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Induction of variability through mutagenesis in opium poppy (*Papaver somniferum* L.)

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Abstract: Opium poppy (*Papaver somniferum* L.), an important plant of the family Papaveraceae, is valued for its extensive medicinal properties due to the presence of more than 80 different alkaloids, which are products of the polygenic interaction of different genes involved in plant metabolism. Broadening the genetic base through induced mutation is a supplementary tool that can lead to the development of genetic variability. The present study was undertaken to generate a broad genetic variability through mutation breeding using physical doses (gamma radiation of kR10 to kR50 at an interval of kR10), chemical doses (EMS of 0.2, 0.4, 0.6, and 0.8% (w/v)), and combined doses (gamma and EMS) of mutagen, and to evaluate the plants' advance generations for different traits as well as for specific alkaloids, especially thebaine and codeine. The kR30 dose, which caused the highest results for all 3 genetic parameters (GCV, h2, and GA%) for 7 traits, was the most effective in NBRI-1. Similarly, the kR10 + 0.4% EMS dose proved to be the best for NBRI-5, affecting 10 different characters. The kR10 + 0.4% EMS dose created positive mutations for high thebaine and codeine content and low morphine content, while the kR40 + 0.6% EMS dose did the same for narcotine. The study also confirmed that the pathway of morphinan alkaloids and narcotine formation was bifurcated at the lower combined dose (kR30 and kR10 + 0.4% EMS), which was effective in causing micromutation in morphinan alkaloid pathways. The higher combined dose (kR40 + 0.4% EMS) affected narcotine production.

Key words: Alkaloids, genetic variability, induced mutation, *Papaver somniferum*, thebaine

Introduction

The creation of genetic variability in a base population of any crop becomes essential when breeding objectives are complex. Plant breeders mostly remain concerned with the genetic improvement of quantitative traits of the crop, which are controlled by polygenic interactions. Alkaloid biosynthesis in alkaloid-bearing plants is also a result of polygenic interaction. Opium poppy (*Papaver somniferum* L.), an important plant of the family Papaveraceae, is valued for its extensive medicinal properties due to the presence of more than 80 different alkaloids. Phenanthrene (morphine, codeine, thebaine), benzylisoquinoline (papaverine), and phthalide isoquinoline (narcotine) are the major alkaloid groups in opium latex (Singh et al. 1995; Pushpangadan and Singh 2001; Ziegler et al. 2005). Among these alkaloids, morphine, despite being a narcotic, is the most important alkaloid for medicinal purposes. It is used as a painkiller and as a sedative for conditions such as anxiety and insomnia, and it is also valuable in the treatment of disorders such as biliary and renal colic, myocardial infarction, and congestive heart failure, as well as for severe trauma

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such as hemorrhaging. In addition to morphine, thebaine is also used as an analgesic and sedative, but is a nonnarcotic and nonaddictive constituent of poppy, found in a lesser quantity (1%-2%) in opium latex (Singh et al. 1995; Hevel et al. 2001; Shukla and Singh 2004). In recent years, the global demand for thebaine has increased multifold, as it can be easily converted into codeine and morphine. Therefore, it is necessary to develop opium poppy varieties rich in thebaine to meet this rising global demand. The traditional varieties of opium poppy have low potentiality and restricted variability with respect to economic characteristics. To broaden the genetic base of the existing varieties and to develop strains with opium latex rich in specific alkaloids, induced mutagenesis is a quick and early approach (Fist 2001). Various classes of physical and chemical mutagens differing in efficiency can be used to induce mutations. The combination of different mutagens, if their mutagen induction processes are independent and capable of interaction, may increase the mutation frequency and can alter the mutation spectrum. Even though ionizing radiation is thought to be the most suitable agent for inducing genetic variability (Irfaq and Nawab 2003; Joseph et al. 2004; Sangsiri et al. 2005; Tah 2006), recent studies have shown that a number of chemicals have been found to be equally and even many times more effective and efficient as mutagens (Solanki 2005; Rekha and Langer 2007; Basu et al. 2008; Dhanavel et al. 2008; Ganapathy et al. 2008). The present study is an effort to generate broad genetic variability in 2 varieties of opium poppy, especially for specific alkaloids, through mutation breeding by using physical, chemical, and combined doses of mutagen followed by the evaluation of advance generations for specific alkaloids to identify the plant lines rich in thebaine content.

Materials and methods

The material used in the present experiment comprised 2 promising high-yielding commercial varieties of opium poppy (*Papaver somniferum L*), NBRI-1 and NBRI-5. Seeds of these varieties were presoaked for 12 h in distilled water before being subjected to different doses of a chemical mutagen ethyl methyl sulfonate ($CH_3SO_2OC_2H_5$) solution (EMS) prepared in phosphate buffer at pH 7.0 (FAO/IAEA Technical Report Series No. 119, 1977). For both varieties, 10 g were standardized at a 12% moisture content and irradiated with gamma radiation through a ^{60}Co source with a dose rate of 63 s/kilorad (kR) from a gamma chamber 9000 at NBRI, Lucknow, India. Administered doses ranged from kR10 to kR50 at an interval of kR10. The doses used for chemical mutagenesis were of 0.2, 0.4, 0.6, and 0.8% (w/v) EMS concentrations and were given using the seed-soaking method. In this method, the seeds were presoaked in distilled water followed by a series of EMS solutions of different concentrations. The NBRI-5 variety was subjected to chemical and combined doses of both mutagens in different concentrations. For the combined effect of both mutagens, dry seeds were irradiated with the same doses of gamma rays followed by presoaking in distilled water and a series of EMS doses (0.2, 0.4, 0.6, and 0.8 (w/v)).

