

Mutagenic Sensitivity in Early Generation in Black Gram (*Vigna mungo* L. Hepper).

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Abstract: The investigation was conducted with the aim of artificial induction of mutation by sodium azide (SA) to overcome the limitation of variability in black gram and to induce specific improvement without disturbing their better attributes. Seeds of two varieties of urdbean viz. KU92-1 and KU300 were treated with SA of different concentrations (0.5%, 1.0%, 1.5%) to determine their mutagenic sensitivity in terms of seed germination, seedling height, number of branches/plant, number of leaves/plant, root length, root length number/plant etc. One part of the mutagen treated seeds as well as the control of both the varieties were sown in plastic petri dishes and grown under room temperature and other part was planted in the experimental field following randomized block design (RBD) layout to raise first mutant generation (M_1). With increase in the dose of SA, germination percentage, seedling growth and number of leaves/plant etc. were decreased. Both the parental varieties differed significantly between themselves for both percentage seed germination, seedling height and most of the characters at seedling stage for SA treatments. KU300 were highly sensitive and did not germinate in all treatments under field conditions. Variances among treatments were also significantly high.

Key words: Urdbean, sodium azide, mutagen, random block design, mutant generation

I. Introduction

Black gram (*Vigna mungo* (L.) Hepper) is an erect, fast-growing annual, herbaceous legume reaching 30-100 cm in height. It has a well-developed taproot and its stems are diffusely branched from the base. It is valued for its high digestibility and freedom from flatulence effect [1]. It is a summer pulse crop with short duration (90–120) days and high nutritive value [2]. Black gram is used for human food, green manure, a cover crop, forage, silage, hay and chicken pasture [3]. Although, India has the distinction of being the world's single largest producer of pulses, the difference in production and population ratio is significant. The increase in population has pushed up demand of pulses while the fall in availability has pushed up their prices. Although a large area is under different pulse crops including urdbean, their production is more or less stagnant for the last four decades [4].

Most of the traits of a plant breeder's interests are quantitative traits, which are controlled by polygenic interactions. A series of experiments carried out with various crops have established that chemical mutagens induce polygenic variability [5-8]. Limited variability is available for its improvement programmes in a self pollinated crop like urdbean, Variability in base population is essential for breeding for more complex traits. Induced mutagenesis thus seems to be an ideal methodology for the induction of desirable genetic variability. Mutation breeding is one of the conventional breeding methods in plant breeding. It is relevant with various fields like, morphology, cytogenetic, biotechnology, and molecular biology etc. Mutation breeding has become increasingly popular in recent times as an effective tool for crop improvement [9] and an efficient means supplementing existing germplasm for cultivar improvement in breeding program's [10]. It was reported that mutagens may cause genetic changes in an organism, break the linkages and produce many new promising traits for the improvement of crop plants [11]. Mutagenesis has been widely used as a potent method of enhancing variability for crop improvement. By varying the mutagenic agent dose, the frequency and saturation of mutations can be regulated [12] and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions [13].

Chemical mutagenesis is an efficient tool used in mutation breeding programmes for improving various vital characteristics in crops. Various chemicals viz. sodium azide, hydrazine hydrate, maleic hydrazide, ethyl methane sulphonate etc. are in use to induce mutation. It can cause allelic mutations, small deletions and other chromosomal rearrangements. These mutations can be used to activate morphometric and reproductive changes in plants; further selection of mutant plants through a number of generations, resulting in introduction of new traits into a treated population [9]. Sodium azide is highly used with seeds to create mutation [14]. The mutagenicity is mediated through the production of an organic metabolite of azide compound [15]. Keeping in view the above facts, the present investigation was undertaken with the objectives: to induce variability and study the effect of SA in seed germination, seedling growth and some other agronomic traits under laboratory and field conditions.

II. Materials and Methods

Uniform healthy seeds of two varieties of black gram (*Vigna mungo* (L.) Hepper) viz., KU92-1 and KU300 were employed to develop the M₁ generation. The seeds were soaked in distilled water for 12 hours and then treated with three different concentrations of sodium azide (0.5%, 1.0%, and 1.5%) for another 12 hours. After 12 hours the treated seeds were left in running tap water for an hour to remove the residual effect of the mutagen sticking to the seed coat. One set of seeds was kept untreated in distilled water to act as control. Treated seeds from both the varieties were finally grown on plastic pots along with both the controls under room temperature to study seed germination, seedling height and root growth. One part of the treated seeds along with the control of both the varieties was planted in the field in randomized block design keeping 20cm seed to seed distance and 30cm row to row distance in two replicates to observe seed germination, seedling height, plant height, number of leaves/plant and number of branch/plant in M₁ generation.

