PHYSICAL AND CHEMICAL MUTAGENESIS IN *JATROPHA CURCAS* L. TO INDUCE VARIABILITY IN SEED GERMINATION, GROWTH AND YIELD TRAITS

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The studies of induced mutation in *Jatropha curcas* were performed by exposing the healthy and dry seeds to gamma rays viz., 5, 10, 15, 20 and 25 Kr doses and ethyl methanesulphonate (EMS) viz., 1, 2, 3 and 4 %. The observations were made for seed germination percentage, growth and yield characters such as root length, shoot length, seedling length and vigour index, plant height, petiole length, days to first flowering, days taken for flowering to fruiting, days taken for fruiting to maturity, total number of flowers per inflorescence, number of fruit bunches per plant and number of fruits per bunch in the treated plants. Seeds treated with 5 Kr dose of gamma rays and 1 % EMS revealed a stimulatory effect while 25 Kr dose of gamma ray except seed germination and 4 % EMS treatment showed an inhibitory effect for all the characters studied when compared to other treatments. Data obtained in this study were statistically significant at 5 % level. The results conclude that treatments of gamma rays were found to be greater compared to those of EMS treatments. Based on the variation in flowering and yield traits of gamma rays and EMS treated plants, superior strain will be screened by PCR-RAPD marker and published in the near future.

Key words: Biodiesel plant, Ethyl Methanesulphonate, Future bioenergy, Gamma rays, Induced mutagenesis, *Jatropha curcas*.

INTRODUCTION

The genus *Jatropha* belongs to family Euphorbiaceae and includes a large number of species that are distributed all over the world in habitats ranging the tropical to the temperate zone. *J. curcas* is a multipurpose shrub with significant economic importance and having the capabilities to rehabilitate the degraded lands (Ghosh *et al*., 2007). In recent years energy conservation and its alternative production have acquired significant importance in the walk of the world energy crisis. Since the oil crisis of the 1970s and recognition of the limitation of world oil resources, most of the oil importing countries including India have been highly motivated to develop alternative sources of energy to meet their domestic needs

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from natural resources. *J. curcas* has been found a highly promising species which can yield oil seed as sources of energy in the form of biodiesel.

Physical and chemical mutagen studies on experimental mutagenesis in this plant are very limited besides, mutation breeding studies in *J. curcas* carried out in Thailand using fast neutrons and isolated dwarf or early flowering mutants from the M3 generation, but the potential productivity of these variants under intensive cultivation conditions was not proved (Sakaguchi and Somabhi, 1987). Dwemahyani and Ishak (2004) used induced mutations in *J. curcas* for improvement of agronomic characters with irradiation dose of 10 Gy and identified mutant plants with early maturity, 100 seeds weight (30 % over control) and better branch growth and mutation studies undertaken at National Botanical Research Institute (NBRI), Lucknow, India has led to induction of cotyledonary variables in *J. curcas* (Pandey and Datta, 1995). We have taken present investigation on induced mutagenesis in *J. curcas* using gamma rays and EMS as no much reports are available in this plant.

MATERIALS AND METHODS

The physical mutagen (gamma rays) and chemical mutagen (EMS-Ethyl Methanesulphonate) were employed in the present study. Gamma rays treatment was given from the Gamma chamber –900 installed at TamilNadu Agricultural University, Coimbatore and EMS for the present study was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India.

The seeds of *J.curcas* were collected from local former in Chidambaram, Cuddalore District, TamilNadu, India. The seeds treated with different dose of gamma rays viz, 5, 10, 15, 20 and 25 Kr and EMS viz., 1, 2, 3 and 4 % along a control group were sown at 1 cm depth in plastic trays (23×27 cm in height) filled with river sand: red soil and farm yard manure in the ratio of 3:2:1. Water was applied manually on alternative day and germination was observed periodically on fifth, tenth, twenty fifth days after sowing. After a month of germination, twenty five plants from each treatment were transferred into poly bags containing 4 kg mixture of river sand, red soil and farm yard manure in the ratio of 3:2:1 and maintained up to 3 months. Thereafter, ten plants from each treatment transplanted in experimental field which was ploughed well before planting to ensure good growth of plants.

