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Analysis of Seed Yield and its Contributing Traits Inheritance in M_3 Generation of EMS Induced Black Gram (*Vigna mungo* L. Hepper) Mutants

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ABSTARCT: The present study was carried to analyse the EMS induced on seed yield and its contributing characters of blackgram (accession IC-436524) when raised during M_3 generation in Kharif season in the month of mid June-mid September, 2022 in the field located Kodad, Suryapet, Telangana. Demand for grain legumes has been increased recently due to their high protein and nutritional values. This resulted for the production of huge quantity of grains to meet the demand for increasing population like India. Genetic variability is prerequisite for isolating high yield blackgram genotypes. Mutation breeding is the simple, cheap and best practise to genetic variability in self pollinated crop like blackgram. In this study, EMS induced blackgram (accession IC-436524) mutants was used to identify the inheritance of high yielding mutants of M_2 progeny during M_3 generation along with Untreated seeds were used as (Control) and T-9 (as check, collected from ICAR-CRIDA, Hyderabad). Investigation revealed that top 5 mutants with high morphological variations in Plant height, Branches, leaves, Clusters, Pods, Seeds, Seed yield, root length and root nodules were observed. It clearly indicated that there are adequate amount of variability among the blackgram mutants due to EMS treatments.

Keywords: Blackgram, mutants, M_3 generation, Seed yield.

INTRODUCTION

Blackgram locally called as Urdbean, is an important legume crop (Family: Leguminacae) rich in proteins (about 26%). It is known as "Poor man's meat" due to its high nutritional value of proteins, minerals, vitamins etc. It is being used as dietary protein for most of the vegetarian population. Blackgram is an excellent alternative for high quality protein with good digetability in nature.

This is one of the important crops cultivated in South East Asia including India. Most favorable climate for its cultivation is semi arid region (Abraham *et al.,* 2013) and used as mixed crop along with rice, wheat, maize etc. It helps in atmospheric nitrogen fixation through the soil. It is also used as fodder for animals. Among the pulses it has high culinary values and high content of carbohydrates, proteins, minerals, fats, vitamins, potassium, calcium, iron, amino acids. Optimum temperature required for black gram plant growth is 27^oC-30^oC. Mostly cultivated in Kharif season throughout India. Black gram requires relatively heavier soil than other pulse grams. It can be grown on a variety of soils ranging from sandy soils to heavy cotton soil. The most ideal soil for cultivation of black gram crop is well drained loamy soil with a pH of 6.5 to 7.8. Average seed yield of blackgram is 604 Kg/ha which was low when compared to other pulse crops like field pea (912 Kg/ha, Singh *et al*. (2015), chick pea (889 Kg/ha, Raina *et al.*, 2019), lentil (705 Kg/ha, Laskar *et al*., 2018) (Annual Report 2016–2017). In order to break the gap and to meet the growing demand for production of black gram, efforts are needed to develop the high yielding varieties through crop improvement programmes.

The scheme of crop improvement programme is to create variabilities in crops such as high yielding, disease resistant, drought resistant etc. As the blackgram is a self pollinated crop which is having lesser variability, it is very important to create variability (Arvind Kumar *et al.*, 2007; Ramya *et al.*, 2014).

Genetic variability

Genetic variability through mutations

Crop plants have been recognized for their human health benefits because of rich in bioactive compounds like phenols, sugars, oils, minerals, vitamins, flavonoids, carbohydrates, etc. (Zia-ul-Haq *et al.*, 2014; Unlukara, 2019). Mutations played very significant source in increasing the crop productivity and simultaneously enhanced world food security, as an effective tool in supplementing the existing germplasm for cultivar improvement *i.e,* new food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production (Vanniarajan *et al.,* 2017; Barshile, 2006; Singh and Singh 2001; Sanjay, 2012; Patil *et al*., 2003). Mutagenic agents are used to induce mutations for creating variability particularly for isolating mutants with desirable characters of economic importance. Mutagens cause's genetic changes in an organism, break linkages and produces many new promising characters (Shah *et al.*, 2008; Usharani and Kumar 2015). Mutations provide an opportunity to create hitherto unknown alleles, so that the plant breeder does not remain handicapped due to limited allelic variation at one or more gene loci of interest (Goyal and Khan 2010).

