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Efficacy of physical and chemical mutagenic treatments for developing superior mutants in greengram (*Vigna radiata*)

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ABSTRACT

Greengram [*Vigna radiata* (L.) Wilczek] varieties Sujata and OBGG 52 were treated with gamma rays (20, 40 and 60 kR), EMS (0.2, 0.4 and 0.6%), NG (0.005, 0.010 and 0.015%) and MH (0.01, 0.02 and 0.03%) individually and combine mutagens of 40 kR gamma rays coupled with 0.4% EMS or 0.010% NG or 0.02% MH. Best three high yielding progenies of M_3 generation from each treatment were assessed for yield and eight yield attributing traits in M_4 and M_5 generation during 2009-10. Ten of Sujata and twelve of OBGG 52 mutant cultures exhibited significantly higher yield over their respective parent variety. High frequency of positive mutations was observed for pod length, pods/plant, seeds/pod and 100-seed weight in both the varieties. Single mutagenic treatments were more effective in both varieties than the combine treatments. Mutagenic treatments dose of 20 and 60 kR gamma rays; 0.2, 0.4, and 0.6% EMS; 0.005 and 0.015% NG, 0.01% MH and gamma rays (40 kR) followed by NG (0.01%) or MH (0.02%) were most effective in induction and isolation of more useful mutants.

Key words: Chemical mutagens, Gamma-rays, Greengram, Induced mutations, *Vigna radiata*

Induction of mutation by physical and chemical mutagens provides a powerful means of creating new and useful variability in crop plants (Das *et al*. 2006). Physical and chemical mutagenic agents cause genes to mutate at rates above the spontaneous base line, thus producing a range of novel traits and broadening of the genetic diversity of plants (Lagoda 2007). In most breeding programme, major emphasis is placed on the improvement of seed yield and its related component traits, which are mostly complex and controlled by many genes (Sharma *et al*. 2008, Baisakh *et al.* 2011). Gregory (1956) suggested that micro-mutations affecting polygenic characters and each having small effects on the parental genotype might be more useful than macro-mutants because of their high buffering ability to the growing conditions of the parent variety. Successful use of micro-mutation as a method of crop improvement requires information on the efficiency of mutagens and mutagenic treatments for inducing micromutations, direction and magnitude of induced variation. Further, effective and efficient methodology for identification and selection of desirable micromutants is also required. The 'efficacy' of mutagenic treatment can be assessed by the potential to produce more of useful mutations (Gregory 1961). Therefore, the present investigation was undertaken to assess the effects of physical and chemical mutagens and their synergistic effects on yield attributes of greeengram [*Vigna radiata* (L.) Wilczek].

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MATERIALS AND METHODS

The material for the present study comprised two morphologically distinct varieties of greengram, viz. Sujata—a pedigree selection from the cross (L 24-2 × Pusa Baisakhi) having characters like small pod with shining green colour small seeds and OBGG 52, a mutant of K 851 (EMS at 0.375% to K 851) having lodging resistance and non-shattering ability. Both the varieties have maturity period of 55-65 days and are suitable for cultivation in *kharif*, pre-*rabi, rabi* and summer seasons. Dry and well filled seeds of these two varieties were administered with single mutagenic treatments of gamma rays (20, 40 and 60 kR), ethyl methane sulphonate (EMS) (0.2, 0.4 and 0.6%), nitroso guanidine (NG) (0.005, 0.010 and 0.015%) and maleic hydrazide (MH) (0.01, 0.02 and 0.03%) and combined mutagenic treatments of 40 kR gamma rays coupled with 0.4% EMS or 0.010% NG or 0.02% MH. The single mutagenic treatments of gamma rays, EMS, NG and MH were coded as G1, G2, G3, E1, E2, E3, N1, N2, N3 and M1, M2 and M3 respectively. Combined

