Indian J. Genet., **54** (4): 430–435 (1994)

FREQUENCY AND SPECTRUM OF INDUCED MUTATIONS FOLLOWING HYBRIDIZATION IN INDIAN MUSTARD

J. N. SAH, U. K. RAI AND S. K. VARSHNEY

Department of Plant Breeding, Tirhut College of Agriculture, Dholi, Muzaffarpur 843121

(Received: February 4, 1993; accepted: May 22, 1995)

ABSTRACT

Frequency and spectrum of γ-ray induced chlorophyll and morphological mutations were studied in F_2M_2 generation of six Indian mustard hybrids. Chlorophyll mutation viridis was most frequent whereas albina was rare. The morphological mutation with fasciated leaves occurred frequently. Maximum mutational events were induced with 100 kR γ-ray treatment in various hybrids. Differences were recorded in the frequency of certain chlorophyll and morphological mutations in the hybrids when compared within their reciprocal cross combinations.

Key words: Indian mustard, *B. juncea*, mutations, hybridization.

The primary objectives of mutation breeding are to enlarge the frequency and spectrum of mutations and increase the incidence of viable mutations as an approach towards directed mutagenesis. Chlorophyll mutations in M_2 generation is a convenient and dependable index for evaluating the genetic effects of mutagenic treatments [1]. Chlorophyll mutations were reported in brown sarson [2] and yellow sarson. Hybridization is also an effective tool for broadening the genetic variability and selection for desirable segregates. The combined effect of induced mutations and hybridization need to be studied in this crop. The present study aims to assess the frequency and spectrum of mutations following hybridization in Indian mustard (*Brassica juncea*).

MATERIALS AND METHODS

The experimental materials comprised three hybrids and their respective reciprocals, viz. DIR 45-8 x B-85, B-85 x DIR 45-8, B-85 x RWC-6 (1/11), RWC-6 (1/11) x B-85, DIR 45-8 x RWC-6 (1/11), and RWC-6 (1/11) x DIR 45-8, involving three genetically pure and diverse parents of Indian mustard. One hundred dry seeds of the 6 hybrids were treated with 25, 50, 75 and 100 kR γ-rays at the Research and Development Isotope Laboratory, FCI, Sindri, Dhanbad, Bihar. The F_1M_1 plants were harvested individually. Plant-to-progeny rows of 30 treatments were grown in F_2M_2 generation at the Research Farm, Tirhut College of Agriculture, Dholi, Muzaffarpur.

RESULTS AND DISCUSSION

Observations were recorded in F_2M_2 generation through the entire growth period of the crop under field conditions for chlorophyll and morphological mutations.

Chlorophyll mutations. The chlorophyll mutants were scored at 4-leaf stage in 15-day-old seedlings. The mutations were classified into the following categories:

Albina: White leaves without chlorophyll (lethal)
Xantha: Yellow to greenish white leaves (lethal)
Viridis: Light green leaves (viable)
Albovirids: Green leaves with white apex (viable)
Virescent: Very light green leaves (viable)

The albina and Xantha mutants did not survive. The mutation frequency and spectrum are presented in Table 1. The mutation rate constantly increased with increase in γ-ray dose in all hybrids. Chlorophyll mutants were most frequent in the 100 kR γ-ray treatment. Over all the doses, the F_2M_2 of the cross DIR 45-8 x B-85 yielded lowest frequency and RWC-6 (1/11) x B-85 exhibited maximum percentage of chlorophyll mutants (Table 2). The mutations viridis, xantha and alboviridis were most frequent, while albina and virescent mutations appeared with the lowest frequency.

In all the hybrids taken together, the different chlorophyll mutants were observed in the following order: viridis > xantha > alboviridis > virescent > albina (Table 2). However, minor deviations from this sequence were observed among the hybrids. Viridis mutants have been reported to be most frequent after γ-ray treatment [3]. Over all the doses, there was some deviation in the frequency of different chlorophyll mutations in the hybrids depending on the direction of cross. This was particularly true for albina, alboviridis and virescent in the crosses DIR 45-8 x B-85 and B-85 x DIR 45-8, for xantha in B-85 x RWC-6 (1/11) and RWC-6 (1/11) x B-85, and for albina in DIR 45-8 x RWC-6 (1/11) and RWC-6 (1/11) x DIR 45-8. No virescent mutation was induced in the cross DIR 45-8 x B-85, while 14 virescent mutants were observed out of total 85 chlorophyll mutations in its reciprocal cross B-85 x DIR 45-8. This indicates that some interaction between nuclear genes and cytoplasmic background influences the frequency of different chlorophyll mutations. Swaminathan et al. [4] and Sree Ramulu [5] suggested that differences in the mutation spectrum and rate in different genotypes may be due to difference in the location of genes in relation to the centromere.