Although earlier studies on mutagenesis in blackgram have been studied (Gautam *et al*., 1992; Sharma *et al*., 2005; Kouser *et al*., 2007; Kumar *et al*., 2019; Raina and Khan 2020; Raina *et al*., 2020); Wani (2017, 2021); Amin *et al*. (2019); Goyal *et al*. (2019), yet limited reports are available on induced viable morphological mutations. Morphological mutations play an important role to change the characteristics of any varieties, to build new ideotype for development of new varieties. Mutants produced through induced mutations when used in cross breeding programmes, found more productive in development of improved new varieties (Pawar *et al*., 2000). Induced mutations are extensively used to create genetic variability for developing new mutants with desirable agro-economical characters (Laskar *et al*., 2019).

In Mutation breeding programmes, choice of an effective and efficient mutagen will certainly increases the possibility of creating desired mutations. The lethal dose (LD50) utilized an assumption that lower doses of treatment which effect minimum impacts on the genome and rarely generates phenotypic changes; whereas high doses may produce multiple impacts on the genome which consistently produces aberrations or negative changes (Ariraman *et al*., 2014). Among the chemical mutagens used for inducing mutational studies, Ethyl methane sulfonate has been found highly reactive in inducing mutations and efficient in creating diversity in agronomic traits of food crops including black gram (Gnanamurthy and Dhanavel 2014).

Major protein portion in blackgram is Lysine (Gill *et al.,* 2017). Inheritance of quantitative characters is regulated by interaction of different genes with additive effects on phenotypic variabilities (Laskar *et al*., 2018). The FAO/IAEA Mutant Variety Database (https://mvd.iaea.org accessed on 24 January, 2021) records show that out of total 466 released mutant varieties of legumes, only 9 have been released in urd-bean till date, indicating that the crop is less exploited for mutation breeding (Goyal *et al*., 2019). Hence, sustained efforts are required to create reproducible mutation protocols for the development of novel genes the regulates economically important traits (Laskar and Khan 2017).

So the present research study was performed to evaluate the EMS induced mutations on Seed yield and yield contributing traits like plant height per plant (cm), number of branches per plant, number of leaves per plant, number of clusters per plant, number of seeds per plant, Seed yield (g) per plant, 100 seed weight(g), root length(cm) and root nodules per plant characters were studied in M_3 generations.

MATERIALS AND METHODS

Collection of Seeds. Black gram accession IC-436524 were obtained from NBPGR regional centre Hyderabad and T9 (check) were obtained from ICAR-CRIDA.

A. Treatment of Seeds using EMS

M_1 generation was raised by treating the seeds with EMS prepared at different known concentration *viz*., 0.2%, 0.3%, 0.4%, 0.5% and Control (untreated EMS) along with T9(check). All agronomic practices were strictly followed during preparation of field, sowing and subsequent management of M_1, M_2 and M_3 generations.

B. M1 generation

Data was prepared accordingly and mutants with high range of morphological characters for high yielding in each row of each concentration like plant height per plant (cm), number of branches per plant, number of leaves per plant, number of clusters per plant, number of seeds per plant, Seed yield(g) per plant, 100 seed weight(g), root length(cm) and root nodules per plant were screened and isolated in M_1 generation (raised by inducing EMS). Seeds of M_1 plants were harvested separately and preserved in paper bags for next generation studies (M_2 generation).

C. M_2 generation

Data was prepared accordingly and mutants with high range of morphological characters for high yielding like plant height per plant (cm), number of branches per plant, number of leaves per plant, number of clusters per plant, number of seeds per plant, Seed yield (g) per plant, 100 seed weight(g), root length(cm) and root nodules per plant in each row of each concentration were screened and isolated in M_2 generation (raised by M_1 progeny). Seeds of M_2 plants were harvested separately and preserved in paper bags for next generation studies (M_3 generation). Fifty seeds from each treatment *viz*., 0.2% mutants, 0.3% mutants, 0.4% mutants, 0.5% mutants, Control and T-9 (totally 300 seeds) were sown in field located at Kodad, Suryapet, Telangana to evaluate the seed yield and yield contributing traits.

