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INVESTIGATION OF LD_{50}, GR_{50} AND SEEDLING DEVELOPMENT IN SODIUM AZIDE AND ETHYL METHANE SULFONATE TREATMENTS OF CHICKPEA (*CICER ARIETINUM* L.)

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Abstract

The effects of different doses of sodium azide (SA) and ethyl methane sulfonate (EMS) on seedling and root characteristics of chickpea variety were investigated. Lethal dose (LD_{50}) and median growth reduction (GR_{50}) values were determined for mutagens. In EMS treatment, Lethal dose was 47.4 mM and median growth reduction was 12.5 mM. Mutation efficiency was not determined at SA doses and it was revealed that higher doses would create mutants. As SA and EMS doses increased, the number of days to germination increased and seedling and root development decreased. Seeds germinated at 40 and 50mM EMS doses but seedlings did not survive. Differences between low and high doses in SA treatments were low. Leaf and plant mutants had 60% mutation efficiency at 20mM dose of EMS.

Introduction

Chickpea (*Cicer arietinum* L.) belongs to Fabaceae. The genus *Cicer* consists of 49 taxa, including 40 wild perennials, eight wild annuals and one cultivated annual species (Smýkal *et al*. 2015). The origin of cultivated chickpea is Turkey and Syria (Ladizinsky and Adler 1976). To feed the future world population and obtain varieties with high adaptation to changing climate conditions, there is a need to improve the genetic progress of chickpea. Genetic progress in plant breeding programs depends primarily on the amount of variability present in the plant (Prasad *et al.* 2022).

New mutant varieties developed by using chemical mutagens are nearly 80% among crops. Ethyl-methane sulphonate (EMS) is widely used as a chemical mutagen to induce quite a large number of heritable mutations in a plant (Jankowicz-Cieslak and Till 2016, Hasan *et al*. 2022). Sodium azide (SA) is one of the most powerful mutagens in crop plants. Sodium azide is a strong mutagen, and growth of plant parts are strongly inhibited with increasing its concentration and treatment duration. The mutational effects of this mutagen has been observed on tomato and it was very effective in inducing mutations with respect to germination percentage, root length, seedling height, and seedling survival (Adamu *et al.* 2007, Khan *et al*. 2009, Turkoglu *et al.* 2022). The present study was aimed to determine effect of different doses of chemical mutagens on germination and seedling traits of chickpea and lethal dose (LD_{50}) and median growth reduction (GR_{50}) for sodium azide and EMS in chickpea.

Materials and Methods

The study was conducted in 2024 at the Southeast Anatolia Project Region, International Agricultural Research and Education Center Application, Diyarbakir, Türkiye. Arda chickpea variety was used. Different doses of sodium azide (NaN_3) (0.5, 1, 1.5, 2, 2.5 and 3%) and ethyl methane sulfonate ($CH_3SO_3C_2H_5$) (10, 20, 30, 40 and 50 mM) were used as mutagens. In ethyl methane sulfonate (EMS) treatment, 8.26 ml EMS was made up to one litre of water in pH 7

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buffer solution. 0.010 molar EMS stock solution was prepared. In sodium azide treatment, 0.37 ml of H_3PO_4 was added to 5.4 g KH_2PO_4 to make it pH 3 and 0.65 g of sodium azide was dissolved with this buffer solution to prepare 100 mM sodium azide stock solution. The solutions were treated in a shaker at 180 rpm for 3 (SA) and 8 (EMS) hrs. Doses were prepared from these stock solutions. The seeds treated with chemicals were washed 4-5 times in tap water to remove chemical residue. The trial soil was made of peat + perlite mixture and seeds were planted as one seed per vial. The trial was arranged in a randomized plot design with 3 replications and 45 seeds per replication. The seeds were germinated in the climate chamber at 25 ± 1ºC growth temperature, 70% humidity and 16/8 hrs day/night conditions. Seed emergence observations were recorded after seed planting and the seedlings were harvested at the end of the 14th day. Measurements were taken on seedling length and root length, seedling and root fresh weights. ANOVA analysis was performed using the JMP-Pro17 package program. Median growth reduction (GR_{50}) was calculated using regression equation (y=ax+b). Probit analysis for LD_{50} was used by Finney's approach (Finney 1971). Germination characteristics, including germination percentage (Erbach 1982), germination rate index (AOSA 1983), mean germination time (Ellis and Roberts 1980), seedling vigour (Kumar *et al.* 2011) and mutation/variation frequency (Khan *et al*. 2009) were determined.

Results and Discussion

The behaviour of the lethal doses (LD_{50}) in SA and EMS treatments is presented in Fig. 1. LD_{50} exhibited a quadratic tendency (P=0.02) in SA treatments and a negative linear tendency (P=0.0037) in EMS treatments. The probit curve analysis shown that the LD_{50} value for SA and EMS were 5.8% and 1.6 mM, respectively. The seedling length exhibited a linear tendency (P=0.005) for SA and EMS. Median growth reduction (GR_{50}) shows the value for SA rays and EMS was 3.9% and 12.5 mM, respectively (Fig. 1).