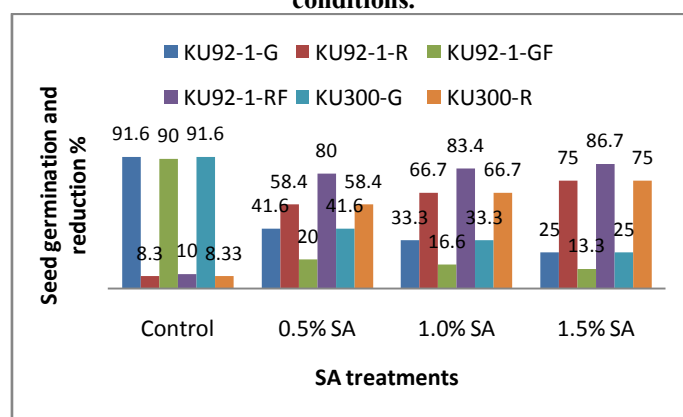
- 1.1. **Evaluation of seed germination:** Seed germination was evaluated under laboratory as well as field conditions. Evaluation was done after one week from day of sowing by counting the number of seedling emerged.
- 1.2. **Seedling height:** Seedling height was observed under laboratory as well as field conditions. The observation was done on 10th day from the day of sowing. The data were compared to that of the control to measure the effect of mutagen under different concentration.
- 1.3. **Root growth:** The seedlings planted in plastic pots in the laboratory were uprooted. It was done by giving water in excess and slowly loosening the soil then pull up each seedling in such a way that there is no damage in root. Number of rootlets and root length were observed and recorded.
- 1.4. **Number of leaves:** Number of leaves was counted on 30th day from the day of sowing and it was compared to that of control.
- 1.5. **Number of branch:** Total numbers of branches were recorded from each plant and added for each treatment separately and divided by the total number of plant scored to calculate the mean.
- 1.6. **Statistical evaluation:** The statistical significance of the effects of NaN₃ on the observed traits was tested by analysis of variance and differences between groups were determined by the least significant differences test, with P < 0.05 considered significant.

III. Results and Discussions

Induced mutagenesis has been accepted as a significant tool to break through the limitations of variability and to create variability in a short period of time. The data recorded for various traits is presented in Tables 1 to 4. It is clear from the tables that the two varieties of black gram KU300 and KU92-1 differed in response to SA treatment in seed germination, root length, number of rootlet/plant and seedling height etc.

- 1.7. **Seed germination:** The extent of reduction in seed germination was more pronounced under field conditions (Table 1). KU300 did not germinate in all the treatments except the control under the field conditions. The highest germination in percent was exhibited by the control in both the varieties in both the environments (Figure 1). Both the varieties were quite sensitive to SA. The reduction and inhibition in seed germination was found to be dose dependent and decreased linearly with an increasing lethal effect of SA concentrations. Similar observations were recorded in many crops like *Vigna radiata* [16-18], barley [19]; pea [20];

Figure 1. Graphical representations of the effect of SA on seed germination under laboratory and field conditions.



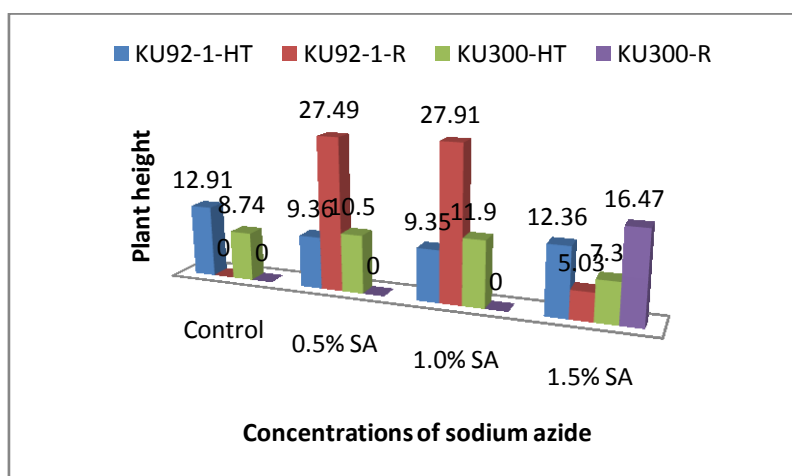
R=Reduction in %; =Germination in %; F=Data from field;

Table 1 Effect of SA on seed germination under laboratory and field conditions.

