American-Eurasian J. Agric. & Environ. Sci., 8 (6): 779-783, 2010
ISSN 1818-6769

Comparative Spectrum of Sodium Azide Responsiveness in Plants

Rajani Prabha, Vineeta Dixit and B.R. Chaudhary

Labortary of Cytogenetics, Department of Botany,
Banaras Hindu University, Varanasi Uttar Pradesh, India
Labortary of Cytogenetics, Department of Botany,
Banaras Hindu University, Varanasi, 221001,Uttar Pradesh, India

Abstract: Seeds of plant species from three different and distantly related genera viz., *Nigella sativa* L., *Plantago ovata* F. and *Trigonella foenum graceum* L. were presoaked and then subjected to 1.5, 3.0 and 4.5 mM concentrations of sodium azide for 4, 6 and 8 hours. The experiment was carried out to find a specific concentration of mutagen which can produce significant effect on distantly related crops. Parameters used to screen biological effect of sodium azide were germination percentage, survivility, seedling height, number of branches, wet to dry weight ratio, 1000-seed weight and mutation frequency. In spite of phytotaxonomically distantly related plant systems most of the traits including mutation frequency showed significant variation at 4.5 mM of sodium azide. The findings have practical utilization as breeders working with random crops can utilize 4.5 mM concentration of sodium azide to bring out induced genetic diversities in the absence of prior reports/works.

Key words: Chemical mutation · Sodium azide · *Nigella sativa* · *Plantago ovata* · *Trigonella foenum graecum*

INTRODUCTION

Breeding for quantitative characters is successful when there exists genetic variability in the population. In some cases genetic progress for yield, which is of a quantitative nature, is difficult to achieve because population variability has already been either intensely exploited or underexploited that no new variety is released [1]. After the discovery of artificial induction of mutations [2-4], various kinds of physical and chemical mutagens had been and are being applied in induced mutagenesis [5-7].

The mutagen of choice here, as in many cultivated plants is HN_3, formed from sodium azide buffered at pH 3 [8,9]. Azide is only marginally mutagenic in humans and animal [10] and *Arabidopsis* [11] and hence quite safe to use. As in any mutagenic study the very first step is to find a very effective dose which can produce significant variations. The present study is confined to plants drawn from three genera-*Nigella, Trigonella* and *Plantago* belonging to family Ranunculaceae, Fabaceae and Plantaginaceae, respectively. According to phylogenetic classification Ranunculaceae (Eudicots) is the most primitive family followed in advancement by Fabaceae (Rosids) and than Plantaginaceae (Asteraceae).

Considering in mind the fact that sodium azide is converted into real mutagen in plant cells and such that mutagenic potentiality of sodium azide is affected by cellular metabolism, special attention is paid to the specificity of behavior with specific dose of the mutagen. This paper outlines an approach for determining a specific concentration which can bring out inheritable changes in different population of plants with various concentrations of mutagen and determines which concentration brings about significant variation at morphological level. Due to lack of uniformity in characters no comparative statistical study can be made in crops of different evolutionary tendencies. With the final aim of finding a specific concentration which can effectively produce morphological changes in distantly related species, the authors here suggest to bring the characters onto a common scale by calculating ratio of the character with its respective control and to analyze them statistically.

Corrosponding Author: B.R. Chaudhary, Labortary of Cytogenetics, Department of BotanyBanaras Hindu University, Varanasi, 221001,Uttar Pradesh, India, Tel: 91-9415812721, E-mail: brchaudhary.bhu@gmail.com.

MATERIALS AND METHODS

Healthy and dry (10-12% moisture) seeds of *Nigella sativa* L., *Plantago ovata* L. and *Trigonella foenum graceum* L. were subjected to three different concentrations (1.5, 3.0 and 4.5 mM) of sodium azide for 4, 6 and 8 hour durations just after presoaking of 14 hour in distilled water. Presoaking facilitates the uptake of mutagen by increasing cell permeability and also initiates metabolism in seeds. The treatments were periodically agitated and the volume of solution was maintained during treatment. The sodium azide (Merck) solution was prepared in 0.1M phosphate buffer at pH 3 and then diluted to each concentration just before treatment.

Immediately after treatment the seeds were thoroughly rinsed overnight under running tap water. A set of 150 seeds were kept in distilled water for varying periods to serve as control.