treatments of 40 kR gamma rays with 0.4% EMS, 0.01% NG and 0.02% MH were coded as GE2, GN2 and GM2, respectively. Dry seeds were administered with gamma rays at Bhabha Atomic Research Centre (BARC), Trombay. For other single mutagenic treatments, the seeds were presoaked in distilled water for 6 hours, blotted dry and then treated with freshly prepared aqueous solution of above chemical mutagens for 6 hours with intermittent shaking. For combined mutagenic treatments, seeds were first irradiated with 40 kR gamma rays and then treated with either 0.4% EMS or 0.01% NG or 0.02% MH solution in the same manner as described above. Treated seeds were thoroughly washed with running water to bleach out the residual chemicals and then dried on blotting paper after treatment. Superior plants were selected from each mutagenic treatment in M_2 and M_3 generation during 2008-09. Best three progenies of each treatment were selected on the basis of higher yield in M_3 generation and were carried forward to M_4 and M_5 generation during March 2010 and September 2010 in RBD with three replications along with their respective parent. The experimental plot size was of three rows of three meter length with a spacing 25 cm × 10 cm. Days to 50% flowering and days to maturity were recorded on plot basis and other seven biometric characters (plant height, cluster per plant, pods per plant, pod length, seeds per pod, 100-seed weight and yield per plant) were recorded on ten random plants per plot in each replication. The mean data were subjected to statistical analysis.

RESULTS AND DISCUSSION

The efficacy of mutagenic treatments in producing high yielding lines could be assessed on M_4 and M_5 generations. Ten cultures of Sujata and twelve cultures of OBGG 52 produced significantly higher yield than respective parents in M_4 and M_5 generation (Table 1). The productive mutants of Sujata included four from EMS, three were from gamma-rays, two were from NG and one was from MH; whereas, OBGG 52 productive mutants included three each from gamma-rays and NG, two each from EMS, MH and one each from combined treatments of gamma-rays with NG and MH. The mutants exhibited diverse changes in most of the characters including flowering, maturity, height and direct yield components like pods/plant, pod length, seeds/pod in both the varieties except for 100-seed weight which was additional to Sujata mutants (Table 2).

The usefulness of any mutagenic agent for crop improvement depends on its ability to induce high frequency of mutations in desirable direction. Evaluation of 45 mutant cultures in M_5 generation indicated that majority of cultures differed significantly from parents for one or more traits in positive or negative direction. In present investigation, the character changes envisaged for improvement of productivity of greengram were early flowering and maturity, short plant height and increase in number of clusters and pods, pod length, seeds/pod, 100-seed weight and yield/plant. The mutant cultures exhibiting significant changes in the desired direction from the parent variety for any character were classified superior or useful mutants for that character. Out of 45 mutant cultures evaluated in each set, 22 cultures in Sujata and 24 cultures in OBGG 52 exhibited superiority over the respective parent variety for one or more of the quantitative characters. Of these, 11 cultures each in Sujata and OBGG 52 (all were significantly higher yielding mutant than their respective parent) were superior for multiple traits, whereas remaining cultures showed superiority for single trait indicating cumulative effects of the micromutation in different quantitative characters causing significantly higher yield in greengram. In Sujata, the mutant culture SE3-2 exhibited superiority for 6 characters, SG1-1and SE1-2 for 5 characters and SG1-2 and SN3-3 for 4 characters and rest 17 mutant cultures recorded significant desirable changes in one to three characters, whereas in OBGG 52, the mutant cultures, OG1-1, ON3-2 and ON3-3 showed superiority in 4 characters and

Table 1 Yield performance of significantly high yielding mutants of Sujata and OBGG-52 in M_4 and M_5 generation