SA Concentration (in %)	No. of seed sown	No. of seeds germinated	Germination (in %)	Reduction (in %)
KU92-1 (in the laboratory)				
Control	12	11	91.6	8.33
0.5	12	5	41.6	58.4
1.0	12	4	33.3	66.7
1.5	12	3	25	75
KU300 (in the laboratory)				
Control	12	11	91.6	8.33
0.5	12	5	41.6	58.4
1.0	12	4	33.3	66.7
1.5	12	3	25	75
KU92-1 (in the field)				
Control	30	27	90	10
0.5	30	6	20	80
1.0	30	5	16.6	83.4
1.5	30	4	13.3	86.7
KU300 (in the field)				
Control	30	25	83.3	16.6
0.5	30	0	-	-
1.0	30	0	-	-
1.5	30	0	-	-

cowpea [21]; *Vigna mungo* [22], *Nigella*, *Plantago* and *Trigonella* [23]; *Stevia rebaudiana* [24].

1.8. Seedling height: Seedling height decreased in all the treatments in KU92-1. The mean height in KU92-1 ranged from 12.91cm in control to 9.36 cm with 0.5%SA treatment. It showed negative sif in mean with elevated value of coefficient of variation (Table 3, Figure 3). The maximum reduction percentage was observed to be 27.91% with 1.0%SA (Figure 2). KU300 exhibited increased height with 0.5% and 1.0% SA treatments while the same variety showed 16.47% reduction with 1.5% SA (Table 2). Reductions in seedling height due to the effect of mutagens have earlier been documented on various crop plants like barley [25], rice [26], tomato [27], *Vicia faba* L. [28], *Pisum sativum* [29] and wheat [30].



HT-Plant height, R-Reduction in % in plant height

Figure 3: Graphical representations of seedling height between the two varieties under laboratory condition.

1.9. Root growth: The two varieties showed inter-varietal differential response to root length. It was decreased at low concentration of mutagen (0.5% SA) but decreased at relatively higher doses in KU92-1. In contrast, in the variety KU300, root length was increased in all the treatments. The highest increment (44.06%) was observed with 0.5%SA. The number of rootlets increased at comparatively lower concentration of SA but it was decreased with 1.5% SA in both the varieties (Table 2). KU92-1 gave elevated values of VC for root growth showing further scope of improvement while KU300 gave lower CV than the control (Table 3). Similar inter and intra varietal differential response of plants in root growth due to SA application were also reported in *Lycopersicon esculentum* [27] and in *Triticum aestivum* L. [30].

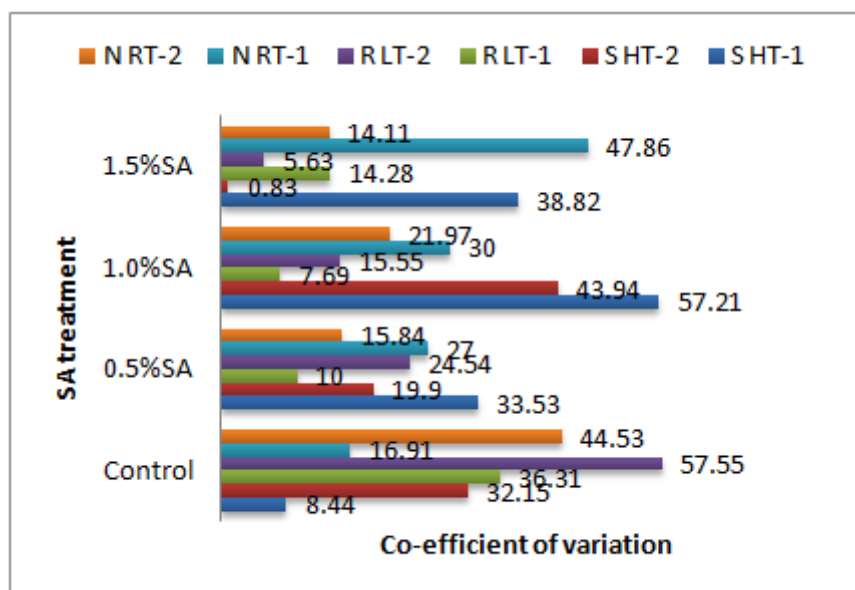
1.10. Plant height : Application SA decreased plant height in all the treatments. The relationship between the quantum of reduction in plant height and dose of SA was not linear in KU92-1. The maximum reduction

was found to be 52.05% with 1.5%SA while the minimum reduction (36.9%) was with 1.0% SA (Figure 3). The present finding is in agreement with the observations in *Vicia faba* L. [28] and *Triticum aestivum* L. [30] where treatment of SA reduced the plant height significantly.