Observations were recorded for growth parameters viz., root length (cm), shoot length (cm), seedling length (cm), vigour index, plant height (cm), petiole length (cm), days to first flowering, days taken for flowering to fruiting, days taken fruiting to maturity, total number flowers/ inflorescence and fruit traits such as number of fruit bunches / plant, number of fruits / bunch. Data were analyzed using one way ANOVA in SPPS software package (17.0).

RESULTS AND DISCUSSION

Table 1 shows seed germination in the control as well as in the treated plants of *J. curcas*. The differences in the values of seed germination due to gamma irradiation and EMS at different time intervals (5, 10 and 25th days after sowing) was highly significant at 5 % level.

In case of seed germination, the data shows that 5 Kr dose (80.66 %) showed a stimulatory effect while all the EMS treatments showed an inhibitory effect as compared to control. In EMS treatments, 1 % EMS treatments showed the highest germination percentage (52.66 %) of other EMS treatments. In both treatments of gamma rays and EMS, minimum seed germination percentage was recorded in the higher dose/ concentration of gamma rays / EMS (37 % and 24.66 % in 15 Kr dose and 4 % EMS respectively).

Table 1

Effect of gamma rays and EMS on germination of *J. curcas*

S. No.	Dose / Concentration	Number of seeds sown	Number of seeds germinated on 5th day	Number of seeds germinated on 10th day	Number of seeds germinated on 25th day	Total number of seeds germinated (%)
1.	Control	100	17.66 ± 2.51	23.66 ± 1.52	17.00 ± 2.64	58.00 ±6.42
2.	5 Kr	100	4.66 ±2.51	42.66 ±1.52	33.33 ± 3.05	80.66 ± 5.50
3.	10 Kr	100	3.33 ± 2.08	24.00 ± 2.64	27.33 ± 1.52	54.66 ± 5.03
4.	15 Kr	100	3.00 ± 2.64	20.00 ± 2.00	14.00 ± 1.00	37.00 ± 4.35
5.	20 Kr	100	2.66 ± 1.52	25.33 ± 2.08	17.00 ± 2.00	44.00 ± 2.00
6.	25 Kr	100	6.33 ± 2.08	28.00 ± 5.56	22.66 ± 1.57	57.00 ± 3.60
7.	1 % EMS	100	3.66 ± 2.08	27.00 ± 3.60	22.00 ± 2.00	52.66 ± 2.08
8.	2 % EMS	100	2.66 ± 1.52	19.66 ± 2.51	17.33 ± 4.93	39.66 ± 5.50
9.	3 % EMS	100	1.66 ± 1.52	15.33 ± 2.51	12.66 ± 3.05	29.66 ± 4.50
10.	4 % EMS	100	1.00 ± 1.00	13.33 ± 2.08	10.33 ± 1.53	24.66 ± 4.55
	F – Value		**17.031**	**24.646**	**23.281**	**38.525**

Significant at 0.05 % level
3 Replicates
± – Standard Deviation

The germination of the treated plants had shown a sharp dose rate relationship, which decreased with the increase in the doses / concentration of mutagenic treatments. Percentage reduction / stimulation in seed germination might have been duo to the effect of mutagens on meristematic tissues of the seed. The decrease in seed germination at higher doses / concentration of the mutagens may be attributed to disturbances at cellular level (caused either at physiological (or) physical level).

Kumar and Mishra (2004) reported that in okra (*Abelmoschus esculentus*) germination percentage generally decreased with increasing doses / concentrations of gamma rays and EMS. Reduced germination percentage with increasing doses of gamma radiation has also been reported in *Pinus* (Thapa, 2004), Rye (Akgun and Tosum, 2004) and Chickpea (Khan *et al*., 2005 and Toker *et al*., 2005). Gradual reduction in germination percentage was also observed with an increase in concentration of mutagen, reaching more than 50 % lethality at 0.5 % EMS in two genotypes of tobacco (Amernath and Prasad, 1998).

In the present study, seeds of *J. curcas* were treated with gamma rays of 5, 10, 15, 20 and 25 Kr and EMS viz., 1, 2, 3 and 4 %. In the laboratory germination test it was found that increase in concentration of gamma rays and EMS had an adverse effect. Similar results have been reported early in *Capsicum annuum* (Alcantara *et al*., 1996 and Jabeen and Mirza 2002) found that seeds treated with 1.5 % EMS in M1 generations had the lowest germination percentage (84 %) among all treatments. Karthika and Lakshmi (2006) the germination percentage was found to be inhibited by gamma irradiation and EMS for two varieties (Co_1 and Co_2) of Soybean.

