine (10-18 nodes). Maximum number of nodes in Mahyco hybrid was found in 0.6 per cent colchicine (14.75 nodes) and minimum number of nodes in Mahyco hybrid was found in 0.8 per cent EMS (14.05 nodes), while control had 13.88 nodes.

Higher range of number of nodes in TCR 2086 variety was / found in 1.2 per cent EMS (9-15 nodes). Maximum number of nodes in TCR 2086 variety was found in 0.8 per cent colchicine (13.12 nodes) and minimum number of nodes in TCR 2086 variety was found in 0.4 per cent colchicine (11.45 nodes), while control had 12.60 nodes.

Wider range of fruit length in Mahyco hybrid was found in 0.4 per cent colchicine (14.8-19.2 cm). Maximum fruit length in Mahyco hybrid was found in 0.8 per cent EMS (16.45 cm) and

minimum fruit length in Mahyco hybrid was found in 0.8 per cent colchicine (15.48 cm), while control had 16.08 cm.

Higher range of fruit length in TCR 2086 variety was found in 0.6 per cent colchicine (12.9-15.9 cm). Maximum fruit length in TCR 2086 variety was found in 1.0 per cent EMS (14.26 cm) and minimum fruit length in TCR 2086 variety was found in 0.8 per cent colchicine (13.40 cm), while control had 14.03 cm.

Wider range of fruit girth in Mahyco hybrid was found in 0.4 per cent colchicine (5.07-6.38 cm). Maximum fruit girth in Mahyco hybrid was found in 0.8 per cent EMS (5.95 cm) and minimum fruit girth in Mahyco hybrid was found in 0.8 per cent colchicine (5.24 cm), while control had 5.53 cm.

Higher range of fruit girth in TCR 2086 variety was found in 0.4 per cent colchicine (5.10-6.21 cm). Maximum fruit girth in TCR 2086 variety was found in 0.4 per cent colchicine (5.68 cm) and minimum fruit girth in TCR 2086 variety was found in 0.8 per cent colchicine (5.24 cm), while control had 5.45 cm.

Wider range of number of fruits in Mahyco hybrid was found in 0.6 per cent colchicine (10-16 fruits). Maximum number of fruits in Mahyco hybrid was found in 0.8 per cent colchicine (12.68 fruits) and minimum number of fruits in Mahyco hybrid was found in 0.8 per cent EMS (11.72 fruits), while control had 12.11 fruits.

Higher range of number of fruits in TCR 2086 variety was found in 0.8 per cent colchicine (8-14 fruits). Maximum number of fruits in TCR 2086 variety was found in 0.8 per cent colchicine (10.79 fruits) and minimum number of fruits in TCR 2086 variety was found in 1.2 per cent EMS (9.30 fruits), while control had 9.00 fruits.

Wider range of number of seeds in Mahyco hybrid was found in 0.8 per cent colchicine (30.6-46.5 seeds). Maximum number of seeds in Mahyco hybrid was found in 0.4 per cent colchicine (40.74 seeds) and minimum number of seeds in Mahyco hybrid was found in 0.6 per cent colchicine (39.68 seeds), while control had 39.62 seeds.

Higher range of number of seeds in TCR 2086 variety was found in 0.8 per cent EMS (35.4-52.6 seeds). Maximum number of seeds in TCR 2086 variety was found in 0.8 per cent EMS (40.85

seeds) and minimum number of seeds in TCR 2086 variety was found in 0.4 per cent colchicine (34.40 seeds), while control had 39.40 seeds.

Wider range of fruit weight in Mahyco hybrid was found in 0.6 per cent colchicine (15.8-20.2 g). Maximum fruit weight in Mahyco hybrid was found in 0.6 per cent colchicine (17.82 g) and minimum fruit weight in Mahyco hybrid was found in 0.8 per cent EMS (17.29 g), while control had 16.56 g.

