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Determination of lethal dose (LD_{50}) and sensitivity of fenugreek (*Trigonella foenum-graecum*) to sodium azide for induction of mutation

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Fenugreek (*Trigonella foenum-graecum* L.), commonly referred to as *Methi* is a desirable nutritious leafy vegetable and an aromatic and spice herb indigenous to Asia and Southern Europe. Fenugreek seeds have also been used for various medical purposes in folk medicine. Crop improvement in fenugreek has been attempted through selection, hybridization and mutation breeding. Since the natural variability present in this crop is very low (Patel *et al.* 2017), the alternate approach to generate variability would be an induced mutation. A dose that induces a higher rate of mutations with less biological damage could be optimal. So, firstly it is essential to identify the LD_{50}, which results in 50% seed mortality or a tolerable dose at which 50% of seeds can survive. Therefore, the present investigation was performed to determine the optimum dose of the chemical mutagen, i.e. sodium azide and also the optimum time for which the chemical treatment should be given to induce the desired variability through mutation in fenugreek crop more accurately.

The present experiment was conducted during 2021 at Sri Karan Narendra Agriculture University, Jobner, Rajasthan. The seeds of fenugreek variety RMt 1 were given chemical treatment with sodium azide solution prepared at room temperature (25±2°C) in 0.1 M phosphate buffer (*p*H = 3) to determine its lethal dose and sensitivity on different growth parameters. 12 different lots pre-soaked in distilled water for 4 h were treated with 12 concentrations, viz. 0.3 mM; 0.6 mM; 1 mM; 2 mM; 3 mM; 4 mM; 5 mM; 6 mM; 7 mM; 8 mM; 9 mM and 10 mM sodium azide solution for 3 h, 6 h and 9 h duration. The seeds were then germinated using paper towels and placed in an incubator. The germination of seeds was observed on the 8th day and after 22 days of germination in pots, observations were recorded on survival %, root length and shoot length. For root and shoot length, the observations were recorded on 5 plants for each treatment and control. Based on the germination % in both the treatment and control sets, the lethal dose (LD_{50}) in each treatment was estimated (Laskar *et al.* 2018) as:

$$\text{Lethal Dose } (LD_{50}) = 100 - \frac{\text{Frequency of treated seedlings}}{\text{Frequency of control seedlings}} \times 100$$

Estimating the lethal dose (LD_{50}) values (Table 1) showed an increase in genotypic lethality to the mutagen doses with an increase in the intensity of sodium azide treatments. The effect of different concentrations and durations of sodium azide treatment on the pooled mean basis in control and mutagen-treated seeds, along with their correlation and regression studies, revealed that the variance due to concentration, duration and concentration × duration was significant (Table 2), which indicates the existence of many variations in the treatments used.

The assessment of the germination percentage of the treated seeds represents the primary concept of mutagenic effectiveness, with germination below 50% being regarded as lethal or non-desirable. Inhibition in seed germination, after the treatment of seed with different mutagens, is a convenient technique for studying the effects of mutagens on plants. It was observed that seed germination gradually decreased with increasing concentration and duration of sodium azide. The decline in germination percentage of the treated seeds could be due to the mutagen-induced disruptions in the genetically controlled bio-physiological and metabolic pathways that play a crucial role in the seed germination process, which may include altered enzyme activity, inhibition of mitotic process and hormonal

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imbalances. Sodium azide's negative effect on germination may also be attributed to azide anions, which are believed to be strong inhibitors of cytochrome oxidase, which in turn inhibits oxidative phosphorylation (Kleinhofs and Sander 1975). The azide anion not only alters the potential of the mitochondrial membrane and is a potent proton pump inhibitor, but it also interacts with enzymes and DNA to cause mutations in cells (Zhang 2000). Combining these elements may prevent ATP from being produced, which would reduce the quantity of ATP available and prevent the pace and percentage of germination. These findings are in close agreement with the earlier reports on reduced germination due to the inhibitory effect of the chemical mutagen by Siddiqui *et al.* (2009) in fenugreek, Laskar *et al.* (2017) in lentil and Singh *et al.* (2022) in rice. It has also been documented that inhibition of DNA synthesis by mutagenic treatments due to induced mutations may be a reason for reducing seed germination (Jahan *et al.* 2021).

