Agriways ISSN (Print) 2321-8614 & ISSN (Online) 2454-2318
(A Multidisciplinary Peer –Reviewed Refereed Journal)
www.agriwaysjournal.com Vol 10 Issue 02 July-Dec 2022

MUTAGENIC EFFECTS: INDUCED VARIABILITY ON DIFFERENT CHARACTERS IN CHICKPEA (*CICER ARIETINUM L.)*

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Abstract:
The success of a mutation breeding programme depends not only on the equality of induced mutation, but also on the screening techniques to identify these mutations, which occur with a very low frequency among a large number of others of little breeding value. An attempt has been made here to develop standard screening techniques for macro mutations affecting the polygenic system. In general, selection for quantitative traits, such as yield, should be preferably carried out in early generations because most of the desired combinations of favorable alleles are likely to be lost in advanced generations due to intensive or even no selection for other traits (Sneepe, 1977).The data on induced variability (CV%) for different polygenic characters. The estimates of coefficient of variance had maximum values for all the characters and treatments compare to their control except days to 50 per cent flowering and plant height treated by EMS and combination of gamma rays + EMS in M_2generation. On the other hand, in M_3generation, the estimates of coefficient of variance showed highest for all the characters compare to their control. The estimates of coefficient of variance (%) have higher for all the characters and mutagens treated population except days to maturity for all mutagens and 100-seed weight for gamma rays and EMS in M_2 generation. However, in M_3generation also estimates of coefficient of variance showed higher most of the characters for all the treatments over their control except plant height, seeds per pod and 100-seedweight.

Key words: *Chickpea, coefficient of variance, M_3 generation, EMS, Gamma rays, Gamma rays+ EMS, BGM-524, KSB-220 and BG-1053*

Date of Submission: 04-12-2022 **Date of Acceptance: 11-12-2022**

Introduction:

Grain legumes occupy a unique position in world agriculture by virtue of their high protein content and capacity to fix atmospheric nitrogen. For developing countries like India, pulses constitute the major source of dietary proteins. In developed countries, grain legumes are also an important indirect source of protein, being animal feed of high biological value. They contain 20-30 per cent protein in their seeds, which is 2 to 3 times more than in the cereals. The proteins from pulses are also nutritionally valuable because of higher lysine content than the cereal proteins. These two groups of

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www.agriwaysjournal.com Vol 10 Issue 02 July-Dec 2022

crops have a complementary relationship in their amino acid composition and their combinedintake cancompensate, to a great extent, for their mutual amino aciddeficiency. Although, a large area about 21-12 million hectare is under different pulse crops in India, their production has remained practically stagnant for the last three decades. Theavailable statistics indicate thatthe pulse production has ranged between 11-15 milliontons of thegrains in different years. The production of pulses reached to its heights 14.91 million tons during 1998-1999. India contributed 11.31 million tones to the total world production of 58.6 million tons of pulses(Anonymous, 2003). Since the growth in pulse production did not keep pace with the increasing population, the per capita availability ofpulseshas progressively declined. In general, pulses give low yield than cereals (Jain, 1975). This led to the assumption that pulses may have lower genetic potential for yield than cereals (Boulter, 1973, Swaminathan, 1973). Chickpea is cultivated globally on about 11.3 million hectares adding8.8 million tons ofgrains annually to the global food basket with an average productivity of 777 kg/ha .Themajor chickpea growing countries in order of their significance are India, Turkey, Pakistan, Iran, Australia, Mexico, Ethiopia, Myanmar, Spain and Bangladesh. India has the distinction of being the largest chickpea producer and accounts for over 64 and 68 per cent of thetotal area and production in the world. It grows chickpea on about 7.5 millionhectaresproducing6.1milliontons of grains,whichrepresents33 and 47 per cent of the national pulse area and production.