For raising M_2 generation total 180 seeds of 30 healthy seeds from each treatment were collected from high yielding mutant and control along with T9 and were sown with 10cm × 30 cm distance between plants per row in a field in RBD (Randomized Block Design) with three replications each along with control. Similar agronomical parameters as M_1 generation were practiced to raise M_2 generation. M_2 population was evaluated for agronomic and morphological characters by phenotypical observations which are yield and yield contributing traits Plants which have high quantitative characters with high yielding in each row of each concentration were separated and seeds from those plants were collected and data was prepared based on the yield produced and yield contributing characters. For raising M_3 generation total 300 seeds of 50 healthy seeds from each treatment were collected from high yielding mutant and control along with T9 and were sown with 10cm × 30 cm distance between plants per row in a field in RBD (Randomized Block Design) with three replications each along with control. Similar agronomical parameters as M_1 generation were practiced to raise M_3 generation.

RESULTS AND DISCUSSION

The Analysis of variance (ANOVA) results in M_3 generation revealed that “Significant genotypic differences were found for all ten characters (plant height (cm), number of branches per plant, number of clusters per plant, number of pods per plant, number of seeds per pod, 100-seed” weight (g), seed yield (g/pl), root length (cm), and number of root nodules) evaluated (Table 1.), indicating that there is a great deal of genetic variability among the mutants evaluated. Indicating that the black gram mutant has sufficient diversity as a result of treatments.

Table 1: The Effect of Ems Mutagenesis on the Yield And Yield-Contributing Features of the M_3 Generation of Black Gram Mutants was Analyzed using Analysis of Variance (ANOVA).

Source of Varaiation	df	“MSSQ Characters									
		Pl.Ht(cm)	No.Br/Pl	No.L/Pl	No.Cl/Pl	No.P/Pl	No S/Pl	S.Y(g)/Pl	100 S.Wt(g)	R.L(cm)/Pl	No.R.N/Pl
Replications	4	84.868	0.233	2.805	7.077	47.993	1541.945	2.312	0.314	18.493	932.209
treatments	5	265.668 **	0.784 **	21.481 **	26.742 **	437.704 **	6312.709 **	10.576 **	0.678 **	28.065 **	1500.80**
Mean		45.39	2.453	12.89	11.1	44.234	179.37	6.49	3.165	21.475	135.73
Error		33.813	0.102	3.57	1.93	43.95	473.599	0.549	0.088	4.674	224.835
SEd		3.678	0.202	1.195	0.879	4.193	13.764	0.469	0.187	1.367	9.483
CV%		12.81	13.03	14.66	12.52	14.99	12.13	11.41	9.36	10.07	11.05

*Significant at 0.05% and ** at 0.01 % level, respectively

Table 2: Performance of Quantitative Characters of Blackgram mutants (0.2, 0.3, 0.4 and 0.5% EMS Concentration) in M_3 generation.

0.2% Mutants	Pl.Ht(cm)	No. Br/Pl	No. L/Pl	No. Cl/Pl	No.P/Pl	No S/Pl	S.Y(g)/Pl	100 S.Wt(g)	R.L (cm)/Pl	No. R.N/Pl
I-IC-436524-M_3	62.52	3.1	18.8	12.9	51.3	215.8	8.59655	3.988	26.35	170.6
VI-IC-436524-M_3	61.17	2.9	17.1	9.90	38.2	213.5	8.132092	3.8019	25.10	176.0
V-IC-436524-M_3	56.86	3.0	16.8	12.9	51.2	188.6	7.57849	3.6095	23.65	154.0
III-IC-436524-M_3	45.75	2.3	13.0	9.9	34.0	168.6	6.44038	3.052	20.36	135.9
VIII-IC-436524-M_3	44.24	2.3	13.8	11.3	44.5	146.2	5.75771	2.7615	18.13	115.9
“0.3% Mutants										
IX-IC-436524-M_3	50.91	3.0	14.2	15.00	62	240.6	9.37003	3.5165	22.51	137.8
V-IC-436524-M_3	50.38	2.6	13.1	16.7	67.1	242.9	9.066475	3.3595	22.68	135.7
II-IC-436524-M_3	44.92	2.6	12.4	12.1	48.5	204.2	7.92666	3.025	20.57	126.2
I-IC-436524-M_3	43.23	2.4	10.6	16.5	67.5	181.5	7.00685	2.703	20.32	133.3
III-IC-436524-M_3	41.76	2.1	11.1	13.6	52.9	160.5	6.6287	2.867	20.16	134.9”
“0.4% Mutants										
III-IC-436524-M_3	49.71	2.5	13.8	10.4	48.4	188.8	6.69358	3.5451	23.088	157.5
V-IC-436524-M_3	45.2	2.4	12.3	10.0	38.5	174.2	6.176376	3.1947	21.841	137.1
IV-IC-436524-M_3	48.47	2.4	12.7	10.2	39.1	161.3	5.767579	3.2127	21.231	136.8
VI-IC-436524-M_3	41.88	2.1	10.5	8.9	46.625	145.2	4.9128	2.709	18.146	140.5
II-IC-436524-M_3	28.56	1.5	7.7	5.6	23.4	102.2	3.517176	2.0658	14.574	88.7”
“0.5% Mutants										
IX-IC-436524-M_3	35.05	2	9.9	9.7	33.2	135.1	4.45569	2.641	20.126	125.9
X-IC-436524-M_3	32.75	1.9	10.6	8.3	30.3	129.4	4.36597	2.698	18.796	117.4
V-IC-436524-M_3	33.71	2	11.1	7.4	31.3	123.5	4.22602	2.738	18.856	116.8
VII-IC-436524-M_3	30.58	1.7	9.7	7.1	28.8	111.3	3.78668	2.381	16.139	103.4
VI-IC-436524-M_3	30.74	1.8	9.5	8.6	32.2	104.7	3.61481	2.416	15.499	112
Control	35.49	1.90	10.32	9.44	36.46	130.10	4.35	2.76	18.73	120.29
T-9	36.46	1.88	10.28	8.82	37.70	128.02	4.39	2.67	17.72	122.04

