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Isolation of Macro Mutants and Mutagenic Effectiveness, Efficiency in Black Gram (*Vigna mungo* (L.) Hepper)

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Abstract: The Present investigation was undertaken to study the frequency and spectrum of macro mutants along with the mutagenic effectiveness and efficiency of different dose/ concentration of gamma rays and EMS in Black gram variety (Vamban-1). The seeds were treated with gamma rays (20, 40, 60, 80 and 100kR) and EMS (5, 10, 15, 20 and 25mM). The biological damage was calculated in M_1 and M_2 generation based on lethality (L) and seedling Injury (I). In this genotypes number of chlorophyll mutants and viable mutants with effectiveness and efficiency were observed. Mutagenic effectiveness and efficiency was calculated based on the biological damage of both generation in chlorophyll mutants and viable mutants. In general the mutation frequency was high on M_1 plant basis than M_2 plant for both the mutagens. The spectrum of chlorophyll mutants (Chlorina, Albino, Xantha, Variegata) and Viable mutants (Dwarf, Tall, Tiny leaf, spreading, Bushy type) were observed in M_1 and M_2 generation. The mutagenic effectiveness decreased with the increased in dose/ concentration of mutagen. Mutagenic efficiency (mutation rate in relation to damage of seedlings) increased at lower dose/ concentration and decreased with higher concentration. Mutagenic efficiency varied depending upon the criteria selected for its estimation. The present investigation the EMS treatments were found more efficient in causing less biological damage and inducing maximum amount of mutations.

Key words: Black gram · Macro mutation · Gamma rays · EMS · Mutagenic effectiveness and efficiency

INTRODUCTION

Black gram (*Vigna mungo* L. Hepper) is important legumes which are widely cultivated and consumed in different states of India. It is rich protein content [1]. A crop plant can be improved in productivity resistance to biotic and abiotic stresses when the genetic variability for the specific trait is available in the considered population or species. Induced mutagenesis has been successfully used to generate variability, portioning for isolating mutants with desirable characters of economic importance such as superior dwarf plant types, synchronous maturity, high grain yield, larger seed size and seed colour etc [2].

Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. The chlorophyll mutation frequency in M_2 generation is the most dependable index for evaluating the genetic effects of mutagenic treatments [3, 4]. The usefulness of a mutagen in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagen), but also on its mutagenic efficiency (mutation in relation to undesirable changes/ damage like lethality, injury etc.). The present investigation was undertaken to study the frequency and spectrum of macro-mutations along with the mutagenic effectiveness and efficiency of different doses of gamma rays and EMS treatments in M_1 and M_2 Generation.

MATERIALS AND METHODS

The experimental seeds of black gram variety vamban-1 were treated with physical and chemical mutagens viz., gamma rays (20, 40, 60, 80and 100kR) in different doses from Co60 source at Sugarcane Breeding Institute at Coimbatore and EMS (5, 10, 15, 20 and 25mM) for 6 hours presoaked the chemicals in the laboratory, Department of Botany, Annamalai University. The treated seeds along with control (untreated seeds) were used and sown immediately in the field with a spacing of 30 × 15cm in a randomized block design (RBD) with 3 replications to raise the M_1 generation. Surviving plants with sufficient seeds in different treatments including control were harvested and threshed individually and their seed yield

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was recorded. The harvested seeds were raised M_2 population along with control (untreated seeds). Necessary cultural operations carried out up to harvesting period. The control and progenies were screened for lethal chlorophyll mutations and viable mutations recorded right from emergence till the age of three weeks after germination, when the seedlings were at four leaf stage in the field as per identification and classification recommended by Gustaffson [5]. Mutation frequency was calculated as the percentage of mutated progenies and plants. Both mutagenic effectiveness and efficiency were determined as per the formulae suggested by Konzak *et al.,* [6].

Mutagenic effectiveness = MP/ tc (or) kR
Mutagenic efficiency = MP/ L, MP/I
MP = Chlorophyll or Viable mutants per 100 M_1 plants
t = Duration of treatment with chemical mutagen in hours
c = Concentration of chemical mutagen in mM
kR = Dose of gamma radiation
L = Percentage of lethality i. e. reduction in survival of 30^{th} day
I = Percentage of injury i. e. reduction in plant height on 30^{th} day.

Mutagenic Effectiveness and Efficiency: The effectiveness and efficiency of mutagenic treatment in mutation breeding of crop plants have been discussed by several workers [7-9]. In this study of the effectiveness and efficiency of mutagenic treatments, the use of one and the same genotype throughout experiments is most important. The term effectiveness however is often used also for biological effects such as seedling growth reduction and chromosome injuries. The effectiveness of treatment with a mutagen is expressed as the magnitude of the effects produced after a particular dose of the mutagen or as the relative doses that produce equivalent effects under different treatments. Nilan *et al*. [7] defined the effectiveness of radiations by mutation rate in relation to dose and the efficiency of radiations by the mutation rate in relation to biological effects.