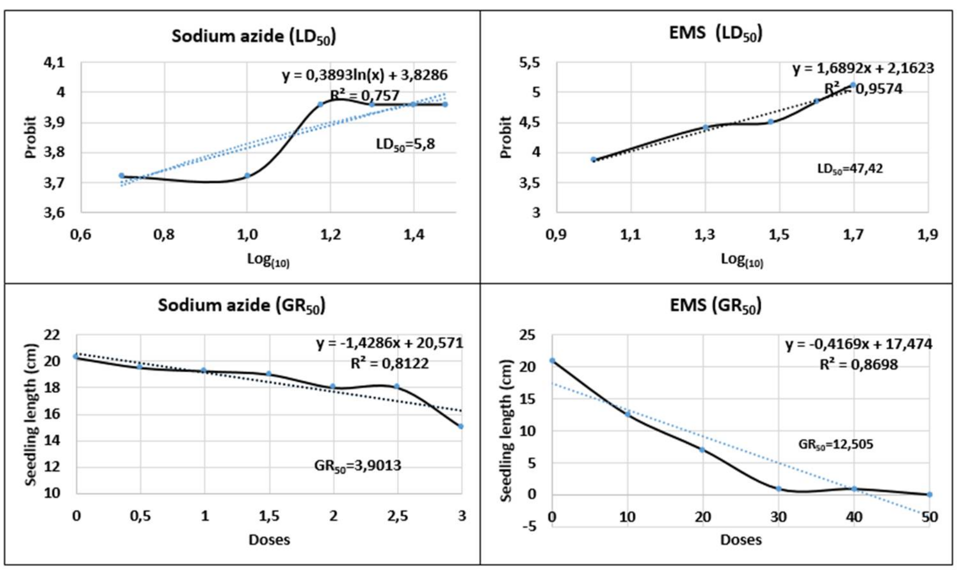

Fig. 1. LD_{50} and GR_{50} on chickpea under sodium azide and ethyl methane treatments.

The difference in LD_{50} and GR_{50} values for EMS is due to the fact that the seeds emerged at all EMS doses except for 50mM, and the emerged seeds were recorded, but since high doses were lethal, the emerged seedlings did not survive. LD_{50} and GR_{50} values for SA showed that the doses used in the experiment were not appropriate and mutations could be induced at higher SA doses (Fig. 1). Conversly, Raina *et al.* (2023) noted that the LD_{50} value in SA-applied plants varied between 0.08 and 0.09% and the probit analysis results showed that low doses of SA were less harmful and low doses could be considered as optimum doses for mutation breeding.

The number of mutant leaves, the number of deformed plants and the percentage of mutant plants were only calculated in EMS concentrations. Increasing EMS doses caused a decrease in germination percentage, germination rate index, mean germination time, seedling vigor 1 and vigor 2 traits. At 20 mM EMS dose, % mutation frequency was as high as 60% in leaf mutations of these mutations, chlorine and simple leaf type, and dwarf and bush type in plant types. Although there was a decrease in germination characteristics, seedling vigor 1 and 2 characteristics at control, 0.5 and 0.1% SA doses, no significant difference was observed at high SA doses (Table 1).

Table 1. Effect of sodium azide and ethyl methane treatments on germination traits of chickpea.

	Germination percentage (%)	Germination rate index	Mean germination time	Seedling vigor1	Seedling vigor2
EMS (mM)					
Control	100 a	15.7 a	0.2 b	3760.0 a	16.0 a
10	100 a	10.0 bc	0.5 a	3100.0 b	13.0 a
20	90 a	15.7 a	0.1 b	2460.6 c	8.1 b
30	80 ab	13.2 ab	0.1 b	999.2 d	1.5 c
40	80 ab	11.5 abc	0.1 b	1064.0 d	0.0 c
50	60 b	7.0 c	0.2 b	0.0 e	0.0 c
P-value	0,04*	0.04*	0.01**	0.01**	0.01**
SA (%)					
Control	90	9.0 ab	0.5	3514.5 a	13.0 ab
0,5	90	9.7 a	0.4	3411.0 a	15.3 a
1,0	90	6.4 bc	0.3	3217.5 b	12.6 abc
1,5	85	5.7 c	0.3	3123.8 bc	11.9 bc
2,0	85	5.7 c	0.3	3094.0 cd	9.4 cd
2,5	85	5.7 c	0.3	2979.3 d	9.9 bcd
3,0	85	5.7 c	0.3	2460.8 e	8.1 d
P-value	0,9ns	0.04*	0.6ns	0.01**	0.01**
EMS dose	Leaf mutant	Plant deformation	% mutant plant habit		
	chlorine	simple leaf	dwarf	bushy	
10mM	1	5	1	-	15,6
20mM	3	15	3	6	60,0

* and **Significant at 5 and 1% probability, respectively, ns, non significant, Means followed by equal letters do not differ by Tukey's HSD test, at 5% probability.

