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INDUCED VIABLE MACROMUTANTS IN *CORCHORUS OLITORIUS* L.

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Eight viable morphological mutants namely, *viridis*, *chloroxantha*, *pigmented stem*, *thick stem* I and II, *broad leaf*, *lax branching* and *late flowering*, were isolated at M_2 (1901 treated plants scored) following gamma irradiations (50, 100, 200, 300, and 400 Gyre doses) and EMS treatments (0.25, 0.50 and 1.00% for 2 and 4h durations) to dry seeds (moisture content: 13.5%) of *Corchorus olitorius* L. var. JRO 524 (Family: Tiliaceae). EMS treatments induced higher mutation frequency (range: 0.56 to 5.88%; total – 1.49%) than gamma irradiations (range: 0.35 to 0.82%; total – 0.52%). Total mutation frequency over the M_2 population was noted to be 0.89% and the mutants occurred in the following order: *viridis* = *chloroxantha* = *pigmented stem* = *thick stem* II > *late flowering* > *thick stem* I = *broad leaf* = *lax branching*. Stem anatomy (base, middle and upper zones) of *thick stem* I and II and *pigmented stem* (with good fibre yield) in comparison to control revealed enhanced fibre zone, higher number of fibre pyramid and increased number of fibre bundles per pyramid. Meiosis (2n = 14) and pollen fertility were studied (control: 95.91%, mutants: 74.91 to 88.95%) in the plant types. Significance of the mutants (*thick stem* I and II, *pigmented stem*, *viridis* and *chloroxantha*) has been highlighted.

Keywords: *Corchorus olitorius*; EMS; Gamma irradiations; Macromutants.

Introduction

Experimentally induced mutation widens the gene pool through creation of genetic variability, and the methodology has been successfully administered for crop improvement and release of elite 'plant type' mutants in India[1].A good number of induced desirable morphological mutants with applied importance were reported in *Corchorus olitorius* L. (Family: Tiliaceae)[2], an important fibre yielding species of Jute. Attempts of mutagenesis in jute species have been most predominantly concentrated on the increase in the variability in fibre yield and yield contributing traits. Present investigation is an additional endeavor in that aspect, and this communication describes M_2 macromutants, in *C. olitorius* L. (var. JRO 524 – reported to be the most high seed yielding variety in India, among *C. olitorius* species[3]), induced following gamma irradiations and EMS treatments. New plant types in jute species from the already existing superior ones is always desirable for crop improvement and enhancement of trade.

Material and Methods

Dry seeds (moisture content 13.5%) of *C. olitorius* L. var. JRO 524 (collected from CRIJAF, Nilgunj, Kolkata) were gamma irradiated (50, 100, 200, 300 and 400 Gyre doses, given from ^{60}CO source at Saha Institute of Nuclear Physics, Salt lake, Kolkata) and EMS (0.25, 0.50 and 1.00% for 2 and 4 h duration; EMS solution prepared in 0.2M phosphate buffer, pH 6.8) treated. EMS treated seeds were thoroughly washed in running water for 3 to 4 h. Control (dry seeds) and treated seeds were sown (50 seeds in each lot of treatment) in the experimental garden of Department of Botany, Kalyani University, at a spacing of 10 cm between plants and 30 cm between lines to raise M_1 generation. Selfed seeds of each surviving M_1 plants were used (plant to row) to grow M_2 population as plant progenies. Frequency of macromutants was estimated at M_2 (1901 plants screened throughout the growth period of treated populations) as per 100 plants. Stem and leaf colour (of identical maturity) in mutants and control were laid with reference to Horticultural Colour Chart I and II (1968). Chlorophyll content (mg/gm of tissue) was assessed quantitatively (leaf tissue of identical maturity) in control and chlorophyll mutant plant types, following the method of Arnon[5]. Meiotic studies were performed in the plant types (PMCs and pollens were stained in 1% propionocarmine solution; fully stained pollens were considered fertile). Photomicrograph was taken from temporary squash preparation.

