

necropsy score and impression smear examination for the presence of acid fast bacilli in the visceral organs. Every animal after death was examined to confirm the specific cause. The typical lesions of the organs provided a reasonable assurance that the animals died due to tuberculosis. The experiment was terminated after 150 days of infection.

The results presented in table 1 show that *M. habana* afforded protection against all *M. tuberculosis* strains used. The main criteria of protection were considered to be prolongation of survival time of vaccinated animals *vis a vis* control animals after being challenged with *M. tuberculosis* strains. Unvaccinated control group of animals challenged with highly and moderately virulent strains started showing deterioration in their general appearance and their body weight dropped considerably as compared to vaccinated animals. These animals died earlier and their visceral organs showed more intense lesions. The impression smears of the organs from these unvaccinated animals had an abnormally large number of acid fast bacilli. A few animals which survived in vaccinated groups challenged with moderately pathogenic strains showed either no lesions or healed lesions on biopsy at 150 days post infection.

The data have been statistically analysed and the *P* values were highly significant. Results show that *M. habana* was able to protect in acute, subacute and chronic types of infection in mice produced by several variants of *M. tuberculosis*. The substantial protection afforded even in chronic situation throws evidence towards the potent immunogenicity of *M. habana*. Varying virulence of *M. tuberculosis* strains tested did not interfere with the protective efficacy of *M. habana*. Hank *et al*<sup>7</sup> reported a similar finding with BCG. *M. habana* has already been found to be non-pathogenic in several species of animals including monkeys<sup>8</sup>. *M. habana* has been found to elicit strong cell-mediated immune responses *in vitro* and *in vivo* against *M. tuberculosis* and *M. leprae* antigens indicating antigenic relationship<sup>9</sup>. The wide spectrum of immunity together with non-pathogenicity afforded by *M. habana* makes it a suitable candidate for future vaccine preparations. Further work is in progress.

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#### INHERITANCE OF INDUCED MUTANT CHARACTERS IN JUTE (*CORCHORUS CAPSULARIS* L.)

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THE dearth of distinct morphological characters in the natural variability seems to be responsible for the few genetic studies in jute (*Corchorus capsularis* and *C. olitorius*). Although the inheritance of some induced mutants was reported in the sixties<sup>1,2</sup>, the data on the genetics of morphological traits are scanty, because the number of true breeding mutants available has been very small. Thus, in the case of *C. capsularis*, inheritance of only 19 mutants has so far been studied<sup>3</sup>. However, a very wide spectrum of mutants (111 mutants in *C. capsularis* and 136 in *C. olitorius*) and trisomic series have been established recently<sup>4-7</sup>. Using these, chromosome mapping and assigning of genes to different linkage groups should be possible. Towards this ultimate goal, the inheritance of 20 induced mutants of *C. capsularis* var. IRC 412 is reported in this paper, and the gene symbols have been proposed.

Eleven mutants were crossed reciprocally to the parent variety IRC 412 (referred as *P* in tables). For nine mutants, only one way crosses could be made. The *F*<sub>2</sub> population consisted of 2 to 10 *F*<sub>1</sub> plant

progenies. All the plants with JRC 412 phenotype from one of the progenies in each cross were individually harvested to study the genotypic segregation in the  $F_3$  generation. Majority of the mutants had more than one altered character and hence the gene symbol proposed denotes the most conspicuous phenotype (table 1) as was previously followed in *Arabidopsis*<sup>8</sup>.

The  $F_1$  phenotype in all the crosses was similar to the JRC 412 parent. In the  $F_2$ , out of the 20 mutants, 15 were inherited as recessive showing a good fit to 3 JRC

412 parent type to 1 mutant type (table 1). Crosses involving three mutants, 'extremely serrated' 'long narrow' and 'short glossy' showed less number of mutants than expected on the basis of monogenic segregation. They showed fitness to a 5:1 ratio of JRC 412 type to mutant type (table 1). However, genotypic segregation in the  $F_3$  indicated a good fit to 1 non-segregating to 2 segregating families (table 2), as expected, on a monogenic recessive basis. The observed recessive deficiency in the mutants could be due

Table 1  $F_2$  segregations in crosses involving jute var. JRC 412 and its induced mutants