Table 1. Spectrum and frequency of chlorophyll mutations in Indian mustard hybrids and their reciprocals

Treatment	No. of F_2M_2 plants	Albina	Xantha	Viridis	Albo-viridis	Viros-cent	Xantho-vidis	Total	Mutant %
DIR 45-8 x B-85	950	—	—	—	—	—	—	—	—
25 kR	700	1	1	1	—	—	—	3	0.41
50 kR	700	5	5	3	—	—	—	13	1.85
75 kR	500	2	5	3	—	—	—	19	3.95
100 kR	500	—	12	12	6	—	—	30	6.00
B 85 x DIR 45-8	850	—	—	—	—	—	—	—	—
25 kR	700	1	1	—	2	—	—	4	0.47
50 kR	800	1	6	5	3	—	—	15	2.18
75 kR	600	—	2	8	8	8	—	26	4.30
100 kR	600	—	12	12	10	6	—	40	6.66
B-85 x RWC-6 (1/11)	1000	—	—	—	—	—	—	—	
25 kR	800	1	1	1	1	—	—	4.0	8.05
50 kR	750	2	—	5	6	2	—	15.0	2.14
75 kR	500	—	2	8	8	8	—	26.0	5.20
100 kR	500	—	10	12	10	8	—	40.0	8.00
RWC-6 (1/11) x B-85	850	—	—	—	—	—	—	—	—
25 kR	850	1	1	—	1	1	—	4	0.47
50 kR	600	2	3	5	3	3	—	16	2.67
75 kR	500	2	7	8	8	2	—	27	5.40
100 kR	500	—	14	10	15	5	—	44	8.80
DIR 45-8 x RWC-6 (1/11)	950	—	—	—	—	—	—	—	—
25 kR	850	1	1	1	1	—	—	4	6.47
50 kR	850	1	5	5	3	2	—	16	1.88
75 kR	500	2	5	5	5	9	1	27	5.40
100 kR	500	—	14	10	15	5	—	44	8.80
RWC-6 (1/11) x DIR 45-8	850	—	—	—	—	—	—	—	—
25 kR	700	1	1	1	—	1	—	4	0.57
50 kR	650	5	5	2	1	3	—	16	2.46
75 kR	600	2	4	6	7	6	—	25	4.17
100 kR	600	2	12	9	9	8	—	40	6.67

Table 2. Frequency of chlorophyll mutations in different mustard hybrids (pooled over all treatments)

Hybrid	No. of F_2M_2 plants	Chloro-phyll mutants	Mutant frequ-ency (%)	Mutation spectrum (No. of events)				
				albina	xantha	viridis	albovi-ridis	vires-cent
DIR 45-8 x B 85	2400	65	2.71	8	28	23	6	—
B 85 x DIR 45-8	2700	85	3.15	2	21	25	23	14
B 85 x RWC-6 (1/11)	2550	85	3.33	3	13	26	25	18
RWC-6 (1/11) x B 85	2450	91	3.71	5	25	23	27	11
DIR 45-8 x RWC-6 (1/11)	2700	90	3.33	4	25	21	24	16
RWC-6 (1/11) x DIR 45-8	2550	85	3.33	10	22	18	17	18
Total	15350	501	3.26	32	134	136	122	77

Morphological mutants. The following morphological mutants were recorded in the F_2M_2 generation:

Dwarf: Short statured plants
Lax: Loose branching pattern
Compactoid: Compact branching pattern
Fasciated leaves: Compressed into a bundle
Bifurcated lamina: Lamina of leaves divided into two parts
Fasciated petiole: Bound or compressed into a bundle

The frequency of morphological mutations also increased with increasing dose of γ rays in all hybrids (Table 3). The frequency of dwarf, lax and compactoid mutation was similar in the three hybrids and their respective reciprocals over all the doses taken together (Table 4). However, for the other three morphological mutants, there was a difference in frequency in hybrids and their reciprocals. This confirms that different cytoplasmic background of the same genotype may influence the frequency of mutations in different genes. The mutation with fasciated leaves was induced most frequently, followed by bifurcated lamina and fasciated petiole. The other three mutants, i.e. dwarf, lax and compactoid, were less frequent and appeared with almost similar frequency. Such morphological abnormalities have been reported earlier in rapeseed [2]. Radiation-induced leaf abnormalities have also been reported in wheat and rice [6–8]. In general, more morphological mutants where recorded in the reciprocals than in the corresponding direct crosses. Among the different morphological mutants, compactoid and dwarf mutations could have economic value and should be used in breeding programmes.

