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Evaluation of Seed Yield and its Contributing Traits in Blackgram (Vigna Mungo L. Hepper) Mutants Raised during M2 Generation

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ABSTRACT:

Identification of plants with higher seed yield and yield contributing characters are the major aspects in crop development programmes.For this purpose a research was carried out by using EMS for inducing mutations in blackgram accession IC- 436524.This experiment was performed by treating the blackgram seeds atdifferent concentrations of EMS viz; 0.2, 0.3, 0.4, 0.5 percent and control(EMS untreated) and T-9(as check) and grown to develop M1 generation seeds.Seeds ofblackgram mutantsfrom each treated mutants from each concentration collected and raised for M2 generation to determine the mutations inherited from M1 generation mutants to its next generation. The observation was recorded for higher seed yield and its contributing characters from M2 generation mutants.Our results demonstrated a large amount of genetic diversity among the black gram mutants due to the EMS treatments for all characteristics during M1 and M2 generation. ANOVA data also revealed there was a significant variability among the treated mutants in studied characters. From the data it was revealed that high yielding blackgram mutants of M1 generation has showed similar trend in higher yielding during M2 generation also.Compared with other treated mutants0.2% treated mutants had higher yield and more significant variability were observed in other selected characters.According to the findings as a whole, the chosen black gram mutant that was grown in an environment with 0.2% EMS concentration demonstrated a significantly better seed output when compared from other mutants and the untreated black gram genotype and T9. It's possible that the mutant will prove valuable in the process of breeding higher-yielding genotypes for future agricultural improvements and for the benefit of farmers.

Keywords: Blackgram, M2 generation, mutants, Seed yield.

INTRODUCTION:

Pulses are knownas “Poor Man’s Meat” and as theyare the sources of vegetable protein containing 21-25 percent. Pulses providenutritive and edible food values and also comprises animportant wellspringof cereals, cookies and other diets in Asia (Kumar et al., 2013). Pulses are potential source of edible protein with an essential aminoacid profilemost probably lysine (Mubarak,2005). Pulses also contains unsaturated fattyacids, whichare beneficial for the human health (Botinestean et al 2012) .Planting in marginal land, indeterminate growth habits, low portioning efficiency, low yield potential ,canopy design, other abiotic &biotic stresses decreases the blackgram yield. The main limiting factors for increasing the higher yields in blackgram are lack of a narrow geneticbase, exploitable genetic variabilityand unavailability of improved seed quality(Kumar et al., 2017).The success of any crop improvement program depends on the strength of variability and diversity. The difference of variation inthe heritable parameters (characters) is important for breeders in crop development programs (Sarkar et al., 2016). Plant growers(breeders)develop variability to select elite genotypes in crop development programs (Sarkar et al., 2014, 2015,Latha et al., 2018). Several papers describing the morphological traits in terms of variability anddiversity were reported, such as agro-economic traits (Hasan et al., 2020), minerals (Sarkar et al.,2020), grain yield(Hasan-Ud-Daula et al.,2020), pigments (Sarkar et al., 2021), proximate compositions, flavonoids content, vitamins (Chakrabarty et al., 2018), phenolics.So, there is an emergencysupport to increase thefood production and productivity and also enchances the nutritional benefits, which improves the crop yield potential of presentgenotypes by modifying their plant characterstics. Finally, plant growers(breeders)need tocreate heterosis for improving the superior plants (Srivastava and Singh, 2013).

