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Research Note

Studies on induced mutations in chickpea (C*icer arietinum* L.) I. Responses of the mutagenic treatments in m_1 biological parameters

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Abstract:
The effect of ethylmethane sulphonate (EMS), sodium azide (SA) and hydrazine hydrate (HZ) on seed germination, pollen fertility and survival at maturity were studied in two varieties *viz.*, Avrodhi and BG- 256 of chickpea. A linear and dose dependant decrease on germination, pollen fertility and survival was observed in all the three mutagenic treatments in both the varieties. The maximum reduction in these parameters was observed to be induced by EMS treatments. Variety Avrodhi proved to be more sensitive to the mutagenic treatments than var. BG-256. Estimated values of LD_{50} for EMS, SA and HZ were observed to be 0.363, 0.045 and 0.052 for Avrodhi and 0.457, 0.058 and 0.070 for BG-256, respectively.

Key words: Chickpea, Biological damage, chemical mutagens, LD_{50} and r-values.

The biological damage, caused by mutagenic treatments, may be used as criteria in determining the effect and mechanism of action of the mutagens in question and also the sensitivity of the biological material. Chickpea (*Cicer arietinim* L.) being a self pollinated crop possesses limited variability. Induction of mutation provides a handy tool to create variability within the genotypes to facilitate effective selection. Limited information is available regarding the use of chemical mutagens for its improvement. However, it is ideal to understand the response of this genotype with respect to mutagen dose to be used for its improvement. The present experiment was designed to assess the response of this genotype to different mutagens and to estimate LD_{50}, correlation and regression analysis among the biological parameters viz., seed germination, pollen fertility and survival in M_1 generation.

Seeds of two chickpea varieties *viz.*, Avrodhi and BG-256, commonly cultivated and widely adapted to the agroclimatic conditions of Uttar Pradesh (India), were presoaked in double distilled water for 12 hours followed by treatments with four concentrations (0.1%, 0.2%, 0.3% and 0.4%) of EMS at pH 7 and 0.01%, 0.02%, 0.03% and 0.04% concentrations of SA and HZ at pH 7 and pH 3 respectively for 6 hours. The treated seeds were thoroughly washed under running tap water and sown in the field in complete randomized block design (CBRD) with three replications of 100 seeds each. Germination was counted as per cent of control. Fifteen flower buds, five from each replicate from each treatment and control were fixed in acetic alcohol (1:3 ratio) for 24 hours and preserved in 70% alcohol. The buds were squashed and stained with 1% acetocarmine to study pollen fertility. Deeply stained pollen grains with regular walls were taken as fertile while unstained pollen grains with irregular walls were counted as sterile. Survival of the plants was recorded at the time of maturity in the field. The data was statistically analyzed to determine the genotypic response to mutagenic doses, germination, LD_{50}, pollen fertility and survival. Correlation coefficient and regression analysis was carried out to understand the relationship between mutagenic treatments and M_1 biological parameters.

The percentage of germination, pollen fertility and survival in both the varieties decreased with the increasing concentration of all the three mutagens (Table 1). The germination in control was 93.66% and 94.00% in var. Avrodhi and var. BG-256 respectively. In treatments, 87.66 to 55.66% with SA treatments and 89.33 to 62.33% with HZ treatment were observed. Similarly in var. BG-256, the range in decreasing order was observed to be 90.66 to 58.33%, 90.33 to 67.33 and 92.66 to 75.66% for EMS, SA and HZ treatments respectively. The reduction in germination after mutagenic treatments may be due to the inhibitory effect of mutagens on germination (Avery and Jain,

1996) or altered enzyme activity (Kurobane *et al*., 1979). Determination of LD_{50} for mutagen is important since low doses may prove ineffective and the higher ones may cause lethality. Furthermore, doses around LD_{50} are believed to induce maximum frequency of mutations (Micke, 1986). A dose dependant increase in pollen sterility may be due to synapses and/or desynapses (Kumar and Gupta, 1978). High sterility in EMS and HZ treatments in both the varieties indicate that these invite a high degree of meiotic abnormalities. Survival of M_1 plants decreased with the increasing mutagen dose. The survival of the plants till maturity depends upon the normal physiochemical balance of the cell metabolism. The mutagens are capable of creating chromosomal and extra chromosomal abnormalities. These abnormalities might have lethal effects on different stages of entogeneisis. The reduction in survival after mutagenic treatments has also been reported in mungbean (Khan *et al*., 1998 a & b).