Results and Discussion

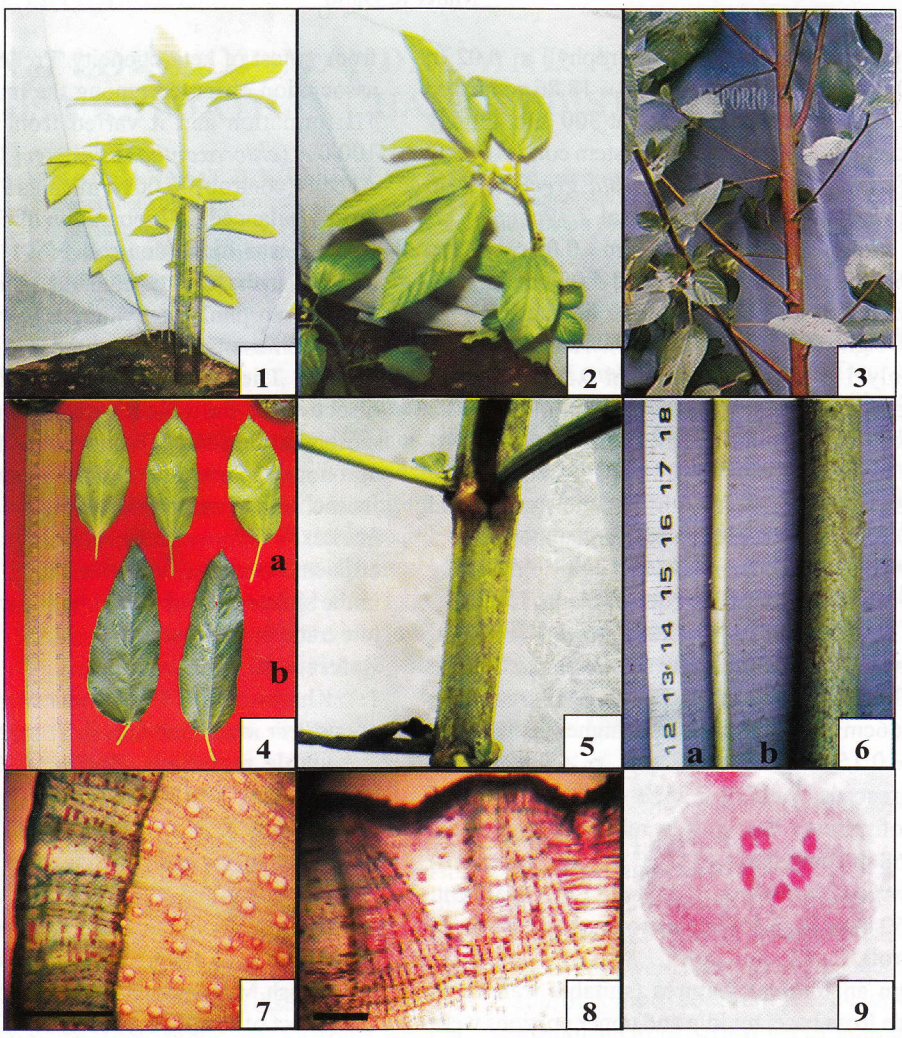
Types of macromutants (8 types - all viable) at M_2 and their frequencies estimated in different mutagen treatments, were presented in Table 1. The viable mutants (Figs. 1- 3, 5) noted (mutant traits confirmed at M_3 from

Table 1. Types and frequency of macromutations in *C. olitorius*.

Doses	No. of plant scored at M_2	Frequency (%)								Total viable mutation frequency
		Viridis	*Chloroxantha*	*Pigmented stem*	*Thick stem I*	*Thick stem II*	*Broad leaf*	*Lax branching*	*Late flowering*	
Gamma rays										
50 Gy	266	0.00	0.00	0.38	0.00	0.00	0.00	0.00	0.00	0.38
100 Gy	288	0.00	0.00	0.35	0.00	0.00	0.00	0.00	0.00	0.35
200 Gy	166	0.00	0.00	0.60	0.00	0.00	0.00	0.00	0.00	0.60
300 Gy	197	0.51	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.51
400 Gy	244	0.82	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.82
Total	1161	0.26	0.00	0.26	0.00	0.00	0.00	0.00	0.00	0.52
EMS										
0.25%, 2h	185	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.08	1.08
0.50%, 2h	108	0.00	0.00	0.00	0.00	0.93	0.00	0.00	0.00	0.93
1.00%, 2h	123	0.00	0.00	0.00	0.00	1.63	0.00	0.00	0.00	1.63
0.25%, 4h	179	0.00	0.00	0.00	0.00	0.00	0.00	0.56	0.00	0.56
0.50%, 4h	68	0.00	2.94	0.00	4.47	0.00	1.47	0.00	0.00	5.88
1.00%, 4h	77	0.00	1.30	0.00	0.00	0.00	0.00	0.00	0.00	1.30
Total	740	0.00	0.40	0.00	0.14	0.40	0.14	0.14	0.27	1.49
Grand Total	1901	0.16	0.16	0.16	0.05	0.16	0.05	0.05	0.10	0.89