Blackgram*(Vigna mungo*(L.) Hepper) is ashort-term crop with great adaptability, less inputs, and the capacity to enhance the “soil fixation” through atmospheric nitrogen(N2). Black gram is native of South Asia, which is often referred to as “urad dal”. This pulse crop is included in a variety of stews, soups, and fermented meals throughout South Asian cuisine. It is chock full of important nutrients, such as protein, minerals, vitamins, dietary fiber, and antioxidants, all of which are useful to the body in a variety of different ways. It is a self-pollinating legume belongs to Leguminacae family, namely the genus *Vigna* and the subgenus Ceratotropis. Blackgram crop has minimal genetic variability (Kumar and Mishra, 2007). As a result, the creation of novel plant types that are suitable for various agro climatic areas is necessary. The absence of high-yielding cultivars was the primary barrier to progress in India's efforts to increase the production of black gram .Blackgram mostly suited for cultivation under different agro-climatic conditions and plays as an important edible proteinsource of many people in India.Blackgram isrich among the pulsesbecause of its highly digestible nature. Cultivation of blackgram has decreased since the last decade and hence, the difference between demand & supply is increased (Imran et al., 2015). As blackgramis a nodulation plant degree of nodulations differs from area to area based on soil and environmental conditions. The most important seed yield and its attributes in blackgram are generally branches per plant, clusters per plant, leaves per plant, pods per plant, seeds per pod, root nodules per plant and test weight etc. Any small changes(improvement) in these traits by implying the proper breeding methods, selection methods in hetrozygotic populations.

The important technique to significantly increase the amount of genetic variability in a population in a shorter period is by inducing mutations in the population artificially (Laskar et al., 2018; Wani et al., 2018,2021; Raina et al., 2016, 2019;). The process of mutagenesis has been utilized extensively for the purpose of increasing crop variety. Ethyl methanesulfonate (EMS) mutagenesis has been shown to be an extremely useful tool in the field of biological study. EMS is the important and significant chemical mutagen, which induces"point mutations". EMS causes mutations randomly in the genetic material (DNA) by nucleotide(nitrogen base, Sugar and Phosphate) replacement. In particular, it causes guanine alkylation (Ausubel, & Borowsky, 2014). The EMS doses are between 0.01 to 0.8%. There are very slight variations in the dose in the EMS treatment to be employed from species to species. The largest numbers of mutations are produced with the least amount of risk at the mutagen's optimal dose. Overdosing of mutagen causes and results in death of plants , whereas lower treatments will result in few mutations. On the other hand, lower frequencies may provide an advantage in that they result in a smaller number of unfavorable changes along with favorable desired mutations.

MATERIALS AND METHODS:

The research study was experimented at farm of Genetics department, Osmania-University,Hyderabadduring *Rabi* season, mid-October, 2021.

Collection of seeds

Black gram seeds of variety IC-436524 were obtained from NBPGR regional centre Hyderabad.

Processing of seeds

This research selected 90 healthy and uniform seeds and soaked in water for duration of 3 to 4 hours. The soaked seeds were cleaned with a tissue paper and dried.

Inducing of mutation

Black gram seeds of a healthy and uniform size were used in the EMS mutagen treatment. Before the treatment, surface sterilization of seeds with a 0.1% $HgCl_2$ (mercuric chloride) solution for about one minute was performed. The seeds were washedproperly and imbibed for five hours in 'distilled water'. The concentration of EMS (Ethyl Methane Sulphonate) mutagen has been prepared in the concentration between 0.2%-0.5percentas per mutagenesis protocol. Under each concentration of EMS, fifteen seeds at each concentration were soaked for 6 hours under rotary shaker at 180rpm in a room temperature of 27±1°C. For uniform and efficient absorption of EMS, it must be noted that the volume of EMS solution must be at the proportion of 10 X of seed volume.

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Control

The untreated 15 seeds were kept as control and T9(check).The untreated seeds,T9 seeds and EMS treated seeds sown in the Randomized Block Design(RBD) field. The sowing in field has been processed with three kinds of replication with the distance of 10cm x 30 cm between the rows and plants respectively.

M2 Generation

M_1 population was evaluated for agronomic and morphological characters by phenotypical observations which are yield and yield contributing characters during *Kharif* season, mid June, 2021.Plants with high quantitative characters with high yielding in each row of each concentration were separated and seeds from those plants were collected. The mean performance data was prepared based on the yield produced and yield contributing characters.

For raising M_2 generation total 120 seeds of 20 healthy seeds from each treatment were collected from high yielding mutant and control along with T9 and were sown with 10cm x 30 cm distance between plants per row in pots in RBD with three replications along with control and T-9(as check).