The experiment was replicated thrice times in RCBD with about 50 seeds per treatment per replication. Fifty seeds of each treatment along with control were germinated in separate petriplates lined with moist filter paper for % germination data. A total of 50 treated seeds from each concentration and treatment duration combinations were planted I n the field along with control blocks comprised of untreated seeds for the comparison of all parameters studied.

Germination % was calculated as per cent of control. % survivility was calculated as per total plant survived at maturity. Height was taken at seedling stage for all the three crops. Chlorophyll was estimated following [12]. Mutation frequency was calculated in M_2 generation following to [13]. Seeds from the sodium azide treated M_1 plants were sown for M_2 generation plants and similar readings were taken as in M_1 generation.

Data collected for various quantitative traits in M_1 and M_2 generation was statistically analyzed using analysis of variance ANOVA and Duncan's multiple range test was used to separate the means on SPSS software.

RESULTS AND DISCUSSION

The biological effects of different concentrations of sodium azide on seed germination and plant survival are tabulated in Table 1, while the frequency of mutation is presented in Fig. 1 for all the three genera studied here. Germination percentages for *Nigella, Plantago* and *Trigonella* differed significantly. All the three crops showed minimum germination at the same concentration (i.e. 4.5 mM) and duration of exposure (i.e. 8 hrs) of sodium azide. With the treatment of sodium azide, *Nigella* exhibited maximum mutation frequency followed by *Trigonella*, whereas it was recorded minimum for *Plantago*. No common observation has been previously recorded, the present author's, however, suggest that since *Nigella* is a member of primitive family Ranunculaceae, it does not seem to have developed defense mechanism against oxidation stress whereas the other two genera belonging to advanced families Fabaceae and Plantaginaceae must have developed it. The highest mutation frequency was induced by 4.5 mM NaN_3 used for 4 hours in *Nigella* and *Trigonella*, where as in *Plantago* the same was achieved by 4.5 mM applied for a period of 6 hours, indicating the said treatments as optimal doses.

LSD analysis (Table 2) for all the three genera did not reveal any general trend with respect to character responses against the applied mutagen doses and has been found to vary unevenly. The study, however has

Table 1: Effect of sodium azide treatments on germination and survival percentage

Treatment		Germination %			Survivility %		
Dur. (hrs)	Conc (mM)	*Nigella*	*Plantago*	*Trigonella*	*Nigella*	*Plantago*	*Trigonella*
control		100.0±1.15	100.0±2.65	100.0±1.73	100.0±1.73	100.0±4.32	100.0±2.40
4	1.5	63.04±2.31	90.15±2.91	81.56±2.33	60.00±4.58	89.64±5.60	87.59±2.79
4	3.0	60.86±2.00	85.61±2.00	80.56±1.73	54.81±2.67	83.20±4.23	83.72±1.34
4	4.5	52.17±3.05	81.06±1.73	76.95±2.02	41.48±2.03	76.80±5.54	81.39±1.34
6	1.5	60.86±3.46	88.63±3.61	80.14±1.76	54.07±0.67	86.40±4.95	82.94±2.05
6	3.0	58.69±3.05	85.61±2.40	78.72±2.08	49.62±1.85	80.80±4.23	79.84±3.87
6	4.5	47.82±2.00	81.06±2.31	75.17±2.33	40.00±3.51	74.40±3.66	72.09±1.34
8	1.5	52.17±4.00	84.09±2.31	78.36±2.03	44.44±1.15	80.00±4.23	81.39±2.34
8	3.0	45.65±2.00	71.21±1.76	76.24±2.18	37.78±1.52	66.40±4.45	74.41±2.67
8	4.5	43.47±1.15	56.81±1.15	74.11±3.17	31.11±2.00	52.00±4.45	65.89±2.34

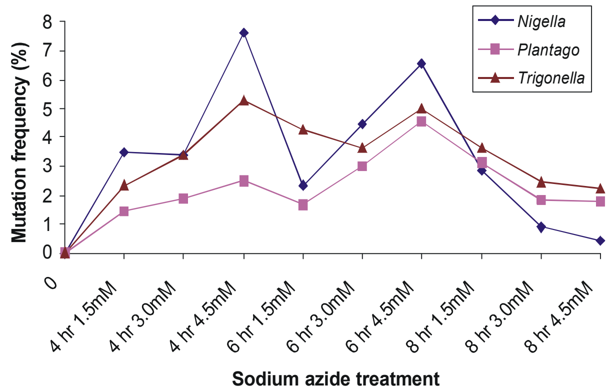

Fig. 1: Effect of sodium azide treatments on mutation frequency

Table 2: Effect of sodium azide on morphological traits of *Nigella, Plantago* and *Trigonella* species in M_1 and M_2 generation