Table 2. Anatomical features in control and mutants of *C. olitorius*.

Traits	Zones	Genotypes				p value of x^2 test of heterogeneity
		Control	Thick stem I	Thick stem II	Pigmented stem	
Fibre zone (cm^2)	Base	0.50±1.14	4.22±1.21	3.08±1.17	2.76±0.32	>0.001
	Middle	0.30±0.69	3.06±1.18	1.48±1.20	1.04±0.65	>0.001
	Upper	0.20±0.57	1.19±1.12	1.12±1.09	0.46±1.71	>0.01
Number of fibre pyramid per T. S.	Base	61.55±3.78	94.60±4.04	84.4±3.94	71.20±3.01	>0.001
	Middle	50.20±3.46	86.20±3.55	78.4±5.76	58.33±2.14	>0.001
	Upper	43.33±4.06	69.00±2.32	64.6±3.32	45.00±1.77	>0.001
Number of fibre bundle per pyramid	Base	40.73±2.48	72.50±2.74	70.4±2.67	61.6±1.65	>0.001
	Middle	26.58±2.20	56.83±2.04	49.4±4.70	54.7±2.01	>0.001
	Upper	16.00±1.50	31.50±1.67	27.6±1.86	30.9±2.04	>0.001
Diameter of fibre cell (μm)	Base	16.50±0.58	17.60±0.73	17.53±0.71	18.02±0.88	0.80-0.90
	Middle	16.78±0.49	18.70±0.80	16.50±1.30	18.15±1.02	0.70 – 0.50
	Upper	18.98±0.87	20.08±0.80	15.58±0.94	14.85±0.94	<0.05



Figs.1-9. *Corchorus olitorius* mutant plant types (1-3, 5), control and mutant traits (4 and 6), stem anatomy (7-8) and meiosis (9). 1. *Chloroxantha*. 2. *Viridis*. 3. *Pigmented stem*. 4. Leaves of *chloroxantha* (a) and control (b). 5.*Thick stem* I. 6. Stem of control (a) and *Thick stem* I mutant (b). 7-8. T. S. of stem of control (7) and *Thick stem* I (8) mutant (middle zone). 9. 7II at MI.

self segregating progenies of M_1 seeds at M_3) have been classified[6-7] on the basis of mutation affecting seedling colour (*chloroxantha* and *viridis*), stem characteristics (*pigmented stem*, *thick stem* I and II), nature of leaf (*broad leaf*), branching pattern (*lax branching*) and maturity (*late flowering*). Mutation frequency was not dose dependent. Frequency of viable mutation varied from 0.38 to 0.82% in gamma irradiations and 0.56 to 5.88% in EMS treatments. EMS has induced (1.49%) higher mutation than gamma irradiations (0.52%). Highest mutation frequency has been observed in 4 hour treatment with 0.50% EMS. Total mutation frequency over the M_3 population was noted to be 0.89% and the mutant types occurred in the following order: *viridis* = *chloroxantha* = *pigmented stem* = *thick stem* II > *late flowering* > *thick stem* I = *broad leaf* = *lax branching*. *Viridis* and *pigmented stem* occurred only in gamma irradiations; while, the other mutant types were scored from EMS.