RESULTS AND DISCUSSION

Chlorophyll mutants are used as markers in genetics, physiological and biochemical investigations. Vanharten [10] report that the chlorophyll synthesis is under the control of nuclear and cytoplasmic genes. The frequency of chlorophyll mutants were scored in M_1 and M_2 seedlings (Table 1). In this present investigation generally chlorophyll mutants occurred in all the dose/ concentration of gamma rays and EMS. The frequency increased from 20kR to 60kR of gamma rays and 5mM to 15mM of EMS on M_1 and M_2 seedling bases and there after reduction in frequency was observed. Chlorophyll mutants frequency per 100 M_1 plants and M_2 plant bases maximum at 60kR of gamma rays and 15mM of EMS In this dose/ concentration maximum chlorophyll mutation frequency observed in M_1 and M_2 seedlings 60kR (46. 66%, 1. 72%) and EMS (53. 33%, 2. 19%). In this study Frequency of chlorophyll mutants was the highest on M_1 generation when compared to M_2 seedlings the similar results were reported by Ahmed John [11] and Vanniarajan [12]. In M_1 and M_2 plant basis the mutants were decreased with increased the dose/ concentration up to certain level of gamma rays and EMS treatments.

Lower doses of EMS were more efficient than higher doses of EMS in producing chlorophyll mutations. Khan [13] gave similar reports in black gram. Chlorophyll mutants are used as tests for evaluation of genetic action of mutagenic factors [14]. The frequency of viable mutants frequency were scored in M_1 and M_2 generations both Physical and chemical mutagenic treatments (Table 1). The maximum mutants at 20kR of gamma rays to 60kR of gamma rays and from 5mM of EMS to 15mM of EMS in M_1 and M_2 plant basis. In this dose / concentration the maximum viable mutants were observed at 60kR of gamma rays (40. 00%, 2. 45%) and 15mM of EMS (40. 00%, 3. 29%). The possible cause of these macro mutations may be chromosomal aberrations like small deficiencies or duplications and most probably gene mutations [15]. Mutagenic effectiveness and efficiency of chlorophyll mutations and viable mutations based on lethality, Injury on M_1 and M_2 plant basis given in Table (2 and 3).

The maximum effectiveness of chlorophyll mutations were observed at 60kR of gamma rays (77. 76%, 2. 81%) minimum at 100kR (20. 00%, 0. 98%) respectively on M_1 and M_2 seedling basis. In EMS the maximum effectiveness of chlorophyll mutations observed at 15mM (74. 06%) in M_1 generation and 15mM (5. 36%) in M_2 plant basis. The viable mutations of effectiveness in physical and chemical mutagens range maximum at 60kR of gammarays (66. 69%, 6. 12%) minimum range 100kR of gamma rays (20. 00%, 0. 84%). In EMS the maximum effectiveness was 15mM (88. 88%, 7. 31%) respectively in M_1 and M_2 generation plants. Generally the effectiveness of chlorophyll and viable mutants were higher in EMS treatment than gamma rays both M_1 and M_2 generation. Reddy *et al*. [16] reported EMS either alone or in

Table 1: Frequency of chlorophyll mutant in M_1 and M_2 generation of blackgram

	Frequency of chlorophyll mutants						Frequency of viable mutants					
	No. of M_1 plants		No. of M_2Plants		Mutant frequency		No. of M_1plants		No. of M_2 plants		Mutant frequency	
Mutagen	Scored	segregated	Scored	segregated	% of M1 plants	% of M2 plants	Scored	Segregated	Scored	Segregated	% of M1 plants	% of m2 plants
Gammarays (kR)												
Control	15	-	654	-	-	-	15	-	654	-	-	-
20	15	1	824	4	13. 33	0. 48	15	2	824	8	13. 33	0. 97
40	15	3	987	15	20. 00	1. 51	15	4	987	20	26. 66	2. 02
60	15	7	1100	19	46. 66	1. 72	15	6	1100	27	40. 00	2. 45
80	15	4	900	9	26. 65	1. 00	15	5	900	9	33. 33	1. 00
100	15	3	710	7	20. 00	0. 98	15	3	710	6	20. 00	0. 84
EMS(mM)												
Control	15	-	600	-			15	-	600	-	-	
5	15	1	720	6	6. 66	0. 69	15	1	720	6	6. 60	0. 83
10	15	3	865	14	20. 00	1. 61	15	4	865	18	26. 66	2. 08
15	15	8	910	25	53. 33	2. 19	15	6	910	30	40. 00	3. 29
20	15	5	754	7	33. 33	0. 92	15	3	754	14	20. 00	1. 85
25	15	4	698	4	26. 66	0. 57	15	2	698	9	13. 33	1. 28

Table 2: Mutagenic efficiency and effectiveness in chlorophyll mutants on M_1 and M_2 seedling bases

