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MICRO-MUTATIONS BY HEAVY METALS IN M_2 GENERATION OF *VIGNA RADIATA*

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ABSTRACT

To conduct this research work the green gram seeds were treated with heavy metals. For this purpose two varieties of green gram (*Vigna radiata*) i.e. Narendra mung-1 (.NM-1) & Pantnagar mung -2 (PTM-2) were obtained from Pantnagar seed department, Pantnagar University, Pantnagar, India. The effluent of city waste water was obtained from the outlet of the B.D.A. colony nalah near Gulabrai Inter college of Bareilly U.P. India. Different type of heavy metals such as copper, zinc, lead, chromium, cadmium etc. is present in city waste water in toxic amount. The concentration of copper, zinc & lead in city waste water was found 7.500 mg/l, 7.270 mg/l & 3.740 mg/l respectively. Different concentrations (80%, 90% & 100%) of the heavy metals were prepared to treat mung bean seeds. In each treatment the data was collected for the different quantitative characters such as number of pods per plant, number of seeds pods, number of seeds per plant, 100 seed weight per plant and total green yield per plant. The shift in mean value and variance worked out for each character.
Keywords : Heavy metals, Micro-mutations, Mung bean, Mean value, Variance.

Introduction

Among the different pulses green gram commonly known as mung been (*Vigna radiata* (L) Wilczek is an important pulse crop of India. Major mung bean producing states are Maharashtra, Rajasthan, Madhya Pradesh, Uttar Pradesh and Gujarat. Almost 50 % of production occur in these five top states of India. Cropping and cultivation period of mung bean crop is very short when used as mixed crop with other.

According to (Dainavizadeh and Mehranzadeh, 2013), the nutrient composition of the seed of mung bean contains 20–24% protein, 9.4% moisture, 2.1% oil, 2.05% fats, 6.4% fiber, 343.5 kcal per 100 gram energy, carbohydrates and a fair amount of vitamin A and B. It is also important because of its fodder value. The states with largest area under Pulses cultivation are Rajasthan (6340 thousand hectares), Madhya Pradesh (4757 thousand ha), Maharashtra (4192 thousand ha), Karnatka (3111 thousand ha) and Uttar Pradesh (2370 thousand ha) (*Source: rbi statistics)*

They contribute substantially to the enrichment of the soil. They probably add many times more nitrogen to occur in soil per year than in added in the form of chemical fertilizers, a fact of great importance in the light of inadequate availability of nitrogenous fertilizers, now and in foreseeable future. Furthermore, pulse crop pay enough debentures to farmers, particularly in view of their market value. Mung bean pulse is cultivated in the adjoining area of Bareilly city of Uttar Pradesh India. Different industries are present around the Bareilly city, some are Indian turpentine and resin corporation Limited Bareilly¸ Camphor and Allied Products, Bareilly, WIMCO Ltd., Bareilly, Synthetic and Chemical Limited, Bareilly, IFFCO Limited, Aonla, Bareilly, Sugar factory, Bareilly and City Waste Nalah, BDA Colony, Bareilly.

Except this many others were also there from which polluted water goes to the adjoining agriculture field of the Bareilly city. This contaminated water has many hazardous heavy metals. Uptake of these heavy metals takes place from the soil by the plants. The elevated concentration of these heavy metals reaches to different organs of plants which affects the plant growth as well human health. (Galal *et al.*, 2021). So it is very essential to protect and care apparently fast damaging plant i.e. quantitative and quantitative aspects, by the hazardous effect of the heavy metals present in city waste water, when farmers use this contaminated water for irrigation purpose, they innocently damage the good genotype of pulses and other crops by mutation. A mutation is a sudden heritable alteration in the genetic material of aliving cell induced by mutagens (Raina *et al.*, 2018).

Micromutations- A mutation with a small effect that can be detected only by the help of statistical analysis such as character, variance, heritability etc. The majority of such mutations are in polygenic traits, they are of the greatest value to plant breeders since most of the economically useful traits are polygenically controlled (Raina *et al.*, 2021).