Higher range of fruit weight in TCR 2086 variety was found in 0.8 per cent colchicine (14.2-19.6 g). Maximum fruit weight in TCR 2086 variety was found in 0.4 per cent colchicine (17.62 g) and minimum fruit weight in TCR 2086 variety was found in 0.6 per cent colchicine (15.61 g), while control had 16.65 g.

Wider range in fruit yield in Mahyco hybrid was found in 0.6 per cent colchicine (189.42-246.45 g). Maximum fruit yield (228.98 g) in Mahyco hybrid was found in 0.8 per cent colchicine and minimum fruit yield in Mahyco hybrid was found in 0.8 per cent EMS (202.60 g), while control had 197.10 g.

Higher range in fruit yield in TCR 2086 variety was found in 0.8 per cent colchicine (143.72-219.41 g). Maximum fruit yield in TCR 2086 variety was found in 0.4 per cent colchicine (176.11 g) and minimum fruit yield in TCR 2086 variety was found in 0.8 per cent EMS (152.75 g), while control had 149.85 g. (Table 3)

CHOICE OF EXPERIMENTAL MATERIALS

In any breeding programme variety is an important aspect. Improvement of crop species through mutation breeding should therefore be pursued with varieties outstanding in their agronomic fitness for practical breeding purpose (Scarascia-Mugnozza, 1966). Popular varieties such as TCR 2086 and Mahyco hybrid commercially cultivated were selected for the study and were subjected to chemical mutagenesis. Though more number of chemicals can be used as mutagens, only a few of them such as EMS and colchicine induced much higher frequency of mutations than others in most of the crops (Kalloo, 1988). Hence, these chemicals which are easily available were used in this investigation.

In the present investigation, mean for different characters were shifted both in positive and negative direction due to the mutagenic treatments. Several explanations were given for the shift in the mean values of quantitative characters due to mutagenic treatments (Brock, 1965).

Mutation in quantitatively inherited characters would depend on the number of genes involved, the relative proportion of genes with positive and negative effects and the degree to which the genes of parental genome operates as a balanced set (Brock, 1965).

A significant positive shift in mean values observed may be due to elimination of detrimental genes in mutations. Borojevik (1971) attributed that positive shift in mean values may be due to the effect of natural selection and elimination of chromosomal aberrants.

For plant height, negative shift over control was observed in most of the treatments in Mahyco hybrid while positive shift was observed TCR 2086. Negative shift has been reported by Hla-Shweet *al* (1993).

For number of branches, Negative shift has been induced by all the treatments in TCR 2086 variety and Mahyco hybrid, SenthilKumar (2000) reported positive shift towards mean value for this character.

For number of nodes per plant, most of the colchicine treatments showed negative shift in TCR 2086. Positive shift has been shown in all the colchicine treatments in Mahyco hybrid. Positive shift has been shown in most of the EMS treatments in TCR 2086. Senthil Kumar (2000) reported negative shift towards the mean values for this character.

For fruit length negative shift for all colchicine treatment was observed in TCR 2086 variety. The negative shift was observed in most of the colchicine treatments in Mahyco hybrid variety. All the EMS treatment in TCR 2086 variety. In Mahyco hybrid showed a positive shift and negative shift observed in most of the EMS in TCR 2086 variety. SenthilKumar (2000) reported positive shift towards the mean value for this character. Whereas in Fruit girth all the colchicine treatments showed a positive shift in Mahyco hybrid. Negative shift has been shown in most of colchicine treatment in TCR 2086 variety. SenthilKumar (2000) reported negative shift towards the mean value for this character.

For number of fruits per plant positive shift has been shown in all the colchicine treatments in TCR 2086 while most of the colchicine treatments showed positive shift in Mahyco hybrid. A

positive shift has been shown in all the EMS treatments in TCR 2086 variety. SenthilKumar (2000) reported positive shift towards the mean value for this character. In number of seeds per fruit a positive shift has been shown in all colchicine treatments in Mahyco hybrid variety and a positive shift has been shown in all colchicine treatments in TCR 2086. SenthilKumar (2000) reported negative shift towards the mean value for this character.