					Effectiveness $\frac{MP\times100}{Tc\,(or)\,kR}$		Efficiency			
		Height	Mutants				M_1 plant basis		M_2plant basis	
Mutagen (dose/conc.)	Survival reduction on 30^{th} day (L)(%)	Height reduction on 30 day th (Injury)(I)	100 M_1 seedlings (MP)	100 M_2 Seedlings (MP)	M_1 plant basis	M_2plant basis	MP× 100/L	MP× 100/I	MP× 100/L	MP×100/I
Gammarays (kR)										
20	20. 08	25. 61	6. 66	0. 48	33. 30	2. 40	33. 16	26. 00	2. 39	1. 87
40	37. 46	42. 45	20. 00	1. 01	50. 00	2. 52	53. 39	47. 11	2. 69	2. 37
60	53. 61	59. 51	46. 66	1. 72	77. 76	2. 81	73. 69	78. 40	3. 94	2. 89
80	64. 16	60. 17	26. 65	1. 00	33. 31	1. 25	44. 29	44. 29	1. 55	1. 66
100	88. 68	71. 46	20. 00	0. 98	20. 00	0. 98	27. 98	27. 98	1. 10	1. 37
EMS(mM										
5	22. 56	19. 24	6. 66	0. 69	44. 40	4. 60	32. 20	26. 00	3. 67	4. 31
10	39. 21	30. 71	20. 00	1. 61	66. 66	4. 86	51. 00	65. 12	4. 10	5. 24
15	60. 62	56. 25	53. 33	2. 19	74. 06	5. 36	87. 94	94. 80	4. 61	5. 89
20	71. 14	44. 12	33. 33	0. 92	33. 33	1. 53	46. 85	75. 54	1. 29	2. 08
25	78. 69	39. 18	26. 66	0. 57	17. 77	0. 76	33. 87	68. 04	0. 72	1. 45

Table: 3 Mutagenic efficiency and effectiveness in viable mutants on M_1 and M_2 seedling bases

					Effectiveness $\frac{MP\times100}{Tc\,(or)\,kR}$		Efficiency			
			Mutants				M_1 plant basis		M_2 plant basis	
Mutagen (dose/conc.)	Survival reduction on 30^{th} day(L) (%)	Height reduction on 30 day th (Injury)(I)	100 M_1 seedlings(MP)	100 M_2 Seedlings(MP)	M_1 plant basis	M_2plant basis	MP× 100/L	MP× 100/I	MP× 100/L	MP× 100/I
Gamma rays(kR)										
20	38. 72	25. 61	13. 33	0. 97	66. 65	4. 85	34. 42	52. 04	2. 50	3. 78
40	45. 43	32. 45	26. 66	2. 02	66. 68	5. 05	58. 68	82. 15	4. 44	6. 02
60	54. 67	40. 51	40. 00	2. 45	66. 69	6. 12	73. 16	98. 74	4. 48	6. 04
80	68. 39	50. 17	33. 33	1. 00	41. 66	1. 25	48. 73	66. 43	1. 46	1. 99
100	88. 69	61. 46	20. 00	0. 84	20. 00	0. 84	22. 55	32. 54	0. 71	1. 30
EMS(mM)										
5	40. 36	29. 24	6. 60	0. 83	44. 00	5. 53	16. 35	22. 57	2. 05	2. 83
10	52. 47	40. 71	26. 66	2. 08	88. 86	6. 93	50. 80	65. 48	3. 96	5. 10
15	60. 51	43. 25	40. 00	3. 29	88. 88	7. 31	66. 10	86. 52	5. 43	7. 60
20	75. 37	34. 12	20. 00	1. 85	33. 33	3. 08	26. 53	58. 61	2. 45	5. 42
25	90. 40	29. 98	13. 33	1. 28	17. 77	1. 70	14. 74	44. 46	1. 41	4. 26

combination more effective than gamma rays. The EMS treatment was more efficiency than gamma rays treatment. In chlorophyll mutations maximum efficiency at 60kR of gamma rays (73. 69%, 78. 40%) in M_1 plants and (3. 94%, 2. 89 %) M_2 plants. The EMS at 15mM (87. 94%, 94. 80%) in M_1 plants and (4. 61%, 5. 89%) in M_2 plants obtained based on lethality and injury.

The efficiency of viable mutants was presented in Table 3. The viable mutant efficiency is more in EMS treatment than gamma rays the maximum efficiency of viable mutants in M_1 and M_2 generation 60kR of gamma rays in (73. 16%, 98. 74%) and (4. 48%, 6. 04%) 15mM of EMS (66. 10%, 86. 52%)and (5. 43%, 7. 60%) was obtained based on the lethality and Injury. In this present investigation EMS was more effectiveness and efficiency of chlorophyll and viable mutants than gamma rays treatments. A number of chemical mutagen have been found to be equally and even many times more effective and efficient mutagens [17-19] M_1 plant basis the frequency was high in viable and chlorophyll mutation compared to M_1 plant basis. Similar results were reported by [20] in *Cyamopsis tetragonoloba*.

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