The seeds treated with different doses of mutagens in 34 treatments, along with 1 control for each variety, were sown in a randomized block design with 3 replications in the experimental field of the Genetics and Plant Breeding Division, NBRI, Lucknow, India. The rows were 3 m long and the plot area was 6 m^2. The plant-to-plant distance was 10 cm and the row-to-row distance was 30 cm. From each treatment, 15 normal stand M_1 plants were randomly selected and selfed to obtain seeds for the M_2 generation. The harvested M_2 seeds were sown in plant-to-progeny rows in the subsequent year. From each M_2 family, selfed seeds of 6 individual plants were further grown to raise an M_3 generation in the following year. In both the M_2 and M_3 generations, selection of individual plants was based on the content of specific alkaloid and opium yields, which were treated as micromutants. Normal cultural practices were followed during the experiments (Yadav et al. 2006). Induced quantitative variability with respect to the following 17 characters were studied: number of days to 50% flowering, plant height (cm), peduncle length (cm), number of leaves, number of branches, number of capsules, stem diameter (cm), capsule size (cm^2), capsule weight (g), seed yield (g), husk yield (g), opium yield (mg), and alkaloid content (morphine, codeine, thebaine, narcotine, and papaverine) per

plant in the M_2 and M_3 generations. Variances in the M_2 and M_3 generations for these different quantitative characters, based on both the experimental and the control data, were statistically analyzed as suggested by Panse and Sukhatme (1978) and Singh and Chaudhary (1985). The 5 major alkaloids in opium latex (morphine, codeine, thebaine, narcotine, and papaverine) were quantified with HPLC as reported by Khanna and Shukla (1986).

Results

Analysis of variance for the combined population of M_2 and M_3 showed significant differences among the physical mutation treatments in NBRI-1 for all traits except for number of branches, number of capsules, and codeine content in M_2 and number of branches, number of capsules, capsule size, and opium yield in M_3. In the M_3 families, significant differences for physical mutagenesis were observed for most of the traits, except number of branches, number of capsules, capsule size, leaf size, stem diameter, seed yield, opium yield, morphine, codeine, thebaine, and narcotine (Tables 1 and 2). In NBRI-5, analysis of variance for the M_2 population showed significant differences among the treatments for all 3 types of mutagenesis. In NBRI-5, significant differences were observed in M_2 as follows: combined mutagenesis showed significant differences for plant height, number of capsules, number of leaves, and leaf size, while chemical mutagenesis showed significant differences for seed weight and papaverine content. Among the M_3 families, significant differences were observed for peduncle length, stem diameter, capsule weight, and seed weight after combined mutagenesis, while significant differences in plant height, number of leaves, and papaverine content were observed after chemical mutagenesis. The capsule size trait showed significant differences due to physical and chemical mutagenesis, while leaf size and husk yield were significantly different due to chemical and combined mutagenesis (Table 2).

In NBRI-1, the highest genotypic coefficient of variability (GCV) was noted for plant height, morphine, and codeine at dose kR10; for number of leaves, leaf size, and papaverine at dose kR40; for peduncle length, opium yield, and thebaine at dose kR50; for capsule weight, seed weight, and husk weight at dose kR30; and for narcotine at dose kR20 (Table 3). The values of genotypic variability also showed an increase over the control group for most of the traits. In NBRI-5, plant height showed the highest GCV at dose kR30; number of leaves at dose kR40; leaf size, capsule weight, opium yield, and husk yield at dose kR50 + 0.4% EMS; peduncle length at dose kR20 + 0.2% EMS; seed weight at dose kR40 + 0.6% EMS; morphine and papaverine at dose kR30 + 0.4% EMS; codeine and thebaine at dose kR40 + 0.2% EMS; and narcotine at dose kR20 + 0.6% EMS (Table 3).

Heritability in the broad sense (h^2) was estimated for all 34 treatments and the controls for all of the traits, including the 5 major alkaloids. The heritability value of NBRI-5 was highest within the population for plant height at dose kR30 + 0.6% EMS, leaves per plant at dose kR40, peduncle length at dose kR20 + 0.2% EMS, capsule weight and seed weight at dose kR30 + 0.2% EMS, husk yield and opium yield at dose kR50 + 0.4% EMS, codeine at dose kR40 + 0.6% EMS, thebaine at dose kR20 + 0.8% EMS, narcotine at dose kR50, papaverine at dose kR30 + 0.8% EMS, and morphine in the control (Table 4).

The maximum heritability value in NBRI-1 was determined for plant height, leaf size, and papaverine at dose kR40; number of leaves, capsule weight, opium yield, morphine, and codeine at dose kR10; peduncle length and seed weight at dose kR30; and husk yield, thebaine, and narcotine at dose kR50 (Table 4).

The genetic advance (GA) in NBRI-5 was highest for plant height at dose kR30; number of leaves at dose kR40; leaf size, capsule weight, and husk yield at dose kR50 + 0.4% EMS; peduncle length at dose kR10 + 0.4%; opium yield at dose kR20 + 0.6% EMS; morphine at dose kR20 + 0.8% EMS; codeine and thebaine at dose kR40 + 0.2% EMS; narcotine at dose kR50; and papaverine at dose kR30 + 0.4% (Table 5). Similarly, GA for NBRI-1 was highest for plant height, morphine, and codeine at dose kR10; for leaves, seed weight, husk yield, and capsule weight at dose kR30; for leaf size and papaverine at dose kR40; for opium yield and narcotine at dose kR50; for thebaine at dose kR20; and for peduncle length in the control (Table 5).