The following parameters were measured and recorded

Plant height(cm), number of leaves per plant number of branches per plant, seed yield per plant (gm) , number of pods per plant, number of clusters per plant, , number of seeds per plant, 100 seed weight (gm),number of root nodules per plant, root length(cm),

RESULTS AND DISCUSSION:

The Analysis Of Variance(ANOVA) was performed to analyze the significant of the difference in the data in M_2 generation by using STAR 1.0 software. The ANOVA data results for Plant Height, Branches per plant, Leaves per plant, Clusters per plant, Pods per plant, Seed Yield per plant were observed highly significant ($p<0.01$) except 100 Seed Weight(g), root Length per plant and root nodules per plant. The analysis of variance results suggest that there is a significant improvement in all traits after treatment of the seeds with EMS (Table 1). It is evident from this that there is a significant degree of heterogeneity among the black gram mutant as a direct result of the treatments.EMS induced mutations for higher seed yield inherited to next generation(M2 generation).

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Table1. ANOVA of black gram mutants for seed yield and its contributing characters.

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	9	34.464	0.477	4.479	1.483	40.684	765.957	0.97	0.423	12.938	789.594
Treatments	5	108.157 **	1.907 **	24.546 **	20.588 **	250.97 **	5980.79 **	12.84 **	0.387 NS	13.077 NS	48.164 NS
Mean		33.19	2.639	12.567	6.245	26.705	123.472	4.135	2.974	19.489	119.322
Error		36.247	0.345	7.458	2.152	40.131	781.797	0.88	0.266	14.329	858.467
SEd		2.692	0.263	1.221	0.656	2.833	12.504	0.42	0.231	1.693	13.103
CV%		18.14	22.27	21.73	23.49	23.72	22.65	22.69	17.35	19.42	24.56

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

Pl.Ht(cm)-Plant Height, No.Br/Pl-Branches Per plant , No.L/Pl- Leaves Per plant, No.Cl/Pl-Clusters Per plant, No S/Pl- Number of seeds Per plant No.P/Pl-Pods Per plant, S.Y(g)/Pl-Seed Yield Per plant, 100 S.Wt(g)-100 Seed Weight, R.L(cm)/Pl-Root Length Per plant and No.R.N/Pl-Root Nodules Per plant

Plant height (cm)

The character plant height (cm) was ranged from 28.75-38.27. Highest Plant height was observed in Control- IC-436524 by 38.27 cm followed by T9 by 35.11 cm, 0.2% mutant (33.53 cm), 0.3% mutant (32.25 cm), 0.4% mutant(31.23 cm) and lowest plant height was observed in 0.5% mutant by 28.75 cm.

Number of branches per plant

Highest number of branches per plant was observed in Control- IC-436524 by 3.4 followed by T9 by 2.8, 0.2% mutant (2.7), 0.3% mutant (2.4), 0.4% mutant by 2.2. Lowest number of branches per plant was observed in 0.5% mutant by 2.2 and the character number of branches per plant was ranged from 2.8-3.4.

Number of leaves per plant

Lowest number of leaves per plant was observed in 0.4% mutant by 10.87 and was ranged from 10.87-14.80. Highest number of leaves per plant was observed in T9 by 14.80 followed by Control-IC-436524 by 13.80, 0.2% mutant (13.00), 0.3% mutant (11.83), and 0.5% mutant by 11.10.

Number of clusters per plant

Highest number of clusters per plant was observed in 0.3% mutant IC-436524 by 7.93 followed by 0.2% mutant (7.67), 0.4% mutant (6.5), Control-IC-436524 by 5.7 and 0.5% mutant by 5.57. The character number of clusters per plant was ranged from 4.10-7.93. Lowest number of clusters per plant was observed in T9 plant by 4.10.

Number of pods per plant

Number of pods per plant were ranged from 21.6-33.30. Highest number of pods per plant was observed in 0.2% mutant by 33.30 followed by 0.3% mutant (32.53), 0.4% mutant (25.9), followed by Control-IC-436524 (23.7) 05% mutant (23.2). Lowest number of pods per plant was observed in T9 by 21.60.