- 1.11. Number of leaves/plant:** Number of leaves/plant was decreased in all the treatments of SA concentrations. The mean number of leaves/plant varied from 10.54 in control to 3.16 with 0.5% SA. The minimum reduction was 54.07% with 1.5% SA and the maximum reduction was 69.25% with 0.5% SA (Table 4). The value of reduction and amount of mutagen is non-linear. Increasing leaf area in musk okra due to treatment with SA was observed [31]. In conformity with the observation in the present findings, reduction in number of leaves/plant due to SA application have been reported in *Lycopersicon esculentum* L. [31].
- 1.12. Number of branches:** Number of branches/plant was decreased significantly in all concentrations of SA in variety KU92-1 (Table 4). The reduction in number of branches in the present investigation can be related to the inhibitory effect of azide ions that binds to the enzymes involved in cellular energy production [32]. Reduction in number of branches due to higher

Table 2 Effect of sodium azide on seedling height and root growth under laboratory conditions.

Characters	Treatments	Reduction (in percent)		Increment (in percent)	
		KU92-1	KU300	KU92-1	KU300
Seedling height (cm)	Control	-	-	-	-
	0.5	27.49	-	-	16.76
	1.0	27.91	-	-	26.55
	1.5	5.03	16.47	-	-
Root length (cm)	Control	-	-	-	-
	0.5	-	-	10.5	44.06
	1.0	9.21	-	-	22.77
	1.5	2.23	-	-	34.74
Number of rootlet/plant	Control	-	-	-	-
	0.5	-	-	8.3	22.81
	1.0	-	-	26.7	23.47
	1.5	13.64	53.36	-	-



S HT=Seedling height; R LT= Root length; N RT=No. of rootlet/plant.
1=KU92-1; 2=KU300;

Figure 3 Graphical representations for co-efficient of variation in a few of the selected traits studied under laboratory conditions.

Table 3 Mean, standard deviation, shift in mean and CV for few of selected traits as observed under laboratory conditions.

Variety	Character	Treatment	Mean ±SD	Shift in mean	CV.
KU92-1	Seedling height (cm)	Control	12.91±1.09	-	8.44
		0.5%	9.36**±3.14	-3.55	33.53
		1.0%	9.35**±5.35	-3.56	57.21
		1.5%	12.26±4.76	-0.65	38.82
	Root length (cm)	Control	3.58±1.3	-	36.31
		0.5%	4.0±0.4	+0.42	10.0
		1.0%	3.25±0.25	-0.33	7.69
		1.5%	3.5±0.5	-0.08	14.28
	No. of rootlets/plant	Control	7.33±1.24	-	16.91
		0.5%	8.0±2.16	+0.67	27.0
		1.0%	10.0**±3.0	+2.67	30.0
		1.5%	6.33±3.03	-1	47.86
KU300	Seedling height (cm)	Control	8.74±2.81	-	32.15
		0.5%	10.5**±2.09	+1.76	19.9
		1.0%	11.9**±5.23	+3.16	43.94
		1.5%	7.3±0.061	-1.44	0.83
	Root length (cm)	Control	2.78±1.633	-	57.55
		0.5%	4.97**±1.225	+2.19	24.54
		1.0%	3.6±0.565	+0.82	15.55
		1.5%	4.26**±0.249	+1.48	5.63
	No. of rootlets/plant	Control	7.14±3.18	-	44.53
		0.5%	9.25**±1.47	+2.11	15.89
		1.0%	9.33**±2.05	+2.19	21.97
		1.5%	3.33**±0.47	-3.81	14.11

CV= Coefficient of variation **Significant at p<0.05

Table 4 Effect of SA on plant height, leaves/plant and branches/plant under field conditions.

Treatment	Concentration	Mean ±S.D.	Reduction (%)
Plant height (cm)			
Control	-	10.95±3.79	-
SA	0.5%	5.75±1.30	47.48
SA	1.0%	6.9±1.14	36.98
SA	1.5%	5.25±0.5	52.05
Number of leaves/plant			
Control	-	10.54±4.36	-
SA	0.5%	3.16±1.16	69.25
SA	1.0%	3.6±1.14	65.46
SA	1.5%	4.75±0.5	54.07
Number of branches/plant			
Control	-	3±1	-
SA	0.5%	2.5±0.57	16.66
SA	1.0%	2.6±0.57	13.33
SA	1.5%	2.5±0.70	16.66

Dose of SA treatment were also reported in M₁ and M₂ generations of *Nigella sativa* L., *Plantago ovata* F. and *Trigonella foenum graecum* L. [23] and in M₁ musk okra [31] and *Lycopersicon esculentum* L. [27].

IV. Conclusion

The two black gram varieties under investigation were quite sensitive to SA treatment in all the concentrations. Most of the traits studied exhibited reduction. There was increment in root growth in both the varieties. Such plants with better root system may be selected with other traits undisturbed. Such mutant plants can be tested for soil with low organic contain such as the site where the study was carried out. The mutant population developed may used to screen for plant with potential for higher yield the next generations.

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