Table 1. Analysis of variance for different characters (MS values) in the M_2 bulked population of NBRI-1 of *P. somniferum* L. for physical mutagenic treatment and NBRI-5 of *P. somniferum* L. for physical, chemical, and combined mutagenic treatments.

Traits	NBRI-1(physical)			NBRI-5 (physical)			NBRI-5 (chemical, EMS)			NBRI-5 (combined)		
	Families (df 14)	Treatments df 5)	Error (df 70)	Families (df 14)	Treatments (df 5)	Error (df 70)	Families (df 14)	Treatments (df 4)	Error (df 56)	Families (df 14)	Treatments (df 19)	Error (df 266)
Plant height (cm)	44.178	391.42**	33.13	56.83	314.71**	38.79	18.78	595.92**	29.391	51.766*	804.65**	43.34
No. of branches/plant	0.76	1.85	0.93	0.5	1.43	1.25	0.356	5.71**	0.67	1.52	1.33	0.79
No. of capsules/plant	0.759	1.851	0.932	0.5	1.43	1.2	0.356	5.71**	0.67	1.518*	1.33*	0.791
No. of leaves/plant	3.017	33.15**	3.12	0.94	3.48*	1.152	1.156	13.94**	1.001	4.316*	18.35**	2.075
Capsule size (cm^2)	9.26**	24.88**	3.65	5.77*	9.11*	3.02	3.057	16.46**	2.07	2.964	70.61**	2.703
Leaf size (cm^2)	66.96	5668.39**	91.39	145.08	5945.16**	126.04	303.52	3623.99**	80.13	219.112**	5000.24**	60.446
Peduncle length (cm)	6.67*	19.03**	3.57	4.55	9.86*	3.45	4.07	5.5	2.81	4.105	13.96**	4.812
Stem diameter (cm)	0.015	0.117**	0.013	0.015	0.129**	0.022	0.007	0.101**	0.011	0.025*	0.18**	0.0137
Capsule weight (g)	6.709	59.38**	7.33	16.81	75.06**	11.21	15.417	119.748**	10.513	15.236	65.36**	11.092
Seed weight (g)	3.853	18.33**	2.98	7.76*	29.57**	3.63	6.011	36.85**	3.885	6.262	23.06**	4.958
Husk weight (g)	0.97	14.15**	1.51	2.22	10.98**	2.94	3.052	24.425**	2.573	3.109	12.86**	2.208
Opium yield (mg)	8828.42**	69,594.2**	3235.21	7207.36	29,758.47**	4148.88	2999.66	13,132.05**	2770.02	4.48.694	37,269.04**	3633.673
Morphine %	0.043	5.89**	0.062	0.016	12.41**	0.019	0.085	32.676**	0.0636	0.0467	13.049**	0.048
Codeine %	12.27	16.83	12.14	0.014	0.386**	0.013	0.017	1.149**	0.01	0.0167	0.7796**	0.0137
Thebaine %	0.006	7.002**	0.007	0.002	8.66**	0.003	0.002	11.049**	0.0032	0.0016	10.3012**	0.0027
Narcotine %	0.005	9.187**	0.0004	0.033	36.99**	0.048	0.0537	49.90**	0.054	0.0279	30.2916**	0.0256
Papaverine %	0	0.000**	0	0.00018	0.9185**	0.00018	0.000**	0.000**	0	0.000024	2.115**	0.000043

* and ** show significance at 5% and 1%, respectively.

Table 2. Analysis of variance for different characters (MS values) in the M_3 bulked population of NBRI-1 of *P. somniferum* L. for physical mutagenic treatment and NBRI-5 of *P. somniferum* L. for physical, chemical, and combined mutagenic treatments.

Traits	NBRI-1 (physical)			NBRI-5 (physical)			NBRI-5 (chemical, EMS)			NBRI-5 (combined)		
	Families (df 14)	Treatments (df 5)	Error (df 70)	Families (df 14)	Treatments (df 5)	Error (df 70)	Families (df 14)	Treatments (df 4)	Error (df 56)	Families (df 14)	Treatments (df 19)	Error (df 266)
Plant height (cm)	140.49**	145.32**	29.52	45.36*	412.66**	23.86	147.84**	2463.68**	28.07	1.27	357.35**	2.9
No. of branches/plant	1.05	0.88	0.59	0.15	0.62	0.5	0.24	1.21*	0.35	0.69	1.64	0.54
No. of capsules/plant	1.04	0.88	0.58	0.15	0.62	0.5	0.24	1.21*	0.34	0.68	1.64**	0.54
No. of leaves/plant	7.51	39.08**	7.3	25.91**	10.36*	3.8	60.57**	16.63*	5.09	1.41	53.68**	1.09
Capsule size (cm^2)	0.64	1.31	1.52	2.92**	5.86**	1.09	9.08**	21.11**	2.66	0.85	14.55**	0.89
Leaf size (cm^2)	68.64	3312.56**	96.78	97.21	3914.21**	142.18	259.67**	2647.46**	92.04	197.52**	3969.35**	79.99
Peduncle length (cm)	11.81*	52.66**	6.16	4.39	23.51**	4.86	3.93	45.88**	2.38	1.35	52.83**	0.99
Stem diameter (cm)	0	0.20**	0	0.005	0.09**	0.003	0.001	0.13**	0	0.005*	0.34**	0
Capsule weight (g)	9.55**	45.41**	3.81	5.64	13.89**	3.41	0.96	53.31**	0.69	11.37**	51.16**	4.71
Seed weight (g)	1.82	11.22**	1.12	1.54	7.11**	1.2	0.26	15.35**	0.28	3.04*	13.94**	1.4
Husk weight (g)	3.27**	11.57**	1.05	1.62*	3.65**	0.85	0.32	11.55**	0.23	2.92**	11.95**	1.27
Opium yield (mg)	316.39	486.96	277.57	58.36	27,555.93**	117.16	166.04	44,877.48**	87.67	65.82	23,091.96**	40.89
Morphine %	0	9.13**	0	0	8.63**	0.001	0	29.36**	0	0	14.34**	0
Codeine %	0	2.19**	0	0	1.25**	0	0.17	6.14**	0.18	0.06	1.70**	0.04
Thebaine %	0.01	7.31**	0.01	0.01	5.38**	0.01	0.01	4.61**	0.01	0	10.55**	0.04
Narcotine %	0	8.66**	0	0	38.79**	0	0	55.79**	0	0	28.33**	0
Papaverine %	0.00**	0.00**	0	0	0.992**	0	0.00**	0.000**	0	0	2.12**	0