Mutation breeding is a strong tool to develop improved crops varieties with desired traits. Induction of micro-mutations in polygenic system controlling the quantitative traits is important for crop improvement. Several authors (Joshi and Verma, 2004; Khan and Wani, 2005; Singh *et al*., 2006; Auti, 2012; Bara *et al*., 2017; Wani, 2017; Patial *et al*., 2017) have reported in various crops thatmicro-mutations result in the release of considerable genetic variability in themutagen treated population.In recent years, mutation breeding has been gaining ground for inducing genetic resources (Datta *et al*., 1993).

Material and Methods

Effluent collection

The effluent of sewage of city waste water (C.W.W.) was used in present study. The effluents of city waste water were obtained from outlet of the B.D.A. colony nalah, situated in the main city near Gulabrai Inter College, Bareilly U.P. India. This polluted city waste water has heavy metals with elevated concentrations which were toxic to plant growth as well as human health.

Heavy Metal Examination

Different heavy metals ,which were found in the city waste water quantitatively analysed in Botany Laboratory, Enviornmental Science Division, NBRI, Lucknow U.P. India by the absorption spectrophotometrer AA.

Varieties of mung bean

Inbreads of two varieties of *Vigna radiata* (L) Millsp. i.e., NM-1& PTM-2 were obtained from Pantnagar Seed Department, Pantnagar University, Pantnagar for the present investigation.

Field Preparation

To ensure adequate plant stand & early vigour, the field should be well levelled free from clods & weeds.

Preparation of solutions

Seeds of both varieties of *Vigna radiate* i.e, NM-1 & PTM-2 were treated with different concentrations (80, 90 & 100%) of freshly prepared solution of Cu^{++}, Zn^{++} & Pb^{++} accordingly with the quantity which was present in the city waste water.

Selection of seeds

Clean, plump & uniform sized seeds were used for experiment & treated with freshly prepared solution (as told previously) for 15 hours & distilled water (control). The M_1 generation was raised by sowing treated seeds in the field.

Sowing of seeds

The seeds of M_1 plants were sown on plant to row progeny basis to raise M_2 generation. Data for different quantitative characters were collected in each treatment given to mung bean seeds.

Results

Concentration of Heavy Metals in C.W.W

Different heavy metals were found in C.W.W such as Cu, Zn, Cd, Ni, Pb etc. After physico-chemical analysis the quantity of copper, zinc& lead in city waste water was found 7.500 mg/l,7.270 mg/l & 3.740 mg/l respectively.

Data was collected for the five different quantitative characters. These were Number of pods per plant, Number of seeds per pod, Number of seeds per plant, 100 seed weight per plant and Total green yield per plant

All the morphological variants were eliminated from the sample taken for the study of these above characters. The shift in mean value and variance worked out for each character. All the morphological mutants were eliminated from the samples taken for the study of these above five characters. The shift in mean and variance were worked out for each character.

1. Number of pods per plant

Shift in mean

By counting number of pods per plant the productive capacity of mung bean plant can be calculated. It is clear from the table-1 that there is a significant change in mean values in all the treatments of heavy metal in both the varieties NM-1 and PTM-2. It is clear from the collected data that shift in mean values were towards negative direction (Table-1)

It is significant in all the treatments except 80% treatment of copper, zinc and lead in NM-1 and 80% treatment of copper and zinc in variety PTM- 2.

Variance

With increasing the concentration of heavy metals there is increased in variance in all the treatment of both the varieties. The variety NM-1 showed the maximum variance in heavy metal treatment i.e. 100% $PbNo_3$ (Lead Nitrate). (Table-1)

Table 1 : Shift in mean and variance for number of pods/ plant in M_2 generation

Treatments	Variety NM-1		Variety PTM-2	
	Mean	Variance	Mean	Variance
Copper Sulphate				
80%	26.00	4.35	25.86	4.05
90%	25.93**	18.08	25.13**	19.73
100%	25.80**	35.14	24.86**	26.90
Zinc Sulphate				
80%	25.66	4.19	25.53	3.96
90%	25.66**	35.07	25.33**	20.02
100%	25.06**	48.20	25.30**	30.19
Lead Nitrate				
80%	25.60	4.96	24.46**	12.06
90%	24.46**	20.02	24.33**	32.09
100%	24.41**	50.33	24.20**	42.06
Control	**30.2**	**3.93**	**28.00**	**3.99**

**Significant at 1% level

2. Number of seeds per pod

Shift in mean

A shift in mean values towards negative direction was noticed in both the varieties. In heavy metal treatment both the varieties showed the shift in mean values towards the negative direction (Table-2).