Table 3: Performance of top five Blackgram mutants based on seed yield and yield contributing traits.

Top Mutants	Pl.Ht(cm)	No.Br/Pl	No.L/Pl	No.Cl/Pl	No.P/Pl	No S/Pl	S.Y(g)/Pl	100 S.Wt(g)	R.L(cm)/Pl	No.R.N/Pl
0.2% I-IC-436524-M_3	62.52	3.1	18.8	12.9	51.3	215.8	8.59655	3.988	26.35	170.6
0.3% IX-IC-436524-M_3	50.91	3	14.2	15	62	240.6	9.37003	3.5165	22.51	137.8
III-IC-436524-M_3	49.71	2.5	13.8	10.4	48.4	188.8	6.69358	3.445	23.088	157.5
0.5% V-IC-436524-M_3	33.71	2	11.1	7.4	31.3	123.5	4.22602	2.738	18.856	116.8
Control	35.49	1.90	10.32	9.44	36.46	130.10	4.35	2.76	18.73	120.29
T-9	36.46	1.88	10.28	8.82	37.70	128.02	4.39	2.67	17.72	122.04

Table 4: Mean performance and % increased/decreased over control (untreated) and T-9 for ten quantitative characters in 0.2, 0.3, 0.4 and 0.5% mutant, control (untreated), T-9 of Blackgram in M_3 generation.

Characters	0.2% Mutant	Control(untreated)	T-9	% increased/decreased over control(untreated)	% increased/decreased over T-9
Pl.Ht(cm)	62.52	35.49	36.46	76.16	71.48
No.Br/Pl	3.10	1.90	1.88	63.16	64.89
No.L/Pl	18.80	10.32	10.28	82.17	82.88
No.Cl/Pl	12.90	9.44	8.82	36.65	46.26
No.P/Pl	51.30	36.46	37.70	40.70	36.07
No S/Pl	215.80	130.10	128.02	65.87	68.57
S.Y(g)/Pl	8.60	4.35	4.39	97.62	95.82
100 S.Wt(g)	3.99	2.76	2.67	44.49	49.36
R.L(cm)/Pl	26.35	18.73	17.72	40.68	48.70
No.R.N/Pl	170.60	120.29	122.04	41.82	39.79

Characters	0.3% Mutant	Control(untreated)	T-9	% increased/decreased over control(untreated)	% increased/decreased over T-9
Pl.Ht(cm)	50.91	35.49	36.46	43.45	39.63
No.Br/Pl	3.00	1.90	1.88	57.89	59.57
No.L/Pl	14.20	10.32	10.28	37.60	38.13
No.Cl/Pl	15.00	9.44	8.82	58.90	70.07
No.P/Pl	62.00	36.46	37.70	70.05	64.46
No S/Pl	240.60	130.10	128.02	84.93	87.94
S.Y(g)/Pl	9.37	4.35	4.39	115.40	113.44
100 S.Wt(g)	3.52	2.76	2.67	27.41	31.70
R.L(cm)/Pl	22.51	18.73	17.72	20.18	27.03
No.R.N/Pl	137.80	120.29	122.04	14.56	12.91