* and ** show significance at 5% and 1%, respectively.

Table 3. Genotypic coefficient of variability (GCV) for different characters in M_2 families in varieties NBRI-1 and NBRI-5 of *P. somniferum* L.

Treatments	PH	LVS	LS	PDL	CWP	SDW	HY	OY	M	C	T	N	P
NBRI-1 (physical)													
Control	1.04	5.01	11.79	0.83	9.09	9.19	10.32	6.79	4.01	0.23	3.52	1.15	0
kR10	13.52	11.29	6.02	5.17	27.98	30.86	24.81	24.26	14.81	18.6	17.96	2.4	0
kR20	9.79	9.28	12.08	8.29	30.29	31.99	28.28	16.39	6.27	12.72	15.56	10.82	0
kR30	11.59	13.58	11.69	2.86	32.59	35.43	30.54	24.03	7.62	6.68	20.79	7.36	0
kR40	13.33	11.36	17.22	8.14	26.11	23.04	28.32	15.73	5.02	8.01	27.14	8.82	302.43
kR50	10.32	7.92	12.52	8.29	28.42	28.16	28.86	36.4	5.45	10.91	30.05	3.17	0
NBRI-5 (physical)													
Control	0.92	4.57	7	2.04	8.56	10.02	9.51	14.94	6.33	1.22	16.82	7.93	0
kR10	7.96	7.84	9.96	6.24	16.76	27.87	15.63	17.44	5.72	11.19	20.91	9.85	191.21
kR20	7.76	7.25	7.17	4.47	20.38	17.39	21.76	21.97	7.23	12.73	24.18	12.81	226.34
kR30	14.33	11.6	10.14	2.51	23.59	21.11	26.28	28.05	5.63	9.54	16.75	11.27	222.84
kR40	0.99	56.74	23.74	7.65	28.21	26.44	28.74	32.79	3.47	8.54	19.26	24.59	0
kR50	4.78	6.28	1.68	7.16	28.26	22.59	31.02	32.28	8.58	11.88	30.32	26.05	80.17
NBRI-5 (chemical, EMS)													
Control	0.92	4.57	7	2.04	8.56	10.02	9.51	14.94	6.33	1.22	16.82	7.93	0
0.2% EMS	8.86	8.41	6.46	4.68	22.16	22.09	23.71	18.75	10.62	18.95	10.39	4.89	298.59
0.4% EMS	4.32	8.59	6.72	4.76	32.19	30.3	34.52	27.33	8.28	14.19	23.87	14.88	294.78
0.6% EMS	10.39	8.22	7.09	3.41	25.27	30.17	19.13	26.25	11.95	13.27	4.36	13.61	0
0.8% EMS	11.41	7.08	19.82	5.05	20.62	27.18	13.95	25.12	7.35	18.92	38.48	15.92	0
NBRI-5 (combined)													
Control	0.92	4.57	7	2.04	8.56	10.02	9.51	14.94	6.33	1.22	16.82	7.93	0
kR10 + 0.2% EMS	5.81	6.43	7.29	1.97	24.41	25.49	22.56	23.55	5.08	9.44	8.78	12.22	120.56
kR10 + 0.4% EMS	4.91	9.29	9.07	1.64	2.94	3.65	6.67	19.29	5.72	12.64	21.44	3.24	0
kR10 + 0.6% EMS	9.76	10.71	11.15	3.21	27.44	36.52	16.64	28.03	6.93	12.85	10.03	4.2	232.59
kR10 + 0.8% EMS	10.69	9.88	14.09	6.56	34.06	32.79	38.45	20.38	7.5	9.57	19.21	10.57	0
kR20 + 0.2% EMS	10.21	7.65	13.74	10.51	23.33	15.59	29.87	36.28	3.77	17.42	26.46	20.24	80.18
kR20 + 0.4% EMS	7.33	5.66	14.41	5.87	13.42	17.45	14.31	32.64	8.51	25.74	23.01	17.49	146.89
kR20 + 0.6% EMS	6.67	4.33	18.08	5.07	36.33	21.35	50.68	35.28	11.16	15.14	21.43	26.64	0
kR20 + 0.8% EMS	6.35	6.92	20.63	5.25	18.98	19.92	19.82	14.39	14.96	19.55	25.18	10.16	299.72
kR30 + 0.2% EMS	8.94	6.89	11.44	5.97	29.61	30.97	28.98	19.58	10.45	21.92	32.39	23.28	305.18
kR30 + 0.4% EMS	9.26	13.15	19.42	3.05	12.38	20.07	5.47	12.47	12.06	23.02	29.74	18.49	325.55
kR30 + 0.6% EMS	13.12	15.42	25.44	1.61	10.63	11.41	7.11	21.67	9.47	16.25	12.13	11.83	46.71
kR30 + 0.8% EMS	7.53	13.95	11.79	3.04	28.14	28.93	25.87	25.27	5.38	10.08	7.15	10.39	163.41
kR40 + 0.2% EMS	11.26	10.75	16.84	4.95	26.97	25.59	28.35	13.87	7.62	32.36	47.76	10.73	269.79
kR40 + 0.4% EMS	10.97	18.01	17.75	9.53	23.74	23.23	24.39	35.79	7.62	9.59	26.53	9.44	263.61
kR40 + 0.6% EMS	9.59	17.1	14.69	8.61	26.36	29.16	24.24	39.38	3.41	25.99	16.59	9.22	133.59
kR40 + 0.8% EMS	9.89	12.99	21.81	9.19	35.33	35.01	34.84	18.26	8.73	20.79	24.89	13.51	0
kR50 + 0.2% EMS	11.21	13.91	14.03	7.33	34.59	37.77	31.66	30.63	2.45	11.76	36.28	19.79	0
kR50 + 0.4% EMS	12.7	13.82	26.49	5.85	45.79	47.78	43.95	42.52	8.81	15.98	18.23	5.51	75.93
kR50 + 0.6% EMS	6.67	4.33	18.08	5.07	36.33	21.35	50.68	35.28	6.94	21.93	30.48	9.39	55.95
kR50 + 0.8% EMS	6.35	6.92	20.63	5.25	18.98	19.92	19.82	14.39	10.87	26.83	42.43	8.54	241.95