Seeds treated with 25 Kr dose also showed a stimulatory effect and recorded 57 % of seed germination as compared to other gamma rays treatments except for 5 Kr dose. Arora *et al*. (1989) and Jamil and Khan (2002) also recorded high percent seed germination at high doses in wheat. Yusuf and Nair (1974) inferred that gamma irradiation interfered with the synthesis of enzymes and at the same time accelerated the degradation existing enzymes involved in the formation of auxins and thus reduces the germination of seeds. Reduced seed germination due to mutagenic treatments may be the result of damage of cell constituents at molecular level or altered enzyme activity (Khan and Goyal, 2009).

Table 2 shows length of root, shoot and seedling and vigour index of gamma rays and EMS treated plants. 5 Kr dose of gamma rays exhibited the highest root length of 21.16 cm as compared to control (14.83 cm) other treatments of 10, 15, 20 and 25 Kr (14.80, 13.85, 11.17 and 8.25 cm respectively), while all the EMS treatments showed a reduced root length as compared to control. Among EMS treatments 1 % EMS showed a maximum root length (13.33 cm) than other treatments of 2 % EMS (12.16 cm), 3 % EMS (11.33 cm) and 4 % EMS (8.66 cm).

A similar trend was observed for shoot length and seedling length. Maximum shoot length of 27.33 cm and a seedling length of 48.83 cm were recorded in 5 Kr dose of gamma rays. In the present study, a decreased trend in length of shoot and seedling was observed with the increase in dose / concentrations of gamma rays and EMS. 4 % EMS treatments recorded 11.20 cm and 19.86 cm for shoot and seedling length respectively when compared to control and other treatments of gamma rays and EMS.

The effect of EMS was more drastic in reducing the seedling vigour than gamma rays. Maximum seedling vigour index was 3924 at 5 Kr dose of gamma

rays and 1600 at 1 % EMS. The minimum vigour index was 1231 and 492 for 25 Kr dose of gamma rays and 4 % EMS respectively as compared to control (1979). This indicates that 5 Kr dose of gamma rays and 1 % EMS treatments have a stimulatory effect on germination rate and growth of seedling (length of root and shoot) as vigour index calculated by germination percentage multiplying with length of seedling.

Table 2

Effect of gamma rays and EMS on seedling traits of *J. curcas* (30 days after sowing)

S. No.	Dose/ Concentration	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Vigour Index
1.	Control	14.83±3.25	18.50±4.09	33.33±7.28	1979±136
2.	5 Kr	21.16±1.04	27.33±1.52	48.83±2.02	3924±282
3.	10 Kr	16.00±1.00	20.66±3.78	36.66±4.72	1980±125
4.	15 Kr	13.80±1.08	18.96±1.05	32.46±1.36	1209±105
5.	20 Kr	10.83±2.02	14.16±1.04	25.00±3.00	1104±182
6.	25 Kr	9.00±2.64	12.50±1.32	21.50±3.90	1231±186
7.	1 % EMS	13.33±3.51	17.33±3.81	30.66±7.32	1600±138
8.	2 % EMS	12.16±2.56	15.93±3.23	28.10±5.78	1110±131
9.	3 % EMS	11.33±1.52	14.00±1.40	25.33±2.91	742±87
10.	4 % EMS	8.66±1.60	11.20±1.58	19.86±3.19	492±52
	F-value	**8.384**	**9.895**	**10.120**	**60.839**

Significant at 0.05 % level
3 Replicates
± – Standard Deviation

Gamma rays and EMS was drastically reduced the length of root, shoot, seedling and vigour index in *J. curcas* at higher doses / concentrations. Similar observations were made by several workers in sunflower (Giriraj *et al*. 1990 and Jayakumar and Selvaraj 2003). The inhibitory effect of mutagens on the length of seedling was evident from the decrease in length of root and shoot with increasing dose / concentration of gamma rays and EMS. The reduction in length of root and shoot was attributed to the effects of mutagens on the physiological system (Gaul, 1977) such a reduction in length of root and shoot arising out of mutagenic treatments was previously reported in crop plants (Reddy and Gupta, 1989; Amarnath and Prasad 1998 and Uma and Salimath, 2001). The stimulatory effect was observed in lower doses / concentrations of gamma rays and EMS on the length of root, shoot and seedling. The hypothetic origin of these stimulations by irradiation and EMS treatments was due to in cell division rates as well as an activation of growth hormone, *e.g*., auxin (Zaka *et al*., 2004 and Gunckel and Sparrow 1961).