Along with germination percentage, the effect of mutagen on survival percentage, root and shoot length to identify the biological influences of chemical mutagen is also utilized as an index for mutagenic lethality. The survival percentage also followed the same inverse trend as the germination percentage. No plants survived in 9 h duration from 3 mM concentration onwards. So, it can be inferred that concentrations above 3 mM at 9 h were utterly lethal in fenugreek. The reduction in plant survival is attributed to cytogenetic damage, physiological disturbances and mutagen's interference with various metabolic pathways of the cell (Sofia *et al.* 2020). The inhibitory effect of sodium azide on seedling survival has already been reported by Prabha *et al*. (2010) and Hassan *et al*. (2018) in fenugreek. The seedlings raised from the treated seeds showed a decreased trend from lower to higher doses of mutagen in root and shoot length. The reduction in plant height by chemical mutagens was ascribed to different factors. It could be due to the uneven damage to the meristematic cells due to gross injury caused at the cellular level, either due to gene-controlled biochemical processes, acute chromosomal aberrations, or both. The badly damaged cells would produce only a few progeny cells and growth would recur from those genetically least damaged cells. Bashir *et al.* (2013) attribute the decrease in seedling height in the treated groups to variations in auxin levels, modifications in the specific activity of several enzymes, and physiological harm incurred by seeds and seedlings. According to Saad-Allah *et al.* (2014), a drop in the mitotic index of the plant cells is the cause of the decrease in plant growth with increasing sodium azide concentrations and treatment duration. Such a reduction in the length of root and shoot arising out of mutagenic treatments was previously reported by Bashir *et al*. (2013) in fenugreek and Raina *et al*. (2018) in cowpea. In summary, we can say that aside from generating resistance against biotic and abiotic stresses, induced mutagenesis in fenugreek using sodium azide is simple and affordable for the development of genetic variability and advancement of agronomic traits that can be utilized to improve the yield and quality traits.

Correlation and regression analysis: Simple correlation coefficients (r) and regression equations worked out to study the extent and type of relationship between survival percentage and germination percentage, root length and

Table 1 Determination of lethal dose (LD_{50}) of fenugreek to sodium azide

	Hours								
	H_1 (3 h)			H_2 (6 h)			H_3 (9 h)		
Treatment (Concentration)	Total treated seeds	Surviving plants (%)	LD_{50}	Total treated seeds	Surviving plants (%)	LD_{50}	Total treated seeds	Surviving plants (%)	LD_{50}
Control (C)	100	99	-	100	96	-	100	92	-
T_1 (0.3 mM)	100	97	2.02	100	95	1.04	100	88	4.34
T_2 (0.6 mM)	100	96	3.03	100	93	3.12	100	85	7.60
T_3 (1 mM)	100	92	7.07	100	89	7.29	100	80	13.04
T_4 (2 mM)	100	91	8.08	100	81	13.54	100	78	15.21
T_5 (3 mM)	100	89	10.10	100	78	18.75	100	0	-
T_6 (4 mM)	100	86	13.13	100	74	22.91	100	0	-
T_7 (5 mM)	100	84	15.15	100	69	28.12	100	0	-
T_8 (6 mM)	100	80	19.19	100	65	32.29	100	0	-
T_9 (7 mM)	100	76	23.23	100	58	39.58	100	0	-
T_{10} (8 mM)	100	59	40.40	100	45	53.12	100	0	-
T_{11} (9 mM)	100	36	63.63	100	31	67.70	100	0	-
T_{12} (10 mM)	100	32	67.67	100	21	78.12	100	0	-

Treatment details are given under Materials and Methods.