For fruit weight all the colchicine treatments showed positive shift in TCR 2086 and Mahyco hybrid. A positive shift has been shown in all the EMS treatments in Mahyco hybrid while negative shift has been shown in most of the EMS treatments in TCR 2086. Senthil Kumar (2000) reported positive shift towards the mean value for this character. In fruit yield per plant a positive shift was seen in all the colchicine treatments in TCR 2086 and Mahyco hybrid. A positive shift has been shown in all the EMS treatments in Mahyco hybrid and TCR 2086 variety. Senthil Kumar (2000) reported positive shift towards the mean value for this character.

The LD₅₀ for EMS was 1.0 per cent, where as it was 0.6 per cent in case of colchicine based on seed germination. A significant reduction in seed germination with the increase of concentration of mutagenic chemicals. In general, the concentration and effect relationship was nonlinear.

In M₂ generations all parameters were in moderate and high value. In height mean values were recorded number of nodes per plant, fruits per plant, fruit length and fruit girth, seeds per plant, fruit weight and fruit yield per plant.

Table 2. Determination of LD₅₀

Treatment	EMS		Treatment	Colchicine	
	Seed germination (%)	Percent of decrease over control		Seed germination (%)	Percent of decrease over control
Control 0.0	90.6	--	Control 0.0	88.9	--
0.2	87.3	-3.65	0.2	76.2	-14.29
0.4	83.4	-7.85	0.4	68.3	-23.17
0.6	74.8	-17.44	0.6	50.9	-42.74
0.8	67.1	-25.94	0.8	42.3	-52.42
1.0	51.6	-43.05	1.0	36.8	-58.61

1.2	43.8	-51.66	1.2	32.3	-63.67
1.4	24.8	-72.63	1.4	27.5	-69.07
1.6	16.3	-82.01	1.6	19.8	-77.73
1.8	8.4	-90.73	1.8	12.7	-85.71
2.0	3.6	-96.03	2.0	5.8	-93.48



Table 3. Effect of mutagens on yield and its components traits of Bhendi in M₂ generation

Treatments (conc. Per cent)	Plant height			Number of branches per plant			Number of nodes per plant		
	Range (cm)	Mean (cm)	Per cent decrease or increase over control	Range	Mean	Per cent decrease or increase over control	Range	Mean	Per cent decrease or increase over control
MAHYCO 0.4% COL	99-124	118.60	+1.36	1-3	1.16	-25.00	10-16	14.20	+2.23
0.6% COL	100-118	115.55	-1.23	1-5	1.20	-22.32	10-18	14.75	+6.19
0.8% COL	98-120	115.95	-0.89	1-3	1.04	-33.03	10-15	14.35	+3.37
0.8% EMS	99-118	114.11	-2.46	1-4	1.18	-23.70	9-15	14.05	+1.20
CONTROL	100-121	117.00	-	1-2	1.55	-	10-15	13.88	-
TCR2086 0.4% COL	99-119	107.37	+1.10	1-5	1.41	-8.92	8-13	11.45	-9.06
0.6% COL	99-116	113.50	+6.87	1-3	1.33	-14.29	9-14	12.20	-3.17
0.8% COL	97-115	111.70	+5.18	1-4	1.37	-11.60	10-15	13.12	+4.16
0.8% EMS	98-110	105.11	-1.02	1-4	1.40	-9.61	10-15	13.06	+3.70
1.0% EMS	99-114	108.60	+2.25	1-3	1.25	-19.14	9-14	12.45	-1.19
1.2% EMS	100-125	108.20	+2.25	1-3	1.45	-6.78	9-15	12.65	+0.39
CONTROL	98-118	106.20	-	1-2	1.55	-	8-15	12.60	-

Table 3. (Conti.)