Treatment		Plant height (cm)						No. of branches						
		Nigella		*Plantago*		*Trigonella*		*Nigella*		*Plantago*		*Trigonella*		
Duration (hrs)	Conc. (mM)	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M2	
0	0.0	$2.6^{b}\pm0.08$	$2.8^{bc}\pm.05$	$4.5^{a}\pm.25$	$4.3^{a}\pm0.21$	$5.6^{a}\pm0.66$	$5.1^{ab}\pm0.15$	$12.0^{a}\pm0.17$	$11.8^{a}\pm0.17$	$4.12^{a}\pm0.06$	$4.01^{ab}\pm0.16$	$5.0^{ba}\pm0.38$	$4.8^{ab}\pm0.20$	mean
		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	mt/mc
4	1.5	$2.8^{a}\pm0.31$	$4.1^{a}\pm1.27$	$4.3^{ab}\pm0.15$	$4.2^{ab}\pm0.11$	$5.33^{ab}\pm0.24$	$5.4^{a}\pm.20$	$11.7^{ab}\pm0.92$	$6.56^{b}\pm0.49$	$4.05^{a}\pm0.05$	$4.13^{a}\pm0.16$	$5.66^{a}\pm0.56$	$5.33^{a}\pm0.49$	mean
		1.4	1.53	0.95	0.97	0.95	1.05	0.97	0.55	0.98	1.02	1.13	1.10	mt/mc
4	3.0	$2.6^{b}\pm0.31$	$4.0^{a}\pm2.71$	$3.93^{abcd}\pm0.24$	$4.0^{abc}\pm0.15$	$4.97^{abcd}\pm0.14$	$5.0^{ab}\pm0.25$	$9.2^{abc}\pm2.65$	$6.43^{bc}\pm1.29$	$3.9^{abc}\pm0.14$	$3.81^{ab}\pm0.18$	$3.83^{bc}\pm0.16$	$3.83^{ab}\pm0.21$	mean
		0.96	1.5	0.87	0.93	0.89	0.98	0.76	0.54	0.93	0.95	0.76	0.79	mt/mc
4	4.5	$2.3^{c}\pm0.35$	$3.4^{abc}\pm0.45$	$3.6^{cde}\pm0.15$	$3.8^{abc}\pm0.10$	$4.03^{d}\pm0.15$	$4.6^{ab}\pm0.21$	7.96c±1.38	$4.56^{bcd}\pm0.94$	$3.84^{abc}\pm0.13$	$3.6^{ab}\pm0.15$	$4.33^{abc}\pm0.48$	$4.53^{ab}\pm0.84$	mean
		0.88	1.21	0.8	0.88	0.72	0..90	0.66	0.38	0.93	0.89	0.86	0.93	mt/mc
6	1.5	$2.5^{b}\pm0.10$	$3.3^{abc}\pm0.32$	$4.13^{abc}\pm0.14$	$4.1^{abc}\pm0.21$	$5.06^{abc}\pm0.13$	$5.0^{ab}\pm0.15$	$7^{c}\pm0.40$	$4.70^{bc}\pm0.63$	$3.97^{ab}\pm19$	$4.0^{ab}\pm0.18$	$4.5^{abc}\pm0.40$	$4.23^{ab}\pm0.67$	mean
		0.9	1.17	0.91	0.95	0.90	0.98	0.58	0.39	0.96	0.99	0.9	0.87	mt/mc
6	3.0	$2.5^{b}\pm0.41$	$3.7^{ab}\pm0.10$	$3.7^{bcde}\pm0.17$	$3.9^{abc}\pm0.26$	$4.53^{bcd}\pm0.35$	$4.7^{ab}\pm0.10$	$7.6^{c}\pm0.21$	$3.83^{d}\pm0.62$	$3.68^{abc}\pm0.16$	$3.68^{ab}\pm0.19$	$3.96^{bc}\pm0.37$	$3.83^{ab}\pm0.44$	mean
		0.73	1.32	0.82	0.92	0.81	0.92	0.63	0.32	0.89	0.91	0.79	0.79	mt/mc
6	4.5	$2.3^{c}\pm0.21$	$3.4^{abc}\pm0.25$	$3.4^{de}\pm0.11$	$3.6^{bc}\pm0.15$	$5.17^{abc}\pm0.37$	$4.4^{b}\pm0.10$	$8.3^{b}\pm0.69$	$4.3^{cbd}\pm0.47$	$3.51^{bc}\pm0.14$	$3.45^{b}\pm0.19$	$3.7^{bc}\pm0.34$	$3.6^{b}\pm0.55$	mean
		0.69	1.21	0.76	0.83	0.97	0.86	0.69	0.36	0.85	0.86	0.74	0.74	mt/mc
8	1.5	$2.5^{b}\pm0.26$	$2.6^{c}\pm0.32$	$3.8^{bcde}\pm0.21$	$3.7^{abc}\pm0.11$	$5.43^{ab}\pm0.37$	$4.9^{ab}\pm0.62$	$8.7^{ab}\pm0.63$	$4.6^{bcd}\pm0.57$	$3.83^{abc}\pm0.19$	$3.87^{ab}\pm0.17$	$4.43^{abc}\pm0.67$	$4.17^{a}\pm0.49$	mean
		0.65	0.93	0.84	0.86	0.97	0.96	0.73	0.38	0.92	0.96	0.88	0.86	mt/mc
8	3.0	$2.4^{bc}\pm0.36$	$4.2^{a}\pm0.21$	$3.43^{de}\pm0.20$	$3.6^{bc}\pm0.25$	$4.66^{abcd}\pm0.13$	$4.7^{ab}\pm0.25$	$8.0^{c}\pm0.72$	$3.5^{d}\pm0.23$	$3.54^{bc}\pm0.11$	$3.57^{ab}\pm0.27$	$3.33^{c}\pm0.33$	$3.4^{b}\pm0.49$	mean
		0.57	1.64	0.76	0.84	0.83	0.92	0.66	0.29	0.85	0.84	0.67	0.70	mt/mc
8	4.5	$2.2^{c}\pm0.25$	$3.3^{abc}\pm0.25$	$3.2^{e}\pm0.26$	$3.5^{c}\pm0.26$	$4.23^{cd}\pm0.18$	$4.5^{b}\pm30.11$	$7.7^{c}\pm0.57$	$4.5^{bcd}\pm0.29$	$3.4^{jc}\pm0.16$	$3.38^{b}\pm0.23$	$3.6^{bc}\pm0.37$	$3.26^{b}\pm0.34$	mean
		0.46	1.17	0.71	0.81	0.75	0.88	0.64	0.38	0.83	0.84	0.74	0.67	mt/mc