Mutagen	Dose	No. of seeds treated	Treatment condition
Control	0.00	100×3	Dry
Gamma rays	20kR	100×3	Dry
	30kR	100×3	Dry
	40kR	100×3	Dry
	50kR	100×3	Dry
	60kR	100×3	Dry
EMS	010%	100×3	
	015%	100×3	
	020%	100×3	Soaking (400ml)
	025%	100×3	
	030%	100×3	
Combined	20kR+0.10	100×3	
treatment	30kR+0.15	100×3	
	40kR+0.15	100×3	Dry + Soaking (400ml)
	50kR+0.15	100×3	
	60kR+0.15	100×3	

Total treatment = 16×3= 48

Total experimental seeds = 48×100=4800

Physical mutagen

The gamma rays obtained from a 2000 curie CO^{60} gamma cell installed in the Division of NRL, IARI New Delhi with a dose rate of 2500 radius per minutes was used as physical mutagen. Gamma rays are electromagnetic type of radiations induce mutations through ionization when a biological material is irradiated a gamma rays photon hits an orbital electron of the atom. The electron gets excited and in turn ejected tremendous energy and is capable of causing further ionization along its

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www.agriwaysjournal.com Vol 10 Issue 02 July-Dec 2022

path. Well dried uniform seeds of each variety size about 10 per cent moisture content were filled in a seed envelope and exposed to gamma rays irradiations in the gamma cell. Different doses of gamma rays were used for irradiation of seeds i.e.10, 20,30,40,50, and 60 kR accordingly. Chemical mutagen Ethyl methane sulphonate (EMS) a potent alkylating agent obtained from Eastman Kodak Chemicals USA was used as chemical mutagen. It is a most widely used chemical mutagen and is also a powerful carcinogen. It induces through alkylation of DNA. Well dried selected seeds of uniform size were presoaked in water for 6 hours and then treated with freshly prepared aqueous solutions of EMS at 25+ 2^0 C. To have maximum absorption of solution, the seeds contained in the conical flask were repeatedly subjected to shaking. Treatments with five different concentrations i.e. 0.10, 0.15, 0.20, 0.25 and 0.30 percent for 4 to 6 hours each were given with intermittent shaking. As soon as treatment is over, seeds were washed in running tap water for 5-10 minutes to remove traces of chemical from the seed surface. Thereafter by spreading the seeds on blotting paper excessive water was removed. The seeds were sown in the field immediately after treatment whereas seeds soaked in tops water for 6 hours were used as control. Combinedmutagen. Seeds irradiated at different doses viz. 20, 30, 40, 50, and 60kR were treated with 010.0.15, 0.20, 0.25, and 0.30 per cent EMS aqueous solution of ethyl methane sulphonate for 6 hours by the method described above.

The investigation was carried out from research farm at Monad University and Janta Vaidic College BarautBaghpat U.P (India) on M_1 to M_3 generations. All generations were grown in well prepared land and data taken very precautionary. Observations were recorded for seven quantitative characters of economic importance. Only families with normal looking plants were included in micro mutations. Those families which were showing segregation for macro mutations (chlorophyll and viable) were treated as a separate class. Ten normal looking plants from eachM_2family thatwas not segregating for macro mutationsand 5-10 normal looking plants from each segregating family for macro mutations, depending on the availability of plants in a family, were chosen randomly to record observations on the seven quantitative characters of economic value. As the interfamily variance was expected to decline in M_3 generation the comparison of mean values was considered as the most important criterion to estimate the effectiveness of M_2selection. Therefore, the mean value of each M_3 family was compared with the highest mean value recorded in the control was considered as "promising" to advance.

Results and discussion:

The effect of range, mean, coefficient of variance, heritability and genetic advance for days to 50 per cent flowering in M_3 generation for BGM-524, BG-1053 and KSB-220 are presented in Table 2. The estimates of coefficient of variance showed wide range with the values ranging from 3.49 (30 kR) to 11.75 (50 kR) for KSB-220 and the values for BGM-1053 ranging from 3.86 (0.10% EMS) to 11.26 (0.20% EMS), whereas for BGM-524 the values ranging from 3.52 (60 kR + 0.30% EMS) to 7.22 (20 kR + 0.10% EMS).The estimates of heritability were highest values 78.23% (20 kR) followed by 75.45% (40 kR), 75.21% (0.30% EMS) and 72.70% (60kR + 0.30% EMS). The estimates of genetic advance were maximum for 11.81 (20 kR) followed by 8.88 (60 kR) and 8.83 (0.20% EMS).