The maximum plant height (Table 3) at maturity of 118.33 cm and 105 cm was recorded in 5 Kr dose and 1 % EMS treatment respectively while minimum plant height was observed in 25 Kr dose of gamma rays (83.00 cm) and 4 % EMS (81.33 cm). In the present study, gamma radiation at lower concentration has shown a stimulatory effect for plant height whereas EMS treatment in both lower and higher concentrations has shown an inhibitory effect as compared to control.

Athwal *et al*., (1970) created variability in plant height in chickpea through gamma radiation. Variability in plant height was observed through EMS treatments in *Capsicum annum* (Jabeen and Mirza, 2002) which supports the present study. Jamil and Khan (2002) found that the radiation doses of 5, and 10 Krad has slightly reduced plant height while other dose had no considerable effect on plant height. Chen and Gottschalk (1970) and Okuno and Kawai (1978) have reported that mutations were affecting the plant height. The result indicated that the mutagens could cause both positive and negative genetic variability in plant height.

Petiole length (Table 3) showed a maximum of 25.50 cm in 10 Kr dose followed by 5 Kr dose (25.46 cm) of gamma rays and in EMS treatments, 1 % EMS showed maximum petiole length of 24.36 cm. The plants treated with 25 Kr dose of gamma rays and 4 % EMS were recorded a reduced petiole length of 18.10 and 15.36 cm, respectively. Except for 5, 10, and 20 Kr doses, all the treatments of gamma rays and EMS revealed an inhibitory effect on the petiole length as compared to control. Tah (2006) observed that two varieties of mungbean (K851 and Sona) were found to have a maximum petiole length in plants treated with a lower dose of gamma irradiation.

Days to first flowering, days taken for flowering to fruiting, days taken for fruiting to maturity were ranged from 255, 6 and 49 to 316, 10.16, and 62.33 respectively, among control and treatments. A minimum decrease in days to first flowering (255), days taken for flowering to fruiting (6) and fruiting to maturity (49) was recorded in 5 Kr dose when compared to control and other treatments of gamma rays and EMS. The plants germinated from seeds which were treated with 4 % EMS took the longest duration for corresponding traits of *J. curcas* (Table 3).

A similar trend was also observed for total number of flowers per inflorescence. Total number of flowers per inflorescence had a high degree of variability as 5 Kr dose of gamma rays had 238 flowers which were almost two times greater than control and other treatments of gamma rays and EMS. The least number of flowers observed in 25 Kr dose of gamma rays and 4 % EMS treatments was 137 and 112, respectively. 1 % EMS treatment showed maximum flower and fruits traits when compared to those of other EMS treatments.

Gamma rays and EMS at a lower concentration induce hormones responsible for flowering, fruit maturity. Early flowering and fruit maturity may be due to the physiological changes caused by gamma irradiation and EMS. Both the mutagens at higher concentration caused a delayed flowering and fruiting might be due to their inhibitory effect. The present study coincides with a previous study in linseed

by George and Nayar (1973). Early flowering was also reported in *Lathyrus sativus* L by Kumar and Dubey (1998) and Girhe and Choudhary (2002). Wani and Khan (2006) in mungbean found early ripening mutants are competitive with or even superior to their mother varieties with regard to seed production.

These findings showed that gamma rays and EMS can change the days to flowering and fruit maturity. Many of the workers reported earlier that gamma ray can change flowering in either positive or negative direction (Karim *et al*., 2008). Mahala *et al.* (1990) found that mutagenesis could widen variability to either positive or negative direction which resulted in a sufficient variability in the treated population that could be utilized for selection of early or late flowering plants.