Characters	0.4% Mutant	Control(untreated)	T-9	% increased/decreased over control(untreated)	% increased/decreased over T-9
Pl.Ht(cm)	49.71	35.49	36.46	40.07	36.34
No.Br/Pl	2.50	1.90	1.88	31.58	32.98
No.L/Pl	13.80	10.32	10.28	33.72	34.24
No.Cl/Pl	10.40	9.44	8.82	10.17	17.91
No.P/Pl	48.40	36.46	37.70	32.75	28.38
No S/Pl	188.80	130.10	128.02	45.12	47.48
S.Y(g)/Pl	6.69	4.35	4.39	53.88	52.47
100 S.Wt(g)	3.45	2.76	2.67	24.82	29.03
R.L(cm)/Pl	23.09	18.73	17.72	23.27	30.29
No.R.N/Pl	157.50	120.29	122.04	30.93	29.06

Characters	0.5% Mutant	Control(untreated)	T-9	% increased/decreased over control(untreated)	% increased/decreased over T-9
Pl.Ht(cm)	33.71	35.49	36.46	-5.02	-7.54
No.Br/Pl	2.00	1.90	1.88	5.26	6.38
No.L/Pl	11.10	10.32	10.28	7.56	7.98
No.Cl/Pl	7.40	9.44	8.82	-21.61	-16.10
No.P/Pl	31.30	36.46	37.70	-14.15	-16.98
No S/Pl	123.50	130.10	128.02	-5.07	-3.53
S.Y(g)/Pl	4.23	4.35	4.39	-2.85	-3.74
100 S.Wt(g)	2.74	2.76	2.67	-0.80	2.55
R.L(cm)/Pl	18.86	18.73	17.72	0.67	6.41
No.R.N/Pl	116.80	120.29	122.04	-2.90	-4.29

Pl.Ht(cm)-Plant Height, No.Br/Pl-Branches Plant^{-1} , No.L/Pl- Leaves Plant^{-1}, No.Cl/Pl-Clusters Plant^{-1} , No S/Pl- Number of seeds Plant^{-1}No.P/Pl-Pods Plant^{-1}, S.Y(g)/Pl-Seed Yield Plant^{-1}, 100 S.Wt(g)-100 Seed Weight, R.L(cm)/Pl-Root length (cm) Plant^{-1} and No.R.N/Pl-Root Nodules Plant^{-10}"

Fig.1. Blackgram mutants M3 generation field and traits observations

Fig. A. Field observation Fig. B. Variability in plant morphology Fig. C. Variability in mutant leaves leaves
Fig. D. Variability in pod number per plant Fig. E. Variability in cluster number per plant Fig. F. Variability in pod numbers/plant in mutants
Fig. G. Observed seven pods per clusters/ plant in 0.2% Fig. H. Observed seven pods size in mutants Fig. I. Observed increased seeds per pods in mutants