PH = plant height (cm), LVS = number of leaves per plant, LS = leaf Size (cm^2), PDL = peduncle length per plant (cm), CWP = capsule weight per plant (g), SDW = seed weight per plant (g), HY = husk yield per plant (g), OY = opium yield per plant (mg), M = morphine %, C = codeine %, T = thebaine %, N = narcotine %, P = papaverine %.

Table 4. Heritability (broad sense) for different characters of the M_2 families in varieties NBRI-1 and NBRI-5 of *P. somniferum* L.

Treatments	PH	LVS	LS	PDL	CWP	SDW	HY	OY	M	C	T	N	P
NBRI-1 (physical)													
Control	77.23	64.78	95.19	16.76	80.37	67.73	68.9	81.42	81.95	0.25	76.96	34.85	0
kR10	90.03	85.37	87.05	56.24	96.87	63.66	67.52	110.45	93.6	80.27	52.68	52.68	0
kR20	87.53	75.16	92.71	80.02	78.67	81.26	70.61	55.35	55.55	66.25	41.8	41.8	0
kR30	89.49	85.88	90.06	23.95	81.26	84.04	71.91	79.29	72.8	18.41	55.94	55.94	0
kR40	92.12	81.78	95.74	68.91	73.71	61.74	75.95	53.55	42.64	65.08	67.93	67.93	0.69
kR50	85.87	71.86	86.48	85.2	75.81	72.75	76.46	82.74	66.25	67.35	84.24	84.24	0
NBRI-5 (physical)													
Control	61.05	74.45	83.27	52.65	86.13	83.56	70.26	99.88	92.79	51.08	82.13	80.88	0
kR10	94.84	85.91	95.43	69.75	42.37	57.82	39.92	67.21	55.33	60.16	74.87	52.95	32.81
kR20	93.66	85.49	91.11	62.39	60.26	41.4	67.01	81.69	72.24	70.32	86.18	71.13	90.35
kR30	97.63	93.08	92.87	27.56	58.65	48.84	62.65	82.29	49.43	58.83	68.29	82.43	70.62
kR40	31.84	99.4	55.08	68.32	71.38	62.81	64.71	79.62	18.53	53.58	69.78	27.83	0
kR50	79.61	72.39	20.67	79.56	62.18	48.86	60.67	80.27	69.47	65.62	90.87	90.54	12.52
NBRI-5 (chemical, EMS)													
Control	61.05	74.45	83.27	52.65	86.13	83.56	70.26	99.88	92.79	51.08	82.13	80.88	0
0.2% EMS	95.37	88.82	78.34	66.39	67.94	66.83	67.26	69.45	75.67	84.23	70.34	35.19	59.44
0.4% EMS	86.85	85.76	75.78	68.55	79.71	70.93	83.19	80.51	81.58	80.77	85.16	85.49	57.93
0.6% EMS	94.96	80.29	82.26	50.49	59.5	61.97	47.16	79.65	80.68	76.46	14.15	79.62	0
0.8% EMS	95.8	79.36	90.27	65.59	55.26	68.96	33.13	72.98	68.47	86.28	82.68	89.51	0
NBRI - 5 (combined)													
Control	61.05	74.45	83.27	52.65	86.13	83.56	70.26	99.88	92.79	51.08	82.13	80.88	0
kR10 + 0.2% EMS	85.72	68.37	87.43	18.82	54.99	46.02	59.39	68.13	53.71	59.2	51.01	84.63	20.3
kR10 + 0.4% EMS	78.98	78.89	81.62	8.79	2.82	3.5	12.14	66.98	70.09	81	81.96	41.18	0
kR10 + 0.6% EMS	96.38	92.03	93.2	38.42	67.73	78.06	36.74	79.4	68.81	78.7	38.16	14.85	68.85
kR10 + 0.8% EMS	95.46	89.72	85.84	74.93	76.35	70.47	78.5	59.12	72.7	63.58	75.92	76.4	0
kR20 + 0.2% EMS	90.4	71.92	91.26	85.36	59.48	31.1	73.02	80.71	28.07	81.18	82.62	83.27	9.23
kR20 + 0.4% EMS	91.23	57.32	91.26	78.27	41.88	54.18	39.5	85.4	72.22	92.58	83.49	83.57	48.69
kR20 + 0.6% EMS	83.36	43.1	97.17	60.13	71.43	40.49	80.99	73.31	82.68	86.62	67.53	85.29	0
kR20 + 0.8% EMS	79.88	67.38	96.44	54.15	52.09	47.43	58.18	61.75	85.14	84.94	89.27	63.29	59.89
kR30 + 0.2% EMS	92.49	81.56	91.79	70.47	83.88	82.46	80.74	55.61	72.71	85.14	90.63	74.54	68.59
kR30 + 0.4% EMS	91.37	86.52	97.91	36.45	29.77	47.37	8.04	42.83	86.52	76.26	78.76	76.59	70.66
kR30 + 0.6% EMS	97.73	92.71	99.15	17.28	29.21	25.35	15.39	70.96	77.58	71.17	50.85	45.48	11.18
kR30 + 0.8% EMS	85.23	83.83	30.7	27.64	63.01	53.54	53.29	87.61	46.28	63.2	24.01	63.73	93.78
kR40 + 0.2% EMS	95.56	92.49	98.31	58.63	73.94	63.28	76.78	61.33	61.58	74.7	88.22	67.46	79.02
kR40 + 0.4% EMS	94.28	93.35	97.57	81.66	52.29	45.67	56.26	86.96	75.32	60.11	69.14	67.97	73.65
kR40 + 0.6% EMS	91.9	93.1	96.86	75.85	66.35	67.81	63.91	91.5	42.25	93.27	48.66	68.93	56.9
kR40 + 0.8% EMS	90.79	84.85	98.58	70.58	82.12	77.97	80.43	60.98	80.54	81.79	71.73	65.95	0
kR50 + 0.2% EMS	93.19	88.49	96.64	50.33	74.53	75.07	70.89	83.51	26.61	70.9	84.11	85.76	0
kR50 + 0.4% EMS	95.75	89.9	99.36	57.55	81.21	77.38	82.35	92.17	79.42	77.89	58.24	35.76	34.47
kR50 + 0.6% EMS	83.36	43.1	97.17	60.13	71.43	40.49	80.99	73.31	66.28	73.18	81.35	54.4	13.13
kR50 + 0.8% EMS	79.88	67.38	96.44	54.15	52.09	47.43	58.18	61.75	86.72	86.48	74.48	63.58	54.98