Plant height

The range, mean, coefficient of variance, heritability and genetic advance for plant height (cm) in M_3generation for BGM-524, BG-1053 andKSB-220 are presented in Table 3.The estimates of coefficient of variance showed wide range with the values ranging from 12.19% (60 kR) to 24.74% (40 kR) for BG-1053, 9.37 (0.10% EMS) to 36.27(0.10% EMS) for BG-1053 and BGM-524 and 9.85

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www.agriwaysjournal.com Vol 10 Issue 02 July-Dec 2022

(30 kR + 0.15% EMS) to 36.10 (60 kR + 0.30% EMS) for BGM-524 and KSB-220,respectively.The highest estimates of heritability (87.50%) were found in treatment 50 kR followed by 86.20% (0.10% EMS) and 80.20% (30 kR + 0.15% EMS). Genetic advance had highest (12.23) in 0.30% EMS for BG-1053 followed by 10.03 (40 kR) and 9.17 (30 kR) in BGM-524.

Days to maturity

The estimates of range, mean, coefficient of variance, heritability and genetic advance for plant height (cm) in M_3 generation for BGM- 524, BG-1053 and KSB-220 are presented in Table 4. The estimates of coefficient of variance showed wide range with the values ranging from 1.62 to 7.06 (30 kR) for the varieties BG-1053 and KSB-220, 3.70(0.30% EMS) to 11.11 (0.20% EMS) for BGM-524 and BG-1053,respectively. However, the values ranging from 2.77 (30 kR + 0.15% EMS) to 6.70 (60 kR + 0.30% EMS) for BGM-524.The heritability was maximum for 75.10% (60 kR + 0.30% EMS) for KSB-220 followed by 73.20% (60 kR) for BGM-524 and 72.30 (0.30% EMS) for BG-1053. The highest estimates of genetic advance (8.00) was observed (0.20% EMS) followed by 7.71 (60 kR + 0.30% EMS)and 6.12 (60 kR) for KSB-220.

Number of pods per plant

The data on range, mean,and coefficient of variance, heritability and genetic advance are presented in Table 4.24. The estimates of coefficient of variance showed wide range with the values ranging from 6.77 (40 kR) to 53.73 (60 kR) for BG-1053 while, 5.71 (0.25% EMS)to 58.24 (0.10% EMS) for BGM-524, whereas 6.34 (60 kR + 0.30% EMS) to 60.21 (50 kR + 0.25% EMS) for BG-1053.The estimates of heritability were maximum (88.22%) in 30 kR followed by 87.36% (40 kR), 85.65% (0.10% EMS) and 75.56% (20kR + 0.10% EMS) for BGM-524, whereas maximum (33.85%) genetic advance in 0.10% EMS followed by 32.86 (40 kR) for BGM-524 and 23.44 (50 kR + 0.25% EMS) for BG-1053.

Number of seeds per pod

The effect of range, mean, and coefficient of variance, heritability and genetic advance for number of seeds per pod in M_3 generation for BGM- 524, BG-1053 and KSB-220 are presented in Table 4.25. The estimates of coefficient of variance showed wide range with the values ranging from 22.79 (20 kR) to 48.23 (60 kR) for the variety BG-1053, 21.29 (0.25% EMS) for BG-1053 to 56.41 (0.10% EMS) for BGM-524. Onthe other hand, 24.64 (20 kR + 0.10% EMS) and 62.50 (60 kR + 0.30% EMS) for BG-1053 and BGM-524, respectively. The estimates of heritability were highest (82.65%) in 20 kR+ 0.10% EMS) for BG-1053 followed by 68.60% (60 kR) for KSB-220 and 60.26% (0.10% EMS) for BG-524. The genetic advance had maximum value 10.13% (60 kR) for BGM-524 followed by 9.06 (20 kR+ 0.10% EMS) for BG-1053 and 4.48 (0.10% EMS) for BGM-524.

100-seed weight

The data for range, mean, coefficient of variance, heritability and genetic advance are presented in Table 4.26. The estimates of coefficient of variance have wide range in the present study with the values ranging from 19.58 (40 kR) and 56.05 (50 kR) for KSB-220 and BGM-524, respectively. Whereas, the values 14.57 (0.30% EMS) for BG-1053 to 50.59 (0.25% EMS) for BGM-524, however, the combined treatment showed 21.03 (30 kR+ 0.15% EMS) for BG-1053 to 46.12 (20 kR + 0.10% EMS) for BGM-524.