The regression equation and correlation studies ascertain the relationship between the dose of the mutagen and M_1 biological parameters, significantly negative correlation was displayed by the two variables (Table 2). Such a negative correlation was also reported earlier in pearl millet (Singh *et al*., 1978) and rye (Reddy *et al*., 1993).

Among, the two chickpea genotypes, var.. Avrodhi was more sensitive to mutagenic treatments than BG-256 in exhibiting more biological damage. This is due to fact that genetic architecture of an organism is an important factor in determining the genotypic difference towards the mutagens. Varietal differenced with respect to mutagenic treatments were also reported earlier in rice (Singh *et al.*, 1998) and cowpea (Ahmed, 1999).

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Table 1. Effect of mutagens on different parameters in M1 generation

Treatment	Var. Avrodhi			Var. BG-256	
	Germination (%)	Pollen Fertility (%)*	Survival (%)	Germination (%)	Pollen Fertility (%)*
Control	93.66	95.25	90.03	94.00	97.80
0.1% EMS	85.66	83.20	79.76	90.66	87.18
0.2% EMS	70.00	75.10	76.66	81.66	79.45
0.3% EMS	56.00	67.75	69.04	64.33	67.93
0.4% EMS	47.00	54.70	67.60	58.33	58.10
0.01% SA	87.66	86.30	81.74	90.33	90.02
0.02% SA	76.33	78.60	78.60	85.00	84.13
0.03% SA	64.33	70.75	76.68	70.00	79.62
0.04% SA	55.66	64.83	70.65	67.33	68.60
0.01% SA	89.33	85.63	85.56	92.66	88.87
0.02% HZ	78.00	76.18	80.76	89.00	80.15
0.03% HZ	69.00	64.88	78.26	77.66	72.19
0.04% HZ	62.33	58.14	71.12	75.66	63.42

*Average of 10 readings of 5 plants randomly selected from each treatment and control.

Table 2. Relationship between dose (x) and different M1 biological parameters (Y) and LD_{50} of EMS, SA and HZ on chickpea (*Cicer a*)

Mutagens	M1 parameters	Var. Avrodhi			Var. BG-256	
		Correlation Coefficient (r)	Regression Equation	Dose (%) for LD-50 (Y=50%)	Correlation Coefficient (r)	Regression Equat
EMS	Germination	- 0.993	Y= 97.16 - 129.98x	0.363	-0.983	Y= 102.32 – 104.32
	Pollen Fertility	-9.990	Y= 93.40 – 92.85x		-0.997	Y= 97.85 – 98.76x
	Survival	-9.968	Y= 84.29 – 44.10x		-0.072	Y= 90.18 – 56.72x
SA	Germination	-0.997	Y= 97.99 – 1080x	0.045	-0.965	Y= 99.16 - 840x
	Pollen Fertility	-0.998	Y= 93.18 – 722.60x		-0.980	Y= 97.78 – 68.77x
	Survival	-0.972	Y= 85.71 – 351.90x		-0.983	Y= 87.08 – 345.40x
HZ	Germination	-0.993	Y= 97.16 – 900x	0.052	-0.963	Y= 99.33 – 623.40x
	Pollen Fertility	-0.995	Y= 94.65 – 937.70x		-0.990	Y= 97.23 – 813.10x
	Survival	-0.982	Y= 90.38 – 458.20x		-0.983	Y= 90.82 – 222.40x