Variance:

With increasing concentration of heavy metals, increase in the variance was observed in both the varieties. The significant reduction was found in all the treatment of variety NM-1 and PTM-2 (Table-2).

Table 2 : Shift in mean and variance for number of seeds/ pod in M_2 generation

Treatments	Variety NM-1		Variety PTM-2	
	Mean	Variance	Mean	Variance
Copper Sulphate				
80%	10.93	4.35	10.33**	4.99
90%	10.66**	11.91	10.13**	12.46
100%	10.06**	14.92	9.86**	15.71
Zinc Sulphate				
80%	10.93	3.23	10.06**	4.06
90%	10.63**	4.63	10.01**	14.96
100%	10.04**	5.55	10.00**	20.12
Lead Nitrate				
80%	10.80	3.26	10.04**	5.04
90%	10.20**	5.69	10.00**	17.33
100%	10.01**	10.17	9.73**	22.09
Control	**11.66**	**0.99**	**11.33**	**1.02**

**Significant at 1% level

3. Number of seeds per plant

Shift in mean

A significant change towards negative direction in mean value was observed in all treatments of heavy metals in variety NM-1 and PTM 2 (Table-3).

Variance

A gradual increase in variance was noticed with the increase in concentration of heavy metals. The maximum variance found in 100% lead treatment in NM-1 and 100% $PbNo_3$ treatment of PTM 2. (Table-3)

Table 3 : Shift in mean & variance for number of seeds/ plant in M_2 generation

Treatments	Variety NM-1		Variety PTM-2	
	Mean	Variance	Mean	Variance
Copper Sulphate				
80%	299.66**	90.69	280.53**	102.73
90%	295.00	135.76	280.46**	222.94
100%	290.00**	495.32	276.87**	402.09
Zinc Sulphate				
80%	296.80**	92.06	297.20**	120.79
90%	290.13**	144.70	272.49**	235.96
100%	289.33**	499.69	270.64**	409.69
Lead Nitrate				
80%	285.80*	120.09	278.09**	199.09
90%	276.87**	185.72	272.07**	294.69
100%	274.20**	499.99	269.99**	492.59
Control	**310.00**	**35.04**	**283.53**	**49.32**

**Significant at 1% level *Significant at 5% level

4. 100 seed weight per plant

Shift in mean

A shift in mean values was observed in negative direction in both the varieties in heavy metal treatment. The significant change was found in all the treatment of variety NM-1 and variety PTM -2 accept 80 and 90% copper sulphate in NM-1 and 80% of copper sulphate treatment in variety PTM-2.(Table-4)

Variance

In both the varieties and increase in variance was not treated in all the treatment with increasing their concentration in heavy metal treatment both the variety showed maximum increase in 100% treatment of Lead Nitrate.(Table-4)

Table 4 : Shift in mean & variance for number of 100 seed weight/ plant in M_2 generation

Treatments	Variety NM-1		Variety PTM-2	
	Mean	Variance	Mean	Variance
Copper Sulphate				
80%	4.35	0.19	4.00	0.41
90%	4.15	0.23	3.64*	0.39
100%	3.98*	0.47	3.06**	0.80
Zinc Sulphate				
80%	4.09*	0.42	3.80*	0.55
90%	4.00**	0.82	3.72**	0.76
100%	3.89**	0.99	3.67**	0.98
Lead Nitrate				
80%	4.07*	0.85	3.50*	0.67
90%	3.99**	0.99	3.30**	0.89
100%	3.79**	1.01	3.13**	1.09
Control	**4.55**	**0.08**	**4.15**	**0.09**

**Significant at 1% level *Significant at 5% level

5. Total grain yield per plant

Shift in mean

A shift in mean value towards negative direction was noticed in all the treatment of heavy metals in both the varieties. It was found significant in all treatments of variety NM-1 and variety PTM-2 i.e. 80% and 90% of copper sulphate and 80% of lead nitrate in NM-1 and 80% and 90% of copper sulphate 80% of $ZnsO_4$ and 80% of in PTM 2 (Table-5).