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www.agriwaysjournal.com Vol 10 Issue 02 July-Dec 2022

The estimates of heritability were maximum (90.23%) in 20 kR for BGM-524 followed by 85.69% (40 kR + 0.20% EMS) and 83.20% (0.30% EMS) for BG-1053. Genetic advance had highest values 10.13 (60 kR) for BGM-524 followed by 9.20 (0.10% EMS) and 8.18 (20 kR+ 0.10% EMS) for BG-1053.

Grain yield per plant

The micro mutationsin plant breeding were best utilized in the extensive and pioneering work of Gregory (1956, 1961, 1965), Gaul (1961, 1965), Scossiroli(1965), Brock (1965b, 1967), Swaminathan(1969) and Lawrence (1968, 1975e). Gregory (1967) demonstrated that mutations affectinga quantitative trait of a crop can be induced by

Irradiationand phenotypic selection can accumulate positive mutations to produce better strains. Gaul (1965) treated seeds of four barley varieties with X-ray and demonstrated that the reduction in mean yield and increase in genetic variance depend on the dose applied. He further reported that even though the major part ofinduced genetic variability is in negative direction, a few lines surpassed the highest yielding control.

The range, mean, coefficient of variance, heritability and genetic advance are presented in Table 4. The coefficient of variance showed wide range with the values ranging from 10.17 (30 kR) in BGM- 524 to 78.10 (40 kR) for KSB-220 whereas, 11.83 (0.10% EMS) for BG-1053 to 40.21 (0.10% EMS) for BGM-524. For combined treatment, the values ranging from 9.28 (40 kR + 0.20% EMS) for KSB-220 to 32.28 (50 kR + 0.25% EMS) for BG-1053.The estimates of heritability were maximum (88.56%) in 0.10% EMS for BGM-524 followed by 85.65% (50 kR + 0.25% EMS) for BGM- 524 and 78.20% (20 kR) for BG-1053. The estimates of genetic advance had maximum values 25.28 (0.10% EMS) for BGM-524 followed by 8.42 (60 kR) for BG-1053 and 8.02 (20 kR + 0.10% EMS) for KSB-220 inthe present study.Except days to maturity for all mutagens and 100-seed weight for gamma rays and EMS in M_2 generation. However, in M_3 generation also estimates of coefficient of variance showed higher most of the characters for all the treatments over their control except plant height, seeds per pod and 100-seed weight.

The estimates on induced variability (CV%) for different polygenic characters in M_2 and M_3generations for KSB-220 and presented in Table 4. The estimates of coefficient of variance have high most of the characters in M generation for the varietyKSB-220. On the other hand, in M_3 generation, coefficient of variance showed highest for all the characters and mutagens compared to their control except days to 50 per cent flowering.All these indicate wide variation for coefficient of variance to their control. The gamma rays showed better performance comparison to both EMS and combined treatments (EMS + gamma rays) in three cultivars.

Table 2. Components of variation, heritability and genetic advance for 100-seed weight (g) in M3 generation of BGM-524, BG-1053 and KSB-220

Statistical Gammarays(kR) Ethyl methane sulphonate(EMS,%) Combination (Gama rays kR+EMS,%)