Number of seeds per plant

The results showed that the trait number of seeds per plant was observed highest in 0.3% mutant (156.67), followed by 0.2% mutant by 151.93, Control IC-436524 by 114.8, T9 plant by 110.1, 0.4% mutant (108.1).The character number of seeds per plant was ranged from 99.2-156.67. Lowest number of seeds per plant was observed in 0.5% mutant by 99.2.

100 seed weight (g)

100 seed weight (g) was ranged from 2.71g-3.19g. Highest 100 seed weight was observed in 0.2% mutant (3.19g) followed by 0.3% mutant and T9 by 3.1g, Control-IC-436524 by 2.98g, 0.4% mutant (2.76g). Lowest 100 seed weight was observed in 0.5% mutant by 2.71g.

Seed yield (g/pl)

Highest seed yield (g/pl) was observed in 0.2% mutant IC-436524 by 5.15g/pl followed by 0.3% mutant by 5.02g/pl, Control-IC-436524 by 3.42 followed by T9 by 3.38 followed by 0.4% mutant (3.2) and was ranged from 2.87g/pl-5.15g/pl. Lowest seed yield(g/pl) was observed in 0.5% mutant by 2.87g/pl.

Root length (cm)

The trait root length (cm) was ranged from 21.15cm-18.16cm. Highest root length was observed in T9 by 21.15 cm followed by Control IC-436524 by 20.31 cm, 0.2% mutant (19.75cm), 0.3% mutant (19.15cm), followed by 0.4% mutant by 18.41cm. Lowest root length was observed in 0.5% mutant IC-436524 by 18.16 cm.

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Table.2. Mean values of quantitative characters in M2 generation of black gram mutants under EMS treatments.

	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% Mutants	33.53	2.70	13.00	7.67	33.30	151.93	5.15	3.19	19.75	120.87
0.3% Mutants	32.25	2.43	11.83	7.93	32.53	156.67	5.02	3.10	19.15	122.83
0.4% Mutants	31.23	2.27	10.87	6.50	25.90	108.13	3.20	2.76	18.41	117.87
0.5% Mutants	28.75	2.23	11.10	5.57	23.20	99.20	2.87	2.71	18.16	116.77
Control	38.27	3.40	13.80	5.70	23.70	114.80	3.42	2.98	20.31	118.50
T-9	35.11	2.80	14.80	4.10	21.60	110.10	3.38	3.10	21.15	119.10

Pl.Ht(cm)-Plant Height, No.Br/Pl-Branches Per plant , No.L/Pl- Leaves Per plant, No.Cl/Pl-Clusters Per plant, No S/Pl- Number of seeds Per plant No.P/Pl-Pods Per plant, S.Y(g)/Pl-Seed Yield Per plant, 100 S.Wt(g)-100 Seed Weight, R.L(cm)/Pl-Root Length Per plant and No.R.N/Pl-Root Nodules Per plant

NUMBER OF ROOT NODULES PER PLANT:

Highest root nodules per plant was observed in 0.3 % mutant IC-436524 by 122.83 followed by 0.2% mutant by 120.87, T9 (119.10), Control-IC-436524 by 118.50, 0.4% mutant by 117.87 respectively. Lowest root nodules per plant was observed in 0.5% mutantby 116.77.

From the results it was revealed that there was a significant increase in mean values among the traits under study viz.,plant height,pods per plant,seed yield,number of branches per plant during M2 generation.Similar reports were reported earlier by Goyal et al., 2021 in blackgram, Khursheed et al 2018 in faba bean,Laskar et al., 2018 in lentil and Raina et al., 2020 in cow pea.Such a increase in seed yield and its contributing traits may be due to induced muatagen for increased mititic divisions,physiological alterations,more interactions of genes with mutagens,biochemical and metabolic pathways(Goyal et al., 2021). For an effective selection, fixation of desirable mutation and subsequent stability of mutant character is imperative in mutational breeding.Similar reports for increase in seed yield in blackgram by inducing EMS has been reported by Ramya et al.(2014), Mohan Dadarwal and Mathur,(2015).Many researchers reported to enhance the yield and its morphological characters in pulses(Wani et al.,2021;Laskar et al., 2019; Raina et al., 2018; Amin, et al., 2015; Goyal et al., 2019; Raina, & Khan, 2019; Tantray et al., 2017).