Variance

The variance increased with the increasing concentration of heavy metal treatment. The maximum increase in variance was noticed in 100% treatment of zinc in variety NM-1 and 80% treatment of zinc in variety PTM- 2 (Table-5).

Table 5 : Shift in mean & variance for Total grain yield / plant in M_2 generation.

Treatment	Variety NM-1		Variety PTM-2	
	Mean	Variance	Mean	Variance
Copper Sulphate				
80%	10.35	0.98	10.29	0.85
90%	10.15	4.20	10.09	4.65
100%	9.99	4.96	9.97**	5.02
Zinc Sulphate				
80%	10.15	1.06	10.06	9.10
90%	10.00	6.25	9.98**	7.65
100%	9.90**	9.59	9.89**	7.95
Lead Nitrate				
80%	10.09	1.09	10.01	2.03
90%	9.99	7.71	9.79**	8.09
100%	9.79	8.92	9.65**	9.02
Control	**10.65**	**0.10**	**10.53**	**0.19**

**Significant at 1% level

Discussion

In the present investigation a great deal of variability has been induced in M_2 generation in mean value of all the characters by all heavy metal treatments i.e. copper sulphate, zinc sulphate and lead nitrate (80, 90 and 100%).A definite shift in mean values was observed in positive as well as negative direction in different treatments. The two varieties responded differently to the same heavy metal treatment and for the same trait.

A significant shift in mean value towards negative direction was observed in 100% lead nitrate treatment in both varieties, for pods /plant in all the treatments of heavy metals in both varieties, for seeds /pod and seeds per/plant and 100 seed weight/plant in both varieties. The 80% treatment of the copper sulphate in both varieties showed the shift in mean value towards the positive direction but it was not significant.

Similar shift in mean values for quantitative character have been reported by (Tripathi, 1990), (Chauhan and Patra, 1993) and (Thakur and Sethi, 1995).

Shift in variance

An increase in variance was noticed in heavy metal treatments in both the varieties as compared to their respective control.

An increase in variance was noticed in all treatments of both varieties except 80% of zinc sulphate in variety NM-1 and PTM-2 for pods/ plant and in all treatments of both varieties for seeds/ pod in all the treatment of copper sulphate, zinc sulphate and lead nitrate in both varieties for seeds / plant.

In 80% copper sulphate treatment of variety and NM-1 for 100 seed weight /plant and in 80% treatment of copper sulphate in both varieties for total green yield.

There are several earlier reports where an increase in variation for quantitative character was observed without significant altering their mean values by (Khan, 1988) (Thakur and Sethi, 1995), (Zhu-B *et al*., 1955). Variance level may be less responsive in one trait and highly responsive in other(Sharma, 1995).

Increased variability in the form of high heritability and genetic advance for different quantitative characters have been reported by many workers (Rao, 1988; Nayeem and Gharim, 1990; Sharma *et al*., 1990; Ignacimuthu and Babu, 1993; Srivastava and Singh, 1993). Gamma ray induced mutagenic variability in chickpea was reported by (Kale *et al*., 1980).

In recent years, the role of mutation breeding in increasing the genetic variability for polygenic characters in a number of crops have been proved beyond doubt (Solanki and Sharma, 1999; Waghmore and Mehra, 2000). The significance of micro-mutations in the evolution was first recognized and emphasized by Baur (1924) and later it has been studied by many workers in different crop plants. Gaul (1965) has emphasized micro-mutations in plant breeding by stating that all the morphological and physiological characters are affected by micro-mutations and they might have higher mutation rates than the macro mutations. Arefrad *et al*. (2012) induced mutation on Glycine max by using different doses of gamma rays.

Conclusion

It is well known that plant breeding is a important tool for improvement of different crop varieties. During the course of evolution crops lost their variability due to various stresses and adaptations. With the help of induced mutations it can be restore and regenerate. It is cost effective and helpful to enhance crop quality and quantitative traits.

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