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parameter	Control	20	30	40	50	60	0.1	0.15	0.2	0.25	0.3	20+0.1	30+0.15	40+0.2	50+0.25	60+0.30
Variance																
V_1	40.34	6.04	12.88	43.94	11.69	30.30	33.82	21.96	91.66	38.93	20.65	16.69	19.86	4.47	38.96	8.20
V_2	17.23	19.18	58.91	30.05	41.61	53.26	25.49	21.40	54.30	23.25	26.25	47.90	25.26	6.86	27.15	46.36
V_3	10.11	7.14	14.98	8.27	22.21	4.39	143.37	7.93	21.97	36.22	5.21	18.83	18.55	33.46	22.74	5.40
GCV																
V_1	18.98	0.24	0.22	19.22	36.56	10.37	13.18	8.99	71.73	28.91	7.52	0.20	0.20	0.23	7.79	7.36
V_2	12.50	0.17	15.05	4.40	20.83	11.34	0.14	0.15	19.08	5.00	7.60	16.73	5.21	6.42	12.66	14.96
V_3	7.88	0.24	6.81	3.46	0.23	0.25	32.85	0.23	0.17	15.98	0.58	11.54	5.34	9.43	10.65	0.59
PCV																
V_1	41.05	22.89	30.92	36.82	56.40	26.28	31.74	23.28	86.12	48.44	29.94	28.33	28.36	21.77	32.04	15.36
V_2	11.53	30.74	25.25	20.30	34.04	26.89	23.49	21.67	26.96	26.68	26.63	25.37	19.55	16.61	21.43	21.25
V_3	19.43	24.77	25.60	19.27	41.14	18.06	56.28	23.59	27.09	28.98	49.74	30.48	27.94	30.36	30.18	44.72
Heritability																
V_1	21.25	78.26	65.23	27.58	42.10	25.65	27.58	24.54	67.56	35.54	63.21	78.56	71.58	50.23	26.58	22.23
V_2	59.00	78.36	36.54	25.65	37.58	27.58	75.56	68.20	50.23	40.23	83.20	43.23	27.56	24.58	35.86	49.58
V_3	26.54	90.23	73.50	42.30	35.69	74.20	34.56	82.65	34.56	30.23	40.26	26.47	24.58	85.69	23.50	23.50
Genetic advance																
V_1	2.34	8.12	7.11	2.99	6.90	1.55	1.78	1.26	8.63	3.50	0.56	8.18	8.00	5.11	0.72	1.12
V_2	9.37	10.12	4.29	0.51	3.76	2.30	9.00	7.13	5.38	3.34	0.23	4.06	0.69	0.71	2.87	4.93
V_3	6.93	7.00	7.67	6.19	6.12	8.13	6.54	9.15	6.12	5.97	6.13	3.14	3.31	4.05	1.10	3.42

V1 = BGM-524 (*desi)* , V2 = BG-1053 (*kabuli)*, V3 = KSB-220 (green)

Table 3. Range, population means, variance, SD and CV for grain yield per plant in M3 generation for BGM-524, BG-1053 and KSB-220

Table 3. Range, population means, variance, SD and CV for grain yield per plant in M_3 generation for BGM-524, BG-1053 and KSB-220

Statistical Parameter		Control	Gamma rays (kR) 20	30	40	50	60	Ethyl methane sulphonate (EMS,%) 0.1	0.15	0.2	0.25	0.3	Combination (Gama rays kR+EMS,%) 20+0.1	30+0.15	40+0.2	50+0.25	60+0.30
Range																	
	V_1	19.3-28.3	11.0-37.3	12.2-22.1	6.3-33.7	9.3-31.5	17.7-57.4	12.5-56.5	19.0-33.6	9.4-29.3	12.1-47.4	21.1-44.6	21.3-43.0	21.6-41.4	27.7-52.3	21.5-57.1	21.1-38.4
	V_2	21.2-30.5	21.0-45.5	21.3-50.4	21.5-47.7	21.0-41.3	21.3-49.9	29.5-49.4	21.3-58.0	21.0-54.0	17.0-38.0	23.0-63.0	21.0-51.1	21.7-57.4	21.1-34.8	11.4-42.2	21.0-47.9
	V_3	14.2-31.0	11.0-37.3	12.4-34.0	21.0-34.2	12.2-39.4	6.3-33.3	11.1-33.7	9.3-31.5	31.3-57.4	12.5-33.6	9.4-29.4	21.1-47.4	21.3-43.6	37.3-57.1	21.0-38.4	11.2-17.0
Mean																	
	V_1	24.03	19.91	17.10	21.71	22.72	42.26	33.47	25.05	19.87	27.44	33.34	30.70	31.60	40.67	42.06	27.14
	V_2	25.75	29.07	33.11	31.79	26.53	36.18	40.83	43.32	33.25	24.79	43.31	32.77	43.40	26.66	21.47	28.53
	V_3	323.13	17.91	22.76	27.50	23.42	19.09	22.55	22.19	45.92	24.26	19.64	31.66	32.49	45.35	25.98	14.00
Variance																	
	V_1	7.46	33.79	3.02	36.72	25.34	99.86	161.27	14.99	32.31	76.76	57.51	38.26	37.60	31.60	91.97	28.91
	V_2	18.17	36.16	68.33	59.15	17.48	72.94	23.33	73.21	65.66	18.63	63.00	87.57	56.22	13.82	47.99	40.99
	V_3	12.06	32.64	25.53	33.40	47.04	39.20	20.03	26.87	46.71	14.78	29.11	54.30	32.91	17.76	20.73	19.07
SD																	
	V_1	2.76	5.81	1.74	6.06	5.03	9.99	13.46	3.86	5.60	8.76	7.56	6.18	5.68	5.62	9.50	5.31
	V_2	4.50	6.01	8.26	7.69	4.18	8.51	4.83	8.55	8.10	4.31	7.90	9.35	7.40	3.44	6.93	6.40
	V_3	3.47	5.71	5.05	5.78	6.85	6.26	4.47	5.10	6.83	3.84	5.33	7.82	5.73	4.21	4.58	3.08
CV																	
	V_1	11.53	29.18	10.17	27.91	22.13	23.63	40.21	15.40	28.18	31.55	22.67	20.13	17.97	13.81	22.58	19.56
	V_2	17.48	20.67	24.95	24.19	15.76	23.52	11.83	19.74	24.36	17.39	18.24	28.53	17.05	12.90	32.28	22.43
	V_3	15.01	31.88	22.18	78.10	29.24	32.79	19.82	22.98	14.87	15.82	27.13	24.69	17.63	9.28	17.62	22.00