Table 3. Spectrum and frequency of morphological mutations in Indian mustard hybrids and their reciprocals

Treatment	No. of F_2M_2 plants	Compactoid	Lax	Dwarf	Fasciated leaf	Bifurcated lamina	Fasciated petiole	Total	Mutant %
DIR 45-8 x B-85	950								
25 kR	700	1	1	1	1	1	1	6	0.86
50 kR	700	3	3	2	2	2	2	14	2.20
75 kR	500	3	4	3	4	3	4	21	4.20
100 kR	500	4	4	5	7	5	5	30	6.00
B-85 x DIR 45-8	850	—	—	—	—	—	—	—	—
25 kR	700	1	2	1	1	1	1	7	1.00
50 kR	800	3	3	4	3	3	3	19	2.79
75 kR	600	3	2	3	4	6	5	23	3.83
100 kR	600	3	4	4	10	8	8	37	6.16
B-85 x RWC-6 (1/11)	1000	—	—	—	—	—	—	—	—
25 kR	800	1	1	1	1	1	1	6	0.75
50 kR	750	2	1	1	4	3	3	14	2.00
75 kR	500	3	2	1	5	6	4	21	4.20
100 kR	500	5	6	5	8	9	7	40	8.00
RWC-6 (1/11) x B-85	850	—	—	—	—	—	—	—	—
25 kR	850	1	1	1	2	1	1	7	0.82
50 kR	600	3	2	2	6	5	6	24	4.10
75 kR	500	3	3	3	8	6	7	30	6.00
100 kR	500	3	4	3	11	10	11	42	8.40
DIR 45-8 x RWC-6 (1/11)	950	—	—	—	—	—	—	—	—
25 kR	850	1	—	1	1	1	1	5	0.58
50 kR	850	2	3	2	4	3	4	18	2.12
75 kR	500	3	3	4	6	5	5	26	5.20
100 kR	500	3	3	5	8	6	6	31	6.20
RWC-6 (1/11) x DIR 45-8	850	—	—	—	—	—	—	—	—
25 kR	700	1	—	1	2	1	1	6	0.85
50 kR	650	2	1	3	7	8	3	18	2.70
75 kR	600	3	3	2	8	6	6	28	4.67
100 kR	600	4	4	3	12	10	10	43	7.16

ACKNOWLEDGEMENTS

The first author (JNS) is grateful to the Rajendra Agricultural University, Pusa, and the Principal, TCA, Dholi for granting study leave and providing facilities, respectively, for the present investigation.

Table 4. Frequency of morphological mutations in different mustard hybrids (pooled over treatments)

Hybrid	No. of plants	Morphological mutants	Mutant frequency (%)	Mutation spectrum (No. of events)					
				dwarf	lax	compactoid	fasciated leaves	bifurcated lamina	fasciated petiole
H_1	2400	71	2.96	11	12	11	14	11	12
H_2	2700	86	3.19	10	11	12	18	18	17
H_3	2550	81	3.18	11	10	8	18	19	25
H_4	2450	103	4.20	10	10	9	27	22	15
H_5	2700	80	2.96	9	9	12	19	15	16
H_6	2550	101	3.96	10	8	9	29	25	20
Total	15350	522	3.40	61	60	61	125	110	105

REFERENCES

1. A. Gustafsson. 1940. The mutation system of the chlorophyll apparatus. Lunds Univ. Arrskr. N. F. Avd., **30**: 1–40.

2. K. Das and P. R. Kumar. 1969–70. Morphological abnormalities in γ irradiated brown sarson. J. Sci. Res. BHU, **7**(1): 118–121.

3. A. Micke. 1970. Type of mutagen and dose. *In*: Mannual of Mutation Breeding. Techn. Report Series No. 119. IAEA, Vienna: 127.

4. M. S. Swaminathan, V. L. Chopra and S. Bhaskaran. 1962. Chromosome aberrations and the frequency and spectrum of mutations induced by EMS in barley. Indian J. Genet., **22**: 192–207.

5. K. Sree Ramulu. 1970. Mutagenicity of radiations and chemical mutagens is sorghum. Theor. Appl. Genet., **40**: 257–260.

6. A. T. Natarajan, S. M. Sikka and M. S. Swaminathan. 1958. Polyploidy and radiosensitivity and mutation frequency in wheat. Proc. 2nd Intern. United Nation Conf. on the Uses of Atomic Energy, vol. 27: 321–331.

7. I. Mashima and T. Kawai. 1959. Mutations of rice induced by radioisotope P^{32}. Proc. Intern. Conf. Peaceful Uses of Atomic Energy, vol. 27: 293–298.

8. B. Majumdar. 1980. Induced leaf mutations of rice. Indian J. Genet., **40**(1): 52–56.