PH = plant height (cm), LVS = number of leaves per plant, LS = leaf Size (cm^2), PDL = peduncle length per plant (cm), CWP = capsule weight per plant (g), SDW = seed weight per plant (g), HY = husk yield per plant (g), OY = opium yield per plant (mg), M = morphine %, C = codeine %, T = thebaine %, N = narcotine %, P = papaverine %.

Table 5. Genetic advance (%) for different characters of the M_2 families in varieties NBRI-1 and NBRI-5 of *P. somniferum* L.

Treatments	PH	LVS	LS	PDL	CWP	SDW	HY	OY	M	C	T	N	P
NBRI 1 (physical)													
Control	1.88	8.29	23.71	69.53	16.79	15.59	17.64	12.64	7.49	0.02	6.37	1.4	0
kR10	26.42	21.5	11.57	7.99	36.66	50.73	41.99	46.68	29.51	34.33	26.85	1.56	0
kR20	18.87	16.56	23.97	15.28	55.36	59.39	48.95	25.13	9.62	21.33	20.73	19.06	0
kR30	22.59	25.93	22.86	2.88	60.51	66.91	53.35	44.07	13.39	5.9	32.05	12.71	0
kR40	26.36	21.17	34.72	13.93	46.18	37.29	50.85	23.72	6.75	13.31	46.07	11.78	518.89
kR50	19.69	13.83	23.98	15.77	50.97	49.48	51.98	68.21	9.14	18.44	56.82	2.51	0
NBRI-5 (physical)													
Control	1.48	8.12	13.17	3.05	16.37	18.87	16.41	30.76	12.55	1.79	31.41	14.68	0
kR10	15.97	14.97	20.05	10.74	22.47	43.65	20.34	29.46	8.76	17.88	37.27	14.77	225.6
kR20	15.46	13.81	14.09	7.28	32.59	23.05	36.69	40.9	12.66	21.98	46.23	22.26	443.19
kR30	29.17	23.06	20.13	2.71	37.21	30.39	42.85	52.42	8.15	15.07	28.52	21.08	385.76
kR40	1.16	116.53	36.29	13.02	49.09	43.16	47.63	60.28	3.08	12.87	33.15	26.73	0
kR50	8.79	11	1.57	13.16	45.91	32.53	49.77	59.58	14.73	19.83	59.53	51.07	58.44
NBRI-5 (chemical, EMS)													
Control	1.48	8.12	13.17	3.05	16.37	18.87	16.41	30.76	12.55	1.79	31.41	14.68	0
0.2% EMS	17.84	16.32	11.79	7.85	37.62	37.2	40.06	32.18	19.04	35.82	17.97	5.98	474.19
0.4% EMS	8.29	16.39	12.06	8.12	59.19	52.57	64.86	50.52	15.42	26.28	45.37	28.35	462.19
0.6% EMS	20.87	15.16	13.25	4.99	40.16	48.93	27.06	48.25	22.13	23.91	3.38	25.02	0
0.8% EMS	23.01	12.99	38.79	8.43	31.57	46.49	16.54	44.21	12.53	36.21	72.08	31.02	0
NBRI-5 (combined)													
Control	1.48	8.12	13.17	3.05	16.37	18.87	16.41	30.76	12.55	1.79	31.41	14.68	0
kR10 + 0.2% EMS	11.09	10.96	14.06	1.76	37.29	35.63	35.81	40.04	7.67	14.96	2.91	23.16	111.89
kR10 + 0.4% EMS	8.99	17.01	16.88	99.93	1.02	3.65	4.79	32.53	9.86	23.44	39.98	4.29	0
kR10 + 0.6% EMS	19.73	21.16	22.17	4.09	46.52	66.46	20.78	80.78	11.85	23.49	12.77	3.34	397.56
kR10 + 0.8% EMS	21.53	19.29	26.89	11.7	61.31	56.72	70.17	32.28	13.18	15.72	34.49	19.03	0
kR20 + 0.2% EMS	20.01	13.37	27.03	19.99	37.07	17.92	52.58	67.14	4.11	32.33	49.55	38.04	50.18
kR20 + 0.4% EMS	14.42	8.82	28.35	10.69	17.89	26.46	18.52	62.13	14.91	51.03	43.31	32.94	211.14
kR20 + 0.6% EMS	12.55	5.86	36.72	8.11	63.25	27.99	93.95	62.23	20.89	29.02	36.29	50.68	0
kR20 + 0.8% EMS	11.69	11.69	41.73	7.95	28.22	28.26	31.15	23.29	28.43	37.11	48.99	16.66	477.81
kR30 + 0.2% EMS	17.7	12.83	22.57	10.32	55.87	57.94	53.64	30.08	18.35	41.66	63.53	41.4	520.67
kR30 + 0.4% EMS	18.24	25.19	39.59	3.79	13.92	28.46	3.19	16.81	23.1	41.41	54.38	33.35	563.72