Cultures	Mutagenic treatments	M_4 generation % increased over parent	M_5 generation % increased over parent
	Sujata		
SG1-1	γ-rays 20kR	44.08	49.53
SG1-2	γ-rays 20kR	41.23	32.08
SG3-3	γ-rays 60Kr	41.71	42.45
SE1-2	EMS 0.2%	48.34	50.00
SE2-2	EMS 0.4%	34.60	33.49
SE2-3	EMS 0.4%	38.39	39.62
SE3-2	EMS 0.6%	48.82	54.25
SN1-2	NG 0.005%	57.35	58.96
SN3-3	NG 0.015%	53.55	43.87
SM1-3	MH 0.01%	37.91	37.74
	OBGG 52		
OG1-1	γ-rays 20kR	27.27	27.69
OG3-2	γ-rays 60Kr	26.82	23.97
OG3-3	γ-rays 60Kr	38.64	27.69
OE1-2	EMS 0.2%	36.36	31.82
OE2-3	EMS 0.4%	35.00	31.40
ON3-1	NG 0.015%	36.82	32.64
ON3-2	NG 0.015%	38.18	36.78
ON3-3	NG 0.015%	31.82	35.95
OM1-3	MH 0.01%	28.64	34.71
OM2-3	MH 0.02%	27.27	23.55
OGN2-3	γ-rays 40 kR + NG 0.01%	36.36	23.55
OGM2-3	γ-rays 40 kR+ MH 0.02%	24.09	23.55

Table 2 Character changes observed in significantly higher yielding mutant cultures of Sujata and OBGG 52 in M_5 generation

Cultures	Yield/plant (g)	Significant changes in characters from respective parent variety
1	2	3
		Sujata
SN1-2	3.37	Increase in pods/plant, and 100-seed weight
SE3-2	3.27	Early maturity, increase in plant height, pods/plant, pod length, seeds/pod and 100-seed weight
SE1-2	3.18	Increase in plant height, pods/plant, pod length, seeds/pod and 100-seed weight
SG1-1	3.17	Early maturity, increase in plant height, pods/plant, pod length, and 100-seed weight
SN3-3	3.05	Increase in pods/plant, pod length and 100-seed weight
SG3-3	3.02	Increase in plant height, pod length and seeds/pod
SE2-3	2.96	Increase in plant height, pod length and 100-seed weight
SM1-3	2.91	Increase in pods/plant and 100-seed weight
SE2-2	2.83	Increase in 100-seed weight
SG1-2	2.80	Early maturity, increase in plant height, pod length, and 100-seed weight
Parent	2.12	
		OBGG 52
ON3-2	3.31	Increase in pods/plant, pod length and seeds/pod
ON3-3	3.29	Increase in pods/plant, pod length and seeds/pod
OM1-3	3.26	Increase in seeds/pod
ON3-1	3.21	Increase in plant height, pods/plant and seeds/pod
OE1-2	3.19	Increase in plant height and seeds/pod
OE2-3	3.18	Increase in seeds/pod only
OG3-3	3.09	Increase in seeds/pod only
OG1-1	3.09	Increase in pods/plant, pod length and seeds/pod
OG3-2	3.00	Increase in pod length and seeds/pod
OGN2-3	2.99	Shorter in plant height and increase in seeds/pod
OM2-3	2.99	Late maturity
OGM2-3	2.99	Increase in seeds/pod only
Parent	2.42	

Table 3 Distribution of cultures with superior mutation for different characters in M_5 generation of Sujata

Tr. code	Days to flowering	Days to maturity	Plant height (cm)	Clusters/ plant	Pods/ plant	Pod length (cm)	Seeds/ pod	100-seed weight (g)	Yield/ plant (g)	Total	%
G1		2			2	2		2	2	10	19.61
G2								3		3	05.88
G3						1	1		1	3	05.88
E1					1	1	1	2	1	6	11.76
E2						1		2	2	5	09.80
E3		2			1	1	1	3	1	9	17.65
N1					1			1	1	3	05.88
N2								2		2	03.92
N3					1	1		1	1	4	07.84
M1					1			1	1	3	05.88
M2										0	00.00
M3										0	00.00
GE2			1							1	01.96
GN2										0	00.00
GM2								2		2	03.92
Total		4	1		7	7	3	19	10	51	
%		7.84	1.96		13.73	13.73	5.88	37.25	19.61		
Mutagens											
Gamma-rays		2			2	3	1	5	3	16	31.37
EMS		2			2	3	2	7	4	20	39.22
NG					2	1		4	2	9	17.65
MH					1			1	1	3	05.88
Combination			1					2		3	05.88