The character plant height (cm) recorded mean value in 0.2% mutant was 62.52, 35.49 in control (untreated) and 36.46 in T-9 (check). The % increase calculated for the character plant height recorded was 76.16 over control (untreated) and 71.48 over T-9 (check). The character number of branches per plant recorded mean value in 0.2% mutant was 3.10, 1.90 in control (untreated) and 1.88 in T-9 (check). The % increase calculated for the character number of branches per plant recorded was 63.16 over control (untreated) and 64.89 over T-9 (check). The character number of leaves per plant recorded mean value in 0.2% mutant was 18.80, 10.32 in control (untreated) and 10.28 in T-9 (check). The % increase calculated for the character number of leaves per plant recorded was 82.17 over control (untreated) and 82.88 over T-9 (check). The character number of clusters per plant recorded mean value in 0.2% mutant was 12.90, 9.44 in control (untreated) and 8.82 in T-9 (check). The % increase calculated for the character number of clusters per plant recorded was 36.65 over control (untreated) and 46.26 over T-9 (check). Significant increment of clusters per plant was also reported earlier by Kumar *et al*. (2009) in black gram; Hakande, (1992) in chickpea. The character number of pods per plant recorded mean value in 0.2% mutant was 51.30, 36.46 in control (untreated) and 37.70 in T-9 (check). The % increase calculated for the character number of pods per plant recorded was 40.70 over control (untreated) and 36.07 over T-9 (check). Our results improvement of pod number per plant were more at lower dose of EMS, meets with earlier reports by Ponaganti *et al*. (2022) in mustard; Raghavendra *et al*. (2021) in sorghum; Ajaz *et al*. (2008); Basu *et al.* (2008) in blackgram; Patil (2009) in cowpea; mung bean by Auti *et al*. (2007), and urd bean by Singh *et al*. (2000). The character number of seeds per plant recorded mean value in 0.2% mutant was 215.80, 130.10 in control (untreated) and 128.02 in T-9 (check). The % increase calculated for the character number of seeds per plant recorded was 65.87 over control (untreated) and 68.57 over T-9 (check). The character seed yield (g/pl) recorded mean value in 0.2% mutant was 8.60, 4.35 in control (untreated) and 4.39 in T-9 (check). The % increase calculated for the character Seed yield (g/pl) recorded was 97.62 over control (untreated) and 95.82 over T-9 (check). Similar yield improvement under induced mutagenic treatment in blackgram was also reported earlier by Tamilzhzrasi *et al.* (2021); Selvam and Elangaimannan (2010); Singh *et al*. (2016); Longnathan *et al*. (2000); Das and Chakraborty (1998); Mehandi *et al*. (2013); Chand (2001). The character 100 seed weight (g) recorded mean value in 0.2% mutant was 3.99, 2.76 in control (untreated) and 2.67 in T-9 (check). The % increase calculated for the character 100 Seed weight (g) recorded was 44.49 over control (untreated) and 49.36 over T-9 (check) (Sinha *et al.*, 2018; Murugan and Nadarajan 2006; Thangavel and Thirugnanakumar 2011)’’. The character root length (cm) recorded mean value in 0.2% mutant was 26.35, 18.73 in control (untreated) and 17.72 in T-9 (check). The % increase calculated for the character root length (cm) recorded was 40.68 over control (untreated) and 48.70 over T-9 (check). The character number of root nodules per plant recorded mean value in 0.2% mutant was 170.60, 120.29 in control (untreated) and 122.04 in T-9 (check). The % increase calculated for the character number of root nodules per plant recorded was 41.82 over control (untreated) and 39.79 over T-9 (check). The character plant height recorded mean value in 0.3% mutant was 50.91, 35.49 in control (untreated) and 36.46 in T-9 (check). The % increase calculated for the character plant height (cm) recorded was 43.45 over control (untreated) and 39.63 over T-9 (check). The character number of branches per plant recorded mean value in 0.3% mutant was 3.00, 1.90 in control (untreated) and 1.88 in T-9 (check). The % increase calculated for the character number of branches per plant recorded was 57.89 over control (untreated) and 59.57 over T-9 (check). The character number of leaves per plant recorded mean value in 0.3% mutant was 14.20, 10.32 in control (untreated) and 10.28 in T-9 (check). The % increase calculated for the character number of leaves per plant recorded was 37.60 over control (untreated) and 38.13 over T-9 (check).The character number of clusters per plant recorded mean value in 0.3% mutant was 15.00, 9.44 in control (untreated) and 8.82 in T-9 (check). Our results meets with Tamilzhzrasi *et al*. (2021); Pathak *et al*. (2017); Gadakh *et al*. (2013); Khan *et al.* (2004). The % increase calculated for the character number of clusters per plant recorded was 58.90 over control (untreated) and 70.07 over T-9 (check). The character number of pods per plant recorded mean value in 0.3% mutant was 62.00, 36.46 in control (untreated) and 37.70 in T-9 (check). The % increase calculated for the character number of pods per plant recorded was 70.05 over control (untreated) and 64.46 over T-9 (check). Many authors have contributed to this field, including Ponaganti *et al*. (2022) in mustard; Raghavendra *et al.* (2021) in sorghum; Singh *et al.* (2006); Panigrahi *et al.* (2015); Wani. (2006) in blackgram. The character number of seeds per plant recorded means value in 0.3% mutant was 240.60, 130.10 in control (untreated) and 128.02 in T-9 (check). The % increase calculated for the character number of seeds per plant recorded was 84.93 over control (untreated) and 87.94 over T-9 (check). The character seed yield (g/pl) recorded mean value in 0.3% mutant was 9.37, 4.35 in control (untreated) and 4.39 in T-9 (check). The % increase calculated for the character seed yield (g/pl) recorded was 115.40 over control (untreated) and 113.44 over T-9 (check). Similar findings were reported earlier by Tamilzhzrasi *et al.* (2021); Bhattu *et al.* (2022); Wani *et al*. (2008); (Makeen *et al*., 2007; Sharma and Ahmed 1997). The character 100 seed weight (g) recorded mean value in 0.3% mutant was 3.52, 2.76 in control (untreated) and 2.67 in T-9 (check). The % increase calculated for the character 100 Seed weight (g) recorded was 27.41 over control (untreated) and 31.70 over T-9 (check). The character root length (cm) recorded mean value in 0.3% mutant was 22.51, 18.73 in control (untreated) and 17.72 in T-9 (check). The % increase calculated for the character root length (cm) recorded was 20.18 over control (untreated) and 27.03 over T-9 (check). The character number of root nodules per plant recorded mean value in 0.3% mutant was 137.80, 120.29 in control (untreated) and 122.04 in T-9 (check). The % increase calculated for the character number of root nodules per plant recorded was 14.56 over control (untreated) and 12.91 over T-9 (check).