Cross		No. of $F_1$ progenies	$F_2$		$\chi^2$ (3:1)	P value
			JRC 412 (P) type	Mutants		
1. P × chlorina 1	(ch1)	3	541	183	0.77	30-50
2. Chlorina 2 × P	(ch2)	9	1480	514	0.03	80-90
3. P × mosaic yellow	(my)	5	627	193	0.94	30-50
4. P × glossy leaf	(gl)	9	1045	382	2.38	10-20
Reciprocal		9	1203	383	0.61	30-50
5. P × leathery crinkled	(lc)	6	978	346	0.91	30-50
Reciprocal		6	1097	354	0.28	50-70
6. P × oblique crinkled	(obc)	9	1174	357	2.31	10-20
Reciprocal		3	350	120	0.07	70-80
7. Involute × P	(in)	6	988	361	2.23	10-20
8. Broad undulate × P	(blu)	6	1043	357	0.19	50-70
9. P × narrow leaf 2	(nl2)	9	1078	582	0.21	50-70
Reciprocal		9	1170	384	0.05	80-90
10. P × Short ovate	(so)	6	842	306	1.68	10-20
Reciprocal		6	765	229	2.04	10-20
11. P × foliaceous stipule	(fol)	6	604	191	0.40	50-70
Reciprocal		6	541	188	2.40	10-20
12. P × brittle stem	(bts)	6	1090	330	2.33	10-20
Reciprocal		10	1508	533	1.55	20-30
13. P × bunchy top	(but)	2	156	52	0	100
Reciprocal		2	207	64	0.27	50-70
14. P × unbranched	(br)	6	759	258	0.07	70-80
Reciprocal		4	475	139	1.83	10-20
15. P × pale veins	(pv)	5	864	274	0.52	30-50
Reciprocal		9	2393	745	2.69	10-20
						$\chi^2$ (5:1)
16. Extremely serrated × P	(ser)	9	2211	421	0.89	30-50
17. P × long narrow 2	(ln2)	6	1087	242	2.28	10-20
Reciprocal		3	810	181	1.81	10-20
18. P × short glossy	(sgl)	9	2590	536	0.52	30-50

(gene symbols given in parenthesis)

**Table 2** Genotypic and Phenotypic Segregations in the  $F_3$  of crosses involving Jute var. JRC 412 and its induced mutants

Cross	Genotypic segregation			Phenotypic segregation				
	Total	(No. of progenies) Homo- zygous	Hetero- zygous	$X^2$ (1:2)	JRC 412 type (P)	Mutants	$X^2$ (5:1)	P value
Extremely serrated $\times$ P	42	16	26	0.42	793	141	1.63	20-30
P $\times$ Short glossy	45	15	30	0	902	180	0	100
Long narrow 2 $\times$ P	53	18	35	0.01	477	164	0.12	70-80
							$x^2(3:1)$	

**Table 3**  $F_2$  segregations in crosses involving jute var. JRC 412 and its induced mutants.

Cross	No. $F_1$ progenies	JRC 412 type (P)	$F_2$			$X^2$ (9:3:3:1)	P value
			Coppary red stem, normal leaf	Normal stem, cordate leaf	Coppary red stem, cordate leaf		
19. Cordate coppery red $\times$ P ( <i>co. CAr</i> )	2	351	109	101	32	2.38	30-50
		JRC 412 type (P)	Coppary red stem, normal leaf	Normal stem, broad leaf	Coppary red stem, broad leaf		
20. P $\times$ Broad coppery red ( <i>bl. CAr</i> )	4	300	99	80	36	3.72	20-30

(gene symbols given in parenthesis)

to the lesser competitive ability of the gametes carrying the mutations. In the remaining two mutants viz 'cordate-coppery red' and 'broad coppery red', the  $F_2$  segregation showed the typical dihybrid ratio of 9:3:3:1 (table 3). The coppery red anthocyanin pigment character was inherited independent of the respective leaf characters. It is likely that two independent mutations are responsible for the observed phenotypes.

The pattern of anthocyanin pigmentation in *C. capsularis* is controlled by three factors C, A and R<sup>9</sup>. The pigmentation of *C. capsularis* variety JRC 412 is classified as green-coppery-red with the genotype *CC AA RR*. Both 'cordate coppery red' and 'broad coppery red' mutants of the present study have coppery red pigmentation of stem, petiole and sepals; and can be grouped under coppery-red pigment type with the genotype *CCAarr* following the classification of Kundu *et al.*<sup>9</sup>. Thus, it is likely that a mutation (or deletion) was induced at R locus in these two mutants.