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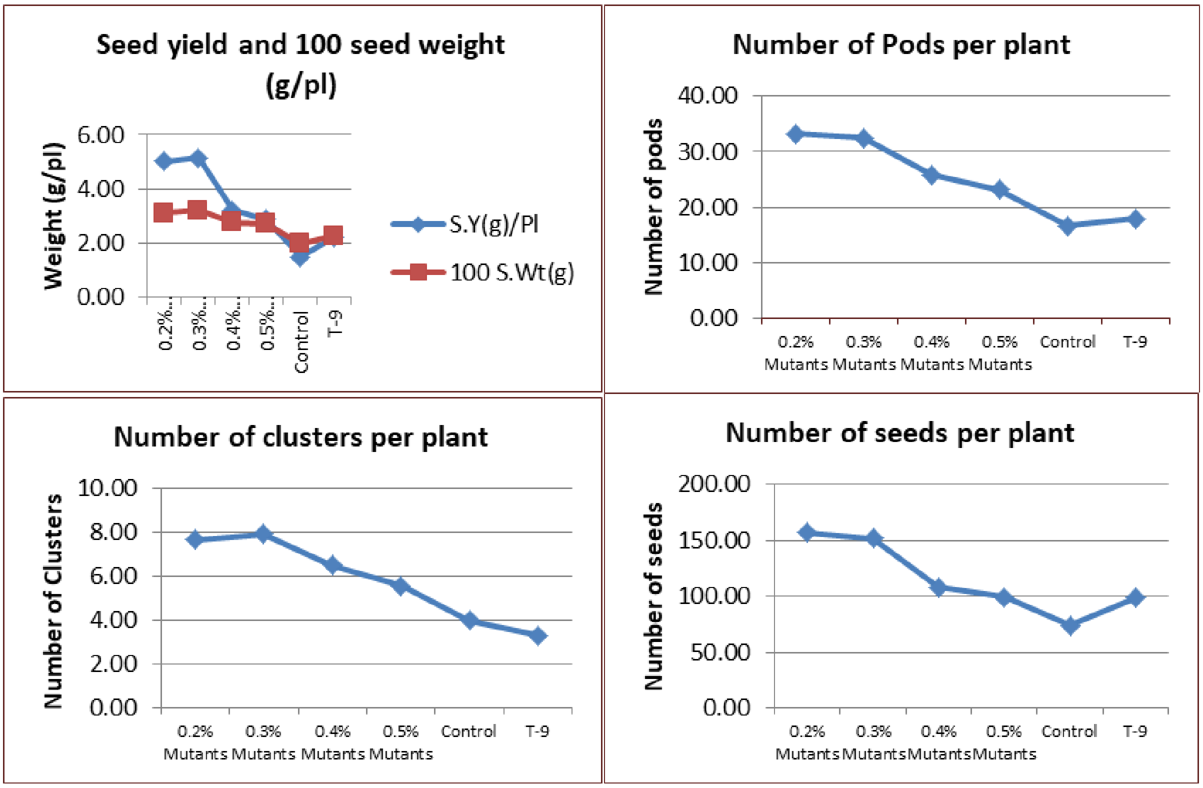
Fig1. Graphical representation of quantitative traits in M2 blackgram generation.



CONCLUSION:

Seed yield is a complex character representing a multiplicative end product of many yield attributes. However, understanding the genetics and inheritance that underlies yield and its component characters pose a prerequisite to attain the actual yield potential of any crop species. The results can be concluded that 0.2% and 0.3% EMS induced mutants which were showed higher seed yield and yield attributes may be advanced to next generation for improving the seed yield in blackgram.

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