The character plant height recorded mean value in 0.4% mutant was 49.71, 35.49 in control (untreated) and 36.46 in T-9 (check). The % increase calculated for the character plant height recorded was 40.07 over control (untreated) and 36.34 over T-9 (check). The character number of branches per plant recorded mean value in 0.4% mutant was 2.50, 1.90 in control (untreated) and 1.88 in T-9 (check). There was a 31.58-percent increase in the reported character-level number of branches per plant compared to the control group (untreated) and 32.98 over T-9 (check). The character number of leaves per plant recorded mean value in 0.4% mutant was 13.80, 10.32 in control (untreated) and 10.28 in T-9 (check). The estimated percentage increase in character leaves per plant was 33.72 over control (untreated) and 34.24 over T-9 (check). The character number of clusters per plant recorded means value in 0.4% mutant was 10.40, 9.44 in control (untreated) and 8.82 in T-9 (check). According to the research of Pathak *et al*. (2017); Khan *et al.* (2004); Gadakh *et al*. (2013) the trait clusters per plant was observed positive results at medium EMS doses. Number of clusters per plant reported a % increase of 10.17 from the control (untreated) and 17.91 from T-9. There were 48.40 pods per plant on average in the 0.4% mutant, 36.46 in the control (untreated), and 37.70 in the T-9 plant (check). Character number of pods per plant increased by 32.75 percent over control (untreated) and by 28.38 percent over T-9 (check). Mean values for character counts of seeds per plant were 188.80 in a 0.4% mutant, 130.10 in a control (untreated), and 128.02 in T-9 (check). The % increase calculated for the character number of seeds per plant recorded was 45.12 over control (untreated) and 47.48 over T-9 (check). The character seed yield (g/pl) recorded mean value in 0.4% mutant was 6.69, 4.35 in control (untreated) and 4.39 in T-9 (check). The % increase calculated for the character Seed yield (g/pl) recorded was 53.88 over control (untreated) and 52.47 over T-9 (check). Significant increment for the trait seed yield at higher mutagenic doses was observed earlier by Raghavendra *et al*. (2021) in sorghum; Parveen *et al*. (2012); Khattak *et al*. (2001); Gupta (2005); Suguna *et al*. (2017); Umaharan *et al*. (1997); Sinha *et al*. (2018); Manivannan (1999). The character 100 seed weight (g) recorded mean value in 0.4% mutant was 3.45, 2.76 in control (untreated) and 2.67 in T-9 (check). The % increase calculated for the character

100 Seed weight (g) recorded was 24.82 over control (untreated) and 29.03 over T-9 (check). The character root length (cm) recorded mean value in 0.4% mutant was 23.09, 18.73 in control (untreated) and 17.72 in T-9 (check). The % increase calculated for the character root length (cm) recorded was 23.27 over control (untreated) and 30.29 over T-9 (check). The character number of root nodules per plant recorded mean value in 0.4% mutant was 157.50, 120.29 in control (untreated) and 122.04 in T-9 (check). The % increase calculated for the character number of root nodules per plant recorded was 30.93 over control (untreated) and 29.06 over T-9 (check).