The mutants included in the present study are being crossed to the trisomics to assign the mutant genes to the different chromosomes.

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### EFFECT OF GAMMA RADIATIONS AND GIBBERELIC ACID ON GROWTH AND SHOOT REGENERATION IN CALLUS CULTURES OF *DATURA INNOXIA*

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STIMULATORY effects of low doses of ionizing radiations on growth and differentiation in cultured plant cells have been reported<sup>1-6</sup>. Some workers have demonstrated a variety of responses of GA<sub>3</sub> on organogenesis. There are evidences of repression of organogenesis by GA<sub>3</sub> in both meristem cultures and callus<sup>7,8</sup> as well as synergistic effect of GA<sub>3</sub> on organogenesis in combination with other plant growth regulators<sup>9-11</sup>. This communication describes the results of investigations carried out on growth and differentiation in the anther derived cultures of *Datura innoxia* that had been subjected to varying doses of gamma-irradiation and transferred to media with and without GA<sub>3</sub>.

Explants of anther-derived haploid plantlets, raised according to the method of Sharma and Chowdhury<sup>12</sup> were used for initiating calli on Murashige and Skoog's<sup>13</sup> medium supplemented with 2 mg/l of 2,4-D (MS<sub>2</sub>). These cultures were irradiated with different doses of gamma rays (source <sup>60</sup>Co; intensity 800 r/min) and transferred to fresh MS<sub>2</sub> and B<sub>3</sub><sup>14</sup> media for callus growth and shoot regeneration respectively. Four cultures were employed for each treatment. All the cultures were kept in the dark at 27 ± 1°C except for shoot differentiation where they were incubated under constant illumination of 4000 lux. Growth rate was measured by recording an increase in the fresh as well as the dry weights after 20 days of incubation.

Growth of callus cultures was stimulated at 0.2 kR dose of gamma-radiation (table 1) but it decreased as radiation dose increased. Cultures exposed to 5 kR dose turned brown, indicating a general inhibition of callus growth. Shoot regeneration, however, was

**Table 1** Growth of callus cultures of *Datura innoxia* after 20 days of incubation.

Rad. Dose (kR) Treatment	% increase in fresh weight		% increase in dry weight	
	MS <sub>2</sub>	MS <sub>2</sub> + GA <sub>3</sub> (2 mg/l)	MS <sub>2</sub>	MS <sub>2</sub> + GA <sub>3</sub> (2 mg/l)
0	301.64	262.24	301.00	228.28
0.2	404.93	360.12	377.00	258.00
1.0	289.34	250.13	220.10	155.95
5.0	112.00	137.04	150.10	139.80

**Table 2** Effect of gamma-irradiation and GA<sub>3</sub> on shoot regeneration (number of shoots regenerated/4 cultures) in callus cultures of *Datura innoxia* after 60 days of incubation.

Rad. Dose (kR) Treatment	Medium	
	B <sub>3</sub>	B <sub>3</sub> + GA <sub>3</sub> (2 mg/l)
0	6	0
0.2	11	0
1.0	19	0
5.0	0	0

stimulated both at 0.2 as well as 1 kR radiation doses (table 2). The frequency of shoot buds formed in cultures irradiated with 0.2 and 1 kR was 2 and 3 times respectively as compared to that in unirradiated cultures. Differentiation in these irradiated cultures occurred 10-12 days earlier than in unirradiated ones. No shoot regeneration was observed in 5 kR irradiated cultures. Low dose (0.2 kR) of irradiation was conducive to active cell proliferation, resulting in exuberant growth of the callus. Also the morphogenetic capacity for regeneration of shoot buds and plantlets in cultures exposed to low doses (0.2 and 1 kR) was augmented over unirradiated control. Higher dose (5 kR) was detrimental to both growth as well as differentiation. The inherent ability for growth and differentiation of the isolated plant cells culminating in plantlet formation, can thus be enhanced by the use of a stimulatory dose of radiation. Addition of GA<sub>3</sub> to the media resulted in the lower growth and it prevented organogenesis completely, both in unirradiated as well as irradiated calli. Thus the stimulatory effects of gamma radiations were not seen in the presence of GA<sub>3</sub>.

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