G1, Gamma-rays 20kR; G2, gamma-rays 40kR; G3, gamma-rays 60kR; E1, EMS 0.2%; E2, EMS 0.4%; E3, EMS 0.6%; N1= NG 0.005%; N2**,** NG 0.01%; N3, NG 0.015%; M1, MH 0.01 %; M2, MH 0.02%; M3, MH 0.03%; GE2, gamma-rays 40kR + EMS0.4%; GN2, gamma-rays 40kR + 0.010%; GM2, gamma-rays 40kR + MH 0.02

Table 4 Distribution of cultures with superior mutation for different characters in M_5 generation of OBGG-52

Tr. code	Days to flowering	Days to maturity	Plant height (cm)	Clusters/ plant	Pods/ plant	Pod length (cm)	Seeds/ pod	100-seed weight (g)	Yield/ plant (g)	Total	%
G1					1	1	1		1	4	9.09
G2											
G3						1	2		2	5	11.36
E1		1					1		1	3	6.82
E2							1		1	2	4.54
E3											
N1						1				1	2.27
N2						2				2	4.54
N3					3	2	3		3	11	25.00
M1			1				1		1	3	6.82
M2							1		1	2	4.54
M3		2								2	4.54
GE2			1							1	2.27
GN2			3				1		1	5	11.36
GM2							1	1	1	3	6.86
Total		3	5		4	7	12	1	12	44	
%		6.82	11.36		9.09	15.91	27.27	9.09	27.27		
Mutagens											
Gamma-rays					1	2	3		3	9	20.45
EMS		1					2		2	5	11.36
NG					3	5	3		3	14	31.82
MH		2	1				2		2	7	15.91
Combination			4				2	1	2	9	20.45

G1, Gamma-rays 20kR; G2, gamma-rays 40kR; G3, gamma-rays 60kR; E1, EMS 0.2%; E2, EMS 0.4%; E3, EMS 0.6%; N1, NG 0.005%; N2, NG 0.01%; N3, NG 0.015%; M1, MH 0.01%; M2, MH 0.02%; M3, MH 0.03%; GE2, gamma-rays 40kR + EMS0.4%; GN2, gamma-rays 40kR + 0.010%; GM2, gamma-rays 40kR + MH 0.02

rest 21 mutant cultures exhibited superiority over parent in one to three characters.

The frequency of superior mutants for nine quantitative traits in different treatments was highest for treatment G1 (19.61%) followed by E3 (17.65%) and E1(11.76%) in Sujata (Table 3) and N3 (25.00%) G3 and GN2 (11.36% each) in OBGG 52 (Table 4). Mutagen-wise distribution of superior mutants frequency was highest in EMS (39.22) followed by gamma-rays (31.37) and NG (17.67) in Sujata, while in OBGG 52, the highest frequency of superior mutants was observed for NG mutagen (31.82%) followed by gamma-rays and combined treatments (20.45% each). Similar differential efficiency of mutagenic treatments were reported earlier in greengram by Kozgar *et al.* (2010).

A comparative study of superior mutants frequency for different characters revealed maximum superior mutants in 100-seed weight (37.25) followed by yield/plant (19.61%) in Sujata and seeds/pod, yield/plant (27.27% each) followed by pod length (15.91%) in OBGG 52.This indicated mutagenic treatments were effective in inducing more positive changes in these characters.

Thus, considering treatment-wise distribution of high yielding mutant progenies /cultures in M_4 and M_5 generations in both the varieties, it was inferred that treatment with gamma rays (20, 60 kR), EMS (0.2, 0.4, 0.6%), NG (0.005, 0.015%), MH (0.01%) and gamma rays (40 kR) followed by NG (0.01%) or MH (0.02%) would be effective in induction and isolation of more useful mutants in greengram.

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