Treatments (conc. Per cent)	Fruit Length			Fruit girth			No. of fruits per plant		
	Range (cm)	Mean (cm)	Per cent decrease or increase over control	Range (cm)	Mean (cm)	Per cent decrease or increase over control	Range	Mean	Per cent decrease or increase over control
MAHYCO 0.4% COL	14.8 - 19.2	16.42	+2.08	5.07-6.38	5.80	+4.78	10-15	12.05	-0.50
0.6% COL	14.5 - 16.3	15.57	-3.21	5.14-6.21	5.62	+1.54	10-16	12.60	+4.03
0.8% COL	14.6 - 16.9	15.48	-3.73	4.88-5.86	5.24	-5.34	10-15	12.68	+4.74
0.8% EMS	14.9 - 18.1	16.45	+2.27	5.55-6.44	5.95	+7.46	9-14	11.72	-3.21
CONTROL	14.5 - 19.0	16.08	-	4.89-6.12	5.53	-	9-15	12.11	-
TCR2086 0.4% COL	12.8 - 15.6	13.71	-2.25	5.10-6.21	5.68	+4.26	8-12	10.00	+11.11
0.6% COL	12.9 - 15.9	13.74	-2.03	4.89-5.86	5.36	-1.65	8-12	9.44	+4.93
0.8% COL	12.6 - 15.5	13.40	-4.48	4.95-5.64	5.24	-3.37	8-14	10.79	+ 19.90
0.8% EMS	12.8 - 15.6	13.63	-2.82	5.02-6.08	5.42	-0.41	8-13	9.45	+5.09
1.0% EMS	13.1 - 15.9	14.26	+ 1.62	4.99-5.85	5.38	-1.14	8-12	9.70	+7.77
1.2% EMS	13.0 - 16.0	13.98	-0.31	5.26-6.18	5.65	+3.72	8-11	9.30	+3.33
CONTROL	12.5 - 15.2	14.03	-	4.81-6.03	5.45	-	8-11	9.00	-

Table 3. (Conti.)

Treatments (conc. Per cent)	Number of seeds per fruit			Fruit weight			Fruit yield per plant		
	Range	Mean	Per cent decrease or increase over control	Range (g)	Mean (g)	Per cent decrease or increase over control	Range (g)	Mean (g)	Per cent decrease or increase over control
MAHYCO 0.4% COL	33.4-48.1	40.74	+2.82	15.5-19.5	17.63	+6.47	183.22-225.46	211.66	+7.38
0.6% COL	32.2-45.3	39.68	+0.15	15.8-20.2	17.82	+7.62	189.42-246.45	223.93	+ 13.1
0.8% COL	30.6-46.5	39.88	+0.67	15.6-19.8	17.42	+4.61	195.63-253.86	228.98	+16.1
0.8% EMS	34.8-48.9	40.45	+2.09	15.1-19.3	17.29	+4.41	190.89-230.19	202.60	+2.79
CONTROL	33.0-50.9	39.62	-	15.0-19.2	16.56	-	162.91-212.14	197.10	-
TCR2086 0.4% COL	28.9-42.1	34.40	-12.67	14.8-18.9	17.62	+5.83	141.18-209.20	176.11	+ 17.5
0.6% COL	32.8-48.3	39.82	+ 1.08	13.8-18.3	15.61	-6.23	133.63-197.22	165.77	+ 10.6
0.8% COL	34.2-50.5	39.46	+0.51	14.2-19.6	16.92	+ 1.66	143.72-219.41	172.18	+ 14.9
0.8% EMS	35.4-52.6	40.85	+3.70	14.5-18.7	16.62	-0.13	131.22-192.83	152.75	+1.93
1.0% EMS	34.7-45.8	39.93	+1.35	14.8-19.3	16.83	+ 1.09	146.24-208.22	163.50	+9.11
1.2% EMS	33.6-48.7	39.54	+0.35	14.9-18.6	16.49	-0.91	120.66-189.61	153.58	+2.49
CONTROL	31.8-46.5	39.40	-	14.7-19.1	16.65	-	116.81-193.86	149.85	-

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