Days to germination were delayed as SA and EMS doses were increased. Seedling length, seedling fresh weight, seedling dry weight, root length, root fresh weight, root dry weight and number of leaflet per leaf were decreased as SA and EMS doses were increased. At EMS doses of 40 and 50 mM, seeds germinated but seedlings did not survive (Table 2). In SA treatment, differences between control and all doses for the number of days to emergence were significant, also doses of 0.5, 1.0 and 1.5% were observed different than high doses. In EMS treatment, control, 10 and 20 mM doses showed the same effect, while higher doses were different (Table 2). In SA treatments, when compared to the control, 0.5, 1.0 and 1.5% dose of the SA had the same effect on seedling height and seedling fresh, while 2 and 3% doses showed different effects. Significant differences for fresh dry weight were between 0.5% and higher doses. In EMS treatments, seedling length, fresh and dry weight between control and 40, 30, 20 mM doses were significantly different, but the difference between the control and the 40 mM dose was greater than the other doses for these traits. Differences between low doses (10 and 20 mM) and high doses (30 and 40mM) for seedling fresh and dry weight were strongly in a decreasing direction, even at 40 mM dose (Table 2). Ali *et al.* (2024) reported that seedling parameters tended to decrease as the concentrations of SA and EMS mutagens increased. A similar trend was also recorded by Handayani *et al.* (2023) and Ravi *et al.* (2023).

Table 2. The effect of sodium azide and ethyl methane treatments on seedling and root traits of chickpea.

EMS (mM)	Days to emergence (days)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	No of leaflet per leaf (n)
Control	3.8 b	21.0 a	1.29 a	0.17 a	16.6 ab	1.04 ab	0.08 ab	86.6 a
10	3.6 b	12.2 b	0.80 b	0.14 a	18.5 a	1.08 a	0.08 a	64.4 b
20	3.9 b	7.0 c	0.60 c	0.09 b	20.3 a	0.93 b	0.07 b	56.4 b
30	5.4 a	0.9 d	0.16 d	0.019 c	11.5 c	0.47 c	0.04 c	-
40	5.8 a	0.9 d	0.10 d	-	12.4 bc	-	-	-
P-value	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**
SA (%)	Days to emergence (days)	Seedling length (cm)	Seedling fresh weight (g)	Seedling dry weight (g)	Root length (cm)	Root fresh weight (g)	Root dry weight (g)	
Control	3.8 c	20.2 a	0.76 ab	0.14 b	18.8 a	0.52 c	0.08 bc	
0,5	5.5 b	19.0 ab	0.89 a	0.18 a	18.9 a	0.99 a	0.11 a	
1,0	5.7 b	19.5 ab	0.77 ab	0.15 b	16.5 ab	0.65 b	0.09 b	
1,5	6.0 b	19.2 ab	0.77 ab	0.14 b	17.4 a	0.39 cde	0.06 d	
2,0	7.4 a	18.0 b	0.63 bc	0.11 cd	18.4 a	0.31 de	0.06 d	
2,5	7.5 a	18.1 b	0.68 b	0.12 c	16.8 ab	0.26 e	0.06 d	
3,0	7.0 a	15.0 c	0.53 c	0.10 d	13.9 b	0.41 cd	0.07 cd	
P-value	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	0.01**	

* and **= Significant at 5 and 1% probability, respectively, ns=non significant, Means followed by equal letters do not differ by Tukey's HSD test, at 5% probability.

The effect of SA doses was the same except 3.0% for root length. For root length, all EMS doses except 30 and 40 mM EMS had the same effect as the control. In SA treatments, 0.5% SA dose showed higher effect than other doses including control for root fresh and dry weight. In EMS treatments, 10 mM dose showed the same high effect as the control for root fresh and dry weight (Table 2). In summary, SA and EMS applications caused negative effects on seedling and root characteristics as they inhibited physiological and enzymatic processes. Similar results were noted by Turkoglu *et al.* (2023) and Ali *et al.* (2024).

In the present study, the Arda chickpea variety showed different LD_{50} and GR_{50} values for different doses of chemical mutagen - SA and EMS. LD_{50} values, which determine the best mutagenic doses, were recorded as 5.8% and 47.42 mM for SA and EMS treatments, respectively. Root and seedling parameters showed significant differences with varying doses of both treatments. With increasing SA and EMS doses, seed germination was delayed and seedling and root development was reduced. The high EMS doses negatively affected plant development compared to SA doses. However, field studies are also needed to confirm the results and obtain useful mutants in subsequent generations.

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