Desirable characteristics of the viable macromutants are: *chloroxantha* (seedling colour *Pea green* 61 Fig. 1 and 4a, compared to *Emerald green* 758 in control, Fig. 4b; chlorophyll content: chlorophyll a 0.003, b 0.02, total 0.03 compared to 0.19, 0.21 and 0.41, respectively, in control; recorded in 0.5 and 1.0%, 4h EMS treatments; plants attained 166.79 cm ± 26.65 height at maturity), *viridis* (seedling colour *Cyprus green*

59, Fig. 2, chlorophyll content : chlorophyll a 0.02, b 0.06, total 0.08; plant height 267.55cm ± 18.86 compared to 259.08 ± 8.81 in control; spotted at 300 and 400 Gy gamma irradiations), *pigmented stem* (stem colour Poppy red 16/1, Fig. 3, compared to *Agathia green* 60/1 in control, Fig. 5; girth of the stem: basal 7.79cm ± 0.17, middle 7.45 cm ± 0.22 and upper 6.27 cm ± 0.08 compared to 7.62cm ± 0.47, 5.77 cm ± 0.85 and 4.00 cm ± 0.43, respectively, in control plants. Fig. 6a; height 367.45 ± 8.96; fibre weight 18.33 g ± 1.67), *thick stem* I (Fig. 5 and 6b – only 1 plant was spotted in 0.50%,4h EMS treatment; girth of the stem : basal 14.2cm, middle 12.2 cm and upper 7.82 cm; plant height 317.5 cm at maturity and yielded 82.0 gm fibre), *thick stem* II (noted in 0.50 and 1.0%, 2h EMS treatments; girth of the stem : basal 11.85cm ± 0.42, middle 8.21cm ± 0.37 and upper 7.37cm ± 0.15; plant height 338.67cm ± 24.45; fibre yield 73.33g ± 1.67 significantly higher than control plants 15.80g ± 2.89 : $t = 22.79$, $DF = 6$, $p > 0.001$), *broad leaf* (leaf colour *Scheeles green* 80; frequency over the mutant population 0.05%, spotted in 0.50%, 4h EMS treatment; leaf area : 9.66cm^2 ± 0.66 significantly higher than control 1.93 cm^2 ± 0.15; $t = 9.34$, $DF = 4$, $p > 0.001$), *lax branching* (recorded only at 0.25%, 4h EMS; angle of divergence of primary branches in relation to main axis 50.8^0 ± 4.68 in comparison to 36.3^0 ± 3.34 in control) and *late flowering* (scored from 0.25%, 2h EMS treatment; flowering 160 days from sowing as compared to 75 to 90 days in control).

Stem anatomical features (suitable transverse sections made from base, middle and upper portions were stained following the method described by Johansen[8]) were analyzed in control and in 3 mutants (*thick stem* I and II and *pigmented stem* – the mutants had thick stem and good fibre yield). Results (Table 2) indicated that fibre zone, number of fibre pyramid/ section and number of fibre bundles per pyramid (Fig. 7 – 8) enhanced significantly($p > 0.01$ to $p > 0.001$) in mutants than control as evidenced from χ^2 test of heterogeneity; however, diameter of fibre cell was random among the plant types. Thus, utility of the mutants has been evident from this study.

Meiotic analysis performed in control (51 PMCs analyzed) and mutants (30 – 93 cells scored) documented $2n = 14$ chromosomes (Fig. 9) always and the chromosomes formed bivalents (control: 7.00/cell; mutants: 6.60/cell to 7.00/cell) mostly and rarely univalents (mutants: 0.00/cell to 0.80/cell). Distribution of bivalent was random among the plant types ($\chi^2 = 2.20$, $df = 8$, $p > 0.95$); however, univalents ($\chi^2 = 152.3$, $df = 8$, $p > 0.001$) were non-randomly distributed as evidenced from χ^2 test of heterogeneity. Predominant chromosome association observed among the mutant plant types was 7II formation and it varied from 72.04% (*viridis*) to 100.0% (*chloroxantha, thick stem* I and *late flowering*). AI cells (control 100.00%; mutants 97.67 to 100.0%) were mostly balanced (7/7 separation of chromosomes). Pollen fertility was high (control: 95.91% mutants: 74.91 to 88.95%) in the plant types. Thus, meiotic analysis revealed that the morphological mutants were not the outcome of any chromosomal disturbances.

The plant types evolved seem to be in the direction of the objective underlined and correspond closely to the ideotype being looked for in the crop. Proper agronomic management of the mutants is most desirable for their future exploration in the field of genetics (genetic marker – *chloroxantha, viridis, pigmented stem*) and efficient breeding (*thick stem* I and II and *pigmented stem*) in the species. *Thick stem* I and II may possibly enhance jute trade in the country.

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