The higher efficiency of a lower concentration of mutagenic agent is due to the fact that biological damage (seedling injury, lethality and sterility) increases with the increase in dose at faster rate than the mutations (Konzak *et al*., 1965). Decrease in efficiency of gamma rays and EMS with the increase in doses has also been observed in Foxtail millet (Gupta and Yashvir 1975) in chickpea (Kharwal 1998). Both kinetin and BA promoted *in vitro* flowering in the responding genotypes but the frequencies varied with cytokinin type, concentration and genotype. Although cytokinins tend to be more supportive of vegetative growth yet, they have the ability to stimulate flowering in a number of plants (Van Staden and Dickens 1991). The influence of cytokinins in promoting flowering in sunflower tissue was reported earlier (Greco *et al*., 1984; Paterson, 1984). Consider the parallel between the effects of low dose irradiation and EMS treatments and the effects of seed size. Both have been reported to influence seedling vigour, flowering date, time to maturity and fruit yield, both have a more pronounced effect on the early stages of plant growth than on fruit yield and both give rise to trends in yield of *J. curcas*. If one assumes that the chemical primary changes resulting from irradiation and chemical treatments of seed, *i.e.* changes in the biochemical and physiological processes of the plant, are short lived, then stimulation of characters such as yield must result from secondary changes of a more permanent nature.

Flower and MacQueen (1972) found that the similarities between the effects of low dose irradiation and seed size suggested the increased seedling vigour could be such a secondary change. Also, because auxiliary bud development and floral differentiation (Friend *et al*., 1963) occur at an early stage in plant development it is possible that any treatment that affects the biochemical process, *e.g.* auxin level (Gunckel and Sparrow 1961; Riza-Zade and Saleimanova 1970 and Sax 1963) regulating the rate and pattern of apical differentiation at these stages could have a direct effect on subsequent agronomic performance of a plant, *e.g.* the number of early flowers.

Table 3

Effect of gamma rays and EMS on growth and fruit traits of *J. curcas* (at maturity)

S. No	Dose/ Concentration	Plant height (cm)	Petiole length (cm)	Total number of flowers / inflorescence	Days to first flowering (days after sowing)	Days taken for flowering to fruiting	Days taken for fruiting to maturity	Number of fruit bunches / plant	Number of fruits / bunch
1.	Control	133.33±3.55	21.76±1.80	220.00±26.90	306±18.02	8.00±1.00	56.66±4.72	11.00±1.00	9.66±1.55
2.	5 Kr	118.33±3.05	25.46±.85	225.00±30.00	255±21.54	6.00±1.00	49.00±3.00	17.00±2.00	15.33±2.08
3.	10 Kr	109.66±5.13	25.50±2.61	238.66±12.66	282±15.71	7.33±1.52	57.33±2.51	13.33±1.52	11.66±2.08
4.	15 Kr	94.33±4.04	21.06±1.05	205.66±18.23	299±10.06	9.00±1.00	59.66±3.80	14.66±6.00	10.66±2.51
5.	20 Kr	100.33±5.13	22.55±4.73	174.00±16.82	294±32.34	8.00±1.00	59.66±6.80	10.33±2.08	7.33±3.21
6.	25 Kr	83.00±3.60	18.10±1.91	137.00±16.37	279±27.79	9.50±0.50	58.66±1.52	6.66±1.52	6.00±1.20
7.	1 % EMS	105.00±5.56	24.36±1.30	232.66±27.06	276±18.52	8.00±2.00	52.66±1.52	14.66±2.08	11.00±2.64
8.	2 % EMS	98.33±5.13	18.13±1.00	159.33±16.01	303±18.02	9.00±1.00	58.66±1.54	9.33±1.53	7.00±1.00
9.	3 % EMS	94.33±5.13	16.76±0.70	135.00±13.22	308±26.07	10.16±0.76	60.66±3.5	8.00±1.00	5.66±0.58
10.	4 % EMS	81.33±3.51	15.36±0.32	112.00±17.08	316±28.08	10.16±1.75	62.33±4.50	6.00±0.80	4.33±0.58
	F-value	**22.312**	**9.688**	**10.650**	**3.021**	**3.369**	**4.681**	**6.470**	**9.190**

Significant at 5 % level
Values are Triplicate
± – Standard Deviation

The number of fruit bunches per plant of treated and untreated plants was counted. Maximum number of fruit bunches (17) was observed in 5 Kr dose of gamma rays treated plants. 1 % EMS among the chemical treatments showed a number of fruit bunches of 14.66. A minimum number of fruit bunches was 6.66 and 6 recorded in 25 Kr dose of gamma rays and 4 % EMS, respectively. Treatments of gamma irradiation revealed a stimulatory effect while EMS treatments exhibited an inhibitory effect on this trait when compared to control. However, a low concentration of EMS (1 % EMS) was shown to increase fruit number when compared to other EMS treatments.