V1 = BGM-524 (*desi)* , V2 = BG-1053 (*kabuli)*, V3 = KSB-220 (green)

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www.agriwaysjournal.com Vol 10 Issue 02 July-Dec 2022

Table4.Componentsof variation, heritability and genetic advance for seed yield per plant (g) in M3 generation of BGM-524, BG-1053 andKSB-220

Statistical		Gammarays (kR)					Ethyl methane sulphonate(EMS, %)					Combination (Gama rays kR+EMS,%)				
parameter	Control	20	30	40	50	60	0.1	0.15	0.2	0.25	0.3	20+0.1	30+0.15	40+0.2	50+0.25	60+0.30
Variance																
V1	21.83	50.78	38.82	56.94	15.73	163.30	53.02	7.50	27.43	86.52	24.89	23.75	16.43	71.58	268.63	26.12
V2	31.23	33.92	37.40	19.97	13.31	26.65	15.67	48.89	37.09	18.93	102.70	89.27	38.73	13.61	73.96	33.28
V3	13.43	28.84	22.47	59.60	56.58	53.10	14.68	17.08	23.22	11.97	18.66	29.93	32.51	27.22	26.04	30.50
GCV																
V1	9.08	14.30	10.33	15.12	0.14	147.61	36.64	0.13	1.72	17.13	4.76	0.10	0.10	10.84	21.66	0.11
V2	0.23	0.11	7.54	0.10	0.12	0.09	0.08	0.07	0.10	0.13	11.36	12.44	0.07	0.12	17.43	0.11
V3	5.09	0.18	6.20	0.10	12.80	15.66	0.13	0.14	0.07	0.13	0.16	0.10	4.23	5.20	6.31	6.02
PCV																
V1	15.42	29.52	68.22	27.18	24.55	22.08	38.97	17.40	26.25	23.70	13.36	17.10	15.42	14.06	23.68	20.26
V2	18.20	20.11	15.12	14.13	16.64	27.18	10.33	18.38	23.64	17.71	23.55	22.93	16.67	14.47	31.57	23.92
V3	14.11	33.36	18.90	79.71	26.53	31.06	18.85	25.01	15.66	16.54	24.33	19.97	16.49	8.80	17.50	28.73
Heritability																
V1	34.23	23.20	22.36	30.30	34.58	43.56	88.56	71.54	25.36	52.56	22.30	42.66	53.54	59.54	85.65	68.20
V2	34.20	78.20	24.58	51.25	42.50	68.50	63.54	43.26	49.20	28.68	23.40	29.54	31.25	43.56	31.25	39.54
V3	23.40	42.65	21.26	74.58	23.58	25.40	30.26	35.67	45.30	70.34	38.81	71.20	66.52	34.58	23.58	24.58
Genetic advance																
V1	2.57	2.84	0.89	3.74	3.98	8.42	25.25	8.11	0.15	7.00	1.17	4.11	5.11	7.00	17.37	7.11
V2	7.30	8.12	2.50	6.13	5.12	7.13	7.00	4.15	5.13	2.00	4.02	4.54	3.13	4.44	4.25	4.12
V3	4.88	6.12	3.95	8.13	2.98	3.11	4.33	5.00	6.13	8.00	5.00	8.02	6.73	4.90	2.22	1.48

V1 = BGM-524 (*desi)* , V2 = BG-1053 (*kabuli)*, V3 = KSB-220 (green)

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