kR30 + 0.6% EMS	26.72	30.58	52.19	1.38	11.84	11.84	5.74	37.61	17.18	28.24	17.83	16.43	32.18
kR30 + 0.8% EMS	14.32	26.32	13.46	3.29	46.02	43.61	38.91	48.73	7.54	16.5	7.22	16.51	326
kR40 + 0.2% EMS	22.68	21.29	34.39	7.81	47.77	41.94	51.18	22.38	12.32	57.62	92.41	18.15	494.04
kR40 + 0.4% EMS	21.94	35.85	36.11	17.73	35.36	32.35	37.61	68.75	13.63	15.33	45.44	16.03	466.01
kR40 + 0.6% EMS	18.94	33.99	29.79	15.44	44.23	49.46	39.92	77.6	4.57	51.69	23.85	15.76	207.6
kR40 + 0.8% EMS	19.42	24.67	44.61	15.91	65.95	63.69	64.37	29.37	16.15	38.74	43.42	22.6	0
kR50 + 0.2% EMS	22.29	26.96	28.41	10.71	61.53	67.42	54.92	57.68	2.6	20.39	68.54	37.76	0
kR50 + 0.4% EMS	25.6	26.98	54.39	9.14	85.01	86.59	82.15	84.09	16.17	29.05	28.65	6.79	91.84
kR50 + 0.6% EMS	12.55	5.86	36.72	8.15	63.25	27.99	93.95	62.23	11.63	26.06	56.62	14.28	41.76
kR50 + 0.8% EMS	11.69	11.69	41.73	7.95	28.22	28.26	31.15	23.29	20.86	51.39	75.43	14.03	369.58

PH = plant height (cm), LVS = number of leaves per plant, LS = leaf Size (cm^2), PDL = peduncle length per plant (cm), CWP = capsule weight per plant (g), SDW = seed weight per plant (g), HY = husk yield per plant (g), OY = opium yield per plant (mg), M = morphine %, C = codeine %, T = thebaine %, N = narcotine %, P = papaverine %.

Discussion

Induced mutation is an important complementary method for the creation of genetic variability for specific characters in a crop when the variability of the crop is completely exhausted and can lead to no further possibilities for genetic improvement of the crop through conventional breeding techniques. In mutation breeding, the possibility exists to change a single gene or only a few genes without altering the total genetic makeup of a specific (outstanding) genotype (Broertjes and Van Harten 1978). This approach can be useful in the development of outstanding varieties of opium poppy rich in specific alkaloids.

The development of early maturing genotypes in any crop primarily depends upon the reduction of days to 50% flowering (Ibrahim et al. 2001; Shadakshari et al. 2001; Jabeen and Mirza 2004; Solanki et al. 2004). In the present study, it is evident that the higher mutagen doses of kR50 and kR50 + 0.8 % EMS reduced the days to 50% flowering to a greater extent than the lower doses of mutagens for both varieties. The doses kR50 for NBRI-1 and kR50 + 0.8% EMS and kR30 + 0.4% EMS for NBRI-5 were effective in reducing days to 50% flowering by up to 5-7 days. Similar findings for various crops were also reported by Sharma (1990), Gupta et al. (1996), Shadakshari et al. (2001), Wang et al. (2003), Jabeen and Mirza (2004), Solanki et al. (2004), and Ramesh and Kumar (2005). Significant variations were noticed for all metric traits except for capsule size of NBRI-1 and papaverine content (%) in both varieties, for all 5 doses given to the mutagen-treated population of NBRI-1 and for all 29 treated populations of NBRI-5. The capsule size of NBRI-1 and the papaverine content (%) of both varieties showed nonsignificant values, so these traits were not considered for further discussion.