A similar trend was observed for the number of fruit per bunch. Number of fruits per bunch was recorded maximum of 15.33 in 5 Kr dose of gamma rays and 11.00 in 1 % EMS among other treatments of gamma rays (10, 15, 20 and 25 Kr doses) and EMS (2, 3, and 4 %). Number of fruits per bunch was reduced for 6 and 4.33 by 25 Kr dose of gamma rays and 4 % EMS treatments respectively. Data obtained in this present study, analyzed by ANOVA (oneway) using SPSS package (17.0) was statistically significant at 5 % level.

Time taken for fruit setting and fruit maturation in the same fruit bunch differed by various time due to mutagenic treatments and genes involved for these traits because these traits are controlled by number of genes that is called as polygenic inheritance. Jabeen and Mirza (2004) stated that this is most probable because of mutations in any one or more than one genes involved in flowering and subsequently in fruit development. Several genes like Leafy and Ap1 involved in flowering have been isolated from model plants like *Arabidopsis* and Tomato (Leandro *et al*., 1990).

Jabeen and Mirza (2002) found that the maximum average number of fruit was recorded in 0.1 % EMS treatment and the minimum average number of fruit was recorded in plants treated with 0.01 % EMS. Promoting effects of low doses of gamma rays and EMS on biological parameters have been reported earlier in *Cicer arietinum* (Mujeeb, 1974 and Mahto *et al.*, 1989) and *Vicia faba* (Vandana and Dubey, 1988). Rubaihayo (1976) obtained high yielding mutants and found significant genetic variability in yield and maturity in soybean plants, grown from seeds irradiated with gamma rays. An increased number of okra fruit per plant and fruit length as a result of gamma irradiation was recorded by many authors (Dubey *et al*., 2007; Mishra *et al*., 2007 and Sharma and Mishra, 2007). Obviously, as a result of an increased number of fruits per plant it reflected positively on seed yield.

McManus *et al*., (2007) stated that because of the extended reproductive cycle of tree species, a traditional approach to mutation induction incorporating seed treatment and setting of seedlings to distinguish between somatic and genetic effects of mutagenic treatment was not feasible. An alternative approach applied extensively in *Zea mays* used mutagenised pollen for artificial pollination. This method, first described by Neufter and Coe (1978), required that pollen soaked in a

suspension of ethyl methane sulfonate (EMS) in inert paraffin oil. Though *J. curcas* is a small tree species (perennial plant), on the contrary, we used seeds as a biological material for induction of mutation using gamma rays and EMS. Results obtained for germination, growth of seedlings and fruit traits in the present study are similar to that of a previous study in tree species through pollen treatment with chemical (McManus *et al*., 2007) and physical mutagens (Yang *et al*., 2004).

CONCLUSIONS

Among the two mutagens used, gamma rays (5 Kr dose) were found to be more effective and showed a stimulatory effect as compared to control and EMS whereas EMS treatments showed an inhibitory effect on all the parameters studied as compared to control and gamma rays treatments. However, low dose of EMS treatments particularly 1 % EMS recorded a maximum germination percentage, growth and fruit traits in *J. curcas*. The stimulatory effect at a lower dose is due to the fact that mutagens at lower concentrations stimulate the role of enzyme and growth hormone responsible for growth and yield while the inhibitory effect is due to the fact that biological damage increased at a faster rate in higher concentrations of mutagens. Hence, genetically improved plants for production of high fruit yield, which results in obtaining economic seed yield in *J. curcas*, can be achieved through induced mutagenesis by gamma rays and EMS. Seed characters, oil content and analysis of genetic variation through PCR-RAPD marker in treated populations yet to be studied will be published in the near future.

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