The character plant height (cm) recorded mean value in 0.5% mutant was 33.71, 35.49 in control (untreated) and 36.46 in T-9 (check). The % decrease calculated for the character plant height recorded was -5.02 over control(untreated) and -7.54 over T-9 (check). The character number of branches per plant recorded mean value in 0.5% mutant was 2.00, 1.90 in control (untreated) and 1.88 in T-9 (check). Number of individual plant branches increased by 5.26 percent as compared to the control group (untreated) and 6.38 over T-9 (check). The character number of leaves per plant recorded means value in 0.5% mutant was 11.10, 10.32 in control (untreated) and 10.28 in T-9 (check). "The % increase calculated for the character number of leaves per plant recorded was 7.56 over control (untreated)" and 7.98 over T-9 (check). The character number of clusters per plant recorded mean value in 0.5% mutant was 7.40, 9.44 in control (untreated) and 8.82 in T-9 (check). Character number clusters per plant showed a % reduction of -21.61 when compared to the control(untreated) and -16.10 when compared to T-9 (check). The 0.5% mutant had a mean pod count of 31.30, 36.46 for controls and 37.70 for T-9 (check). The % decrease calculated for the character number of pods per plant recorded was -14.15 over control (untreated) and -16.98 over T-9 (check). The decreased number of pods per plant at higher doses of mutagen was observed earlier by Barshile, (2006) in chickpea and Sagade (2008) in urdbean. The character number of seeds per plant recorded means value in 0.5% mutant was 123.50, 130.10 in control (untreated) and 128.02 in T-9 (check). The % decrease calculated for the character number of seeds per plant recorded was -5.07 over control (untreated) and -3.53 over T-9 (check). The character seed yield (g/pl) recorded mean value in 0.5% mutant was 4.23, 4.35 in control (untreated) and 4.39 in T-9 (check). The % decrease calculated for the character seed yield (g/pl) recorded was -2.85 over control (untreated) and -3.74 over T-9 (check). The character 100 seed weight (g) recorded mean value in 0.5% mutant was 2.74, 2.76 in control (untreated) and 2.67 in T-9 (check). The % decrease calculated for the character 100 seed weight (g) recorded was -0.80 over control (untreated) and increased (2.55) over T-9 (check). The character root length (cm) recorded mean value in 0.5% mutant was 18.86, 18.73 in control (untreated) and 17.72 in T-9 (check). The % increase calculated for the character root length (cm) recorded was 0.67 over control (untreated) and 6.41 over T-9 (check). The character number of root nodules per plant recorded mean value in 0.5% mutant was 116.80, 120.29 in control (untreated) and 122.04 in T-9 (check). The % decrease calculated for the character number of root nodules per plant recorded was -2.90 over control (untreated) and -4.29 over T-9 (check). The reduction of different quantitative traits used higher dose of mutagens were reported earlier by Raghavendra *et al.* (2021) in sorghum; Pathak *et al.* (2017); Thomas and Sreekumar (2001); Singh *et al*. (1998); Aher *et al*. (2001); Suguna *et al*. (2017); Thangavel and Thirugnanakumar (2011); Srinives *et al*. (1991); Wani, (2006). The overall results revealed that top five mutants based on seed yield (0.2%) I-IC-436524-M3 (8.59g/pl), (0.3%) IX-IC-436524-M_3 (9.370g/pl), (0.4%) III-IC-436524-M_3 (6.693g/pl) and (0.5%) V-IC-436524-M_3 (4.45g/pl) in M_3 Generation were identified and also observed with six pods per cluster, big trifoliate green leaves, average plant height was observed was upto 70cm, 8-10 seeds per pod, black and healthy seeds are abundant per plant, more branches with tendril type and more clusters/plant have been observed in M_3 generation.

CONCLUSIONS

Among top five mutants, two mutants from 0.2% and 0.3% EMS *viz.*, I-IC-436524-M3 and IX-IC-436524-M3 showed highest seed yield (g/pl). The % increase calculated for the character seed yield (g/pl) of I-IC-436524-M3 (0.2%) recorded was 97.62 over control (untreated) and 95.82 % over T-9 (check). The % increase calculated for the character seed yield (g/pl) of IX-IC-436524-M3 (0.3%) recorded was 115.40% over control (untreated) and 113.44% over T-9 (check) in M3 generation. Hence these mutants could be utilized for further crop improvement programme in blackgram.

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