The high genetic variability observed for seed and opium yield and thebaine content in the M_2 generation suggests a scope for effective selection for these traits. GCV values were not consistently high or low in either of the varieties, but rather varied from trait to trait. However, GCV alone is not enough to determine the amount of heritable variability; heritability and GA with GCV are required to assess the heritable portion of the total variation. Heritability, along with high GA, has additive and epistatic gene effects, which are fixable and can provide the desirable gain (Sheeba et al. 2003; Chatterjee et al. 2004). Thus, it becomes necessary to consider all 3 estimates to ensure effective selection for a particular trait. The increased genetic variance in the treated material is a reliable indication of the effects of mutagens. It is evident from the present study that kR30 was the most effective dose for the NBRI-1 variety, which had the highest estimates of all 3 genetic parameters (GCV, h^2, and GA%) for the 7 traits of plant height, number of leaves, capsule weight, seed weight, husk yield, opium yield, and narcotine (%). Dose kR50 was the most effective for peduncle height while dose kR40 was the most effective for thebaine and leaf size, and dose kR10 was the most effective for morphine and codeine. Similarly, for NBRI-5, the treatments of combined mutagenesis showed higher values of GCV, h^2, and GA% for all traits than the other physical and chemical mutagenesis treatments. It was noticed that none of the treatments had high values for these genetic parameters for more than 4 or 5 traits. Dose kR30 + 0.6% EMS was the most effective dose for plant height and leaf size, while dose kR50 + 0.4% EMS was effective for capsule weight and seed weight. Similarly, the kR40 + 0.6% EMS was effective for opium yield and codeine (%). Dose kR20 + 0.6% EMS was effective for husk yield and narcotine content (%), while kR40 + 0.2% EMS was effective for thebaine and codeine (%) with moderately high values of genetic parameters. Based on these genetic parameters, the doses kR20 + 0.2% EMS for peduncle length, kR20 + 0.8% EMS for morphine (%), and kR40 for number of leaves were effective only for a single trait. GCV measures the amount of variation in a character, though it does not provide the amount of heritable variation based on this estimate. GCV, along with heritability estimates, can provide a better idea of the amount of genetic gain expected through phenotypic selection (Burton 1952; Sheeba et al. 2003; Chatterjee et al. 2004). In the present study, heritability estimates of the 17 metric traits for the 2 varieties ranged from 0.303 to 0.836. High estimates of heritability are a reliable guide for any selection program based on the phenotypic performance of induced mutants. Sheeba et al. (2003) reported that heritability estimates in conjugation with expected

advance were more reliable in predicting the resultant effects for selecting the best individuals. It was also reported that broad-sense heritability estimates would be reliable only when accompanied with high GA. In most of the treatments, the magnitude of heritability was high, which suggested that the major contribution of heritable variance for traits such as codeine and thebaine content was additive gene action. Therefore, selection of plant types rich in thebaine and codeine can be easily achieved by simple selection on the basis of a per se performance of the M_2 generation in both varieties. Chatterjee et al. (2004) used these genetic parameters to obtain better selections and to determine the effective doses of mutagens to be used, particularly for selection of economically important traits in opium poppy.

The present study was aimed at enhancing alkaloid content, especially thebaine and codeine, in opium poppy. The selection of plant types rich in thebaine and codeine can be easily achieved by simple selection on the basis of a per se performance of the M_2 generation in both varieties due to a lack of further recombination or segregation in the M_2 and M_3 generations. Selection in the early generation (M_2) followed by confirmation of the enhanced trait in M_3 has been reported by many researchers (Sharma 1977; Tickoo and Jain 1979; Bhadra 1982; Kharkwal 1983; Karavaev et al. 1990; Nicolae and Ilie 1990; Lundqvist 1992; Misra and Momin 2004). The progenies, which confirmed enhancement of the economically important characters of the M_3 subfamilies in comparison with the M_2 families, were rated as elite families or high-ranking families. With thebaine being a viable source for the production of nonaddictive analgesics, the development of high-thebaine and low-morphine elite mutant lines could be advantageous to support the rising world demand for these products (Hevel et al. 2001). These developed elite mutant lines can be utilized in various hybridization programs to further raise thebaine content. The present study concluded that, on the basis of GCV, h^2, and GA%, dose kR30 was the most effective dose for capsule weight, husk yield, and opium yield in NBRI-1 and for husk yield and plant height in NBRI-5, while doses kR10 in NBRI-1 and kR10 + 0.4% EMS in NBRI-5 were most effective for thebaine (%) and doses kR30 + 0.2% EMS and 0.8% EMS were most effective in NBRI-5 for morphine. Dose kR10 + 0.4% EMS was the most effective dose overall, affecting 10 different characters, namely number of capsules, number of leaves, capsule size, leaf size, capsule weight, seed weight, husk yield, opium yield, and thebaine content per plant. Dose kR10 + 0.4% EMS showed good mutability with enhanced effect for high thebaine or codeine content and low morphine content, while dose kR40 + 0.6% EMS showed good results for narcotine content. The study also confirmed that the pathway of morphinan alkaloids and narcotine formation was bifurcated at the lower combined dose (kR30 and kR10 + 0.4% EMS), which is effective for causing micromutation in the pathway of morphinan alkaloids, while the higher combined dose (kR40 + 0.4% EMS) affected narcotine production.

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