Table 2 Combined effect of concentration and duration of sodium azide treatment

Treatment (concentration)	Germination percentage			Survival percentage			Root length (cm)			Shoot length (cm)		
	H_1 (3 h)	H_2 (6 h)	H_3 (9 h)	H_1 (3 h)	H_2 (6 h)	H_3 (9 h)	H_1 (3 h)	H_2 (6 h)	H_3 (9 h)	H_1 (3 h)	H_2 (6 h)	H_3 (9 h)
Control (C)	100	99	96	99	96	92	14.2	13.28	11.36	16.04	14.04	12.8
T_1 (0.3 mM)	99	97	93	97	95	88	12.32	10.6	7.74	13.02	12.14	10
T_2 (0.6 mM)	97	95	91	96	93	85	11.4	8.16	6.24	12.4	9.14	8.24
T_3 (1 mM)	96	95	90	92	89	80	9.66	7.88	6.94	11.48	9.08	8.38
T_4 (2 mM)	95	89	85	91	83	78	8.44	7.38	5.86	10.9	9.14	7.14
T_5 (3 mM)	95	86	70	89	78	0	7.98	6.94	NA	9	8.56	NA
T_6 (4 mM)	93	85	65	86	74	0	7	6.12	NA	8.46	7.1	NA
T_7 (5 mM)	91	83	62	84	69	0	6.44	6	NA	8.18	7.06	NA
T_8 (6 mM)	88	81	57	80	65	0	6.26	5.88	NA	7.3	6.12	NA
T_9 (7 mM)	86	67	52	76	58	0	5.92	5.36	NA	7.04	6.06	NA
T_{10} (8 mM)	66	52	46	59	45	0	5.5	4.92	NA	6.22	5.06	NA
T_{11} (9 mM)	42	37	32	36	31	0	4.78	4.5	NA	5.14	4.96	NA
T_{12} (10 mM)	38	28	21	32	21	0	3.9	3.54	NA	4.02	3.94	NA
S.Em± (Concentration)	0.59			0.56			0.25			0.34		
CD (*P*=0.05)	1.65			1.56			0.70			0.94		
S.Em± (Duration)	0.28			0.27			0.12			0.16		
C.D (*P*=0.05)	0.79			0.75			0.34			0.45		
S.Em± (Concentration × Duration)	1.02			0.96			0.432			0.585		
CD (*P*=0.05)	2.86			2.69			1.208			1.634		

Treatment details are given under Materials and Methods.

NA, Not available; S.Em, Standard error of mean; CD, Critical difference at 5% level of significance.

Table 3 Correlation coefficients (r) and regression equation for survival percentage of fenugreek with germination percentage, root length and shoot length

Independent variable (X)	Correlation (r)	Regression equation
Germination percentage	0.869**	y = 1.3463x - 41.567
Root length (cm)	0.923**	y = 8.5891x + 8.7188
Shoot length (cm)	0.947**	y = 7.7632x + 6.5443

** significant at 1% level of significance

shoot length. A perusal of data revealed that the survival percentage of fenugreek was significantly and positively correlated with germination percentage, root length and shoot length (Table 3). Since the correlation coefficients were found to be highly significant, a linear relationship existed between survival percentage and other parameters. The regression coefficients also worked out to know the quantum of change in survival percentage with the unit change in other parameters. Finally, it indicates that the survival percentage of mutagen-treated fenugreek seeds was markedly influenced by germination percentage, root length and shoot length.

SUMMARY

The present experiment was conducted in the year 2021 at Sri Karan Narendra Agriculture University, Jobner, Rajasthan, to determine the optimum dose (LD_{50}) and duration of the chemical mutagen treatment for the induction of desirable mutation. A set of 100 pre-soaked fenugreek seeds were treated with 12 different concentrations of sodium azide, viz. 0.3 mM, 0.6 mM, 1 mM, 2 mM, 3 mM, 4 mM, 5 mM, 6 mM, 7 mM, 8 mM, 9 mM and 10 mM for three different durations, viz. 3 h, 6 h and 9 h. Results showed that a dose-dependent decreasing tendency was observed in germination percentage, survival percentage, root length and shoot length with increasing concentration and duration of sodium azide treatment. Almost all the mutagenic treatments resulted in decrease in germination percentage, survival percentage and seedling height (root and shoot length) with increasing concentrations and duration of mutagen in laboratory conditions. Also, the LD_{50} value was observed as an 8 mM sodium azide concentration for 6 h in fenugreek. The mutagen treatments given at 9 h duration were detrimental for fenugreek. They cannot be used for mutation induction as they are utterly lethal after a 3 mM sodium azide concentration. So, lower treatments of mutagens have influenced less biological damage and would be suitable for inducing desirable mutations.

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