Treatment		Dry/weight						1000-seed weight (g)						
		Nigella		*Plantago*		*Trigonella*		*Nigella*		*Plantago*		*Trigonella*		
Duration (hrs)	Conc. (mM)	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M_2	M_1	M2	
0	0.0	$7.39^{c}\pm0.15$	$7.18^{a}\pm0.05$	$4.20^{bc}\pm.0.15$	$4.28^{de}\pm0.14$	$4.25^{c}\pm0.04$	$4.28^{de}\pm0.07$	$2.58^{a}\pm0.06$	$2.62^{b}\pm0.07$	$2.36^{c}\pm0.01$	$2.53^{c}\pm0.02$	$17.41^{b}\pm0.07$	$16.80^{b}\pm0.05$	mean
		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	mt/mc
4	1.5	$8.02^{bc}\pm0.39$	$7.25^{a}\pm0.14$	$5.23^{cd}\pm0.10$	$4.61^{bc}\pm0.11$	$4.51^{b}\pm0.06$	$4.61^{cd}\pm0.07$	$1.94^{cd}\pm0.02$	$2.63^{b}\pm0.04$	$2.17^{d}\pm0.07$	$2.32^{d}\pm0.01$	$15.36^{e}\pm0.05$	$14.66^{f}\pm0.09$	mean
		1..09	0.89	1.24	1.25	1.06	1.07	0.75	1.00	0.91	0.91	1.13	0.87	mt/mc
4	3.0	$8.12^{bc}\pm0.22$	$6.26^{b}\pm0.39$	$4.81^{a}\pm0.23$	$4.38^{bcd}\pm0.15$	$3.41^{d}\pm0.07$	$4.87^{bc}\pm0.19$	$2.26^{b}\pm0.07$	$2.27^{c}\pm0.03$	$2.43^{c}\pm0.01$	$2.57^{c}\pm0.02$	$12.70^{h}\pm0.07$	$15.17^{d}\pm0.10$	mean
		1.23	0.87	1.14	1.15	0.80	1.13	0.87	0.86	1.03	1.01	0.76	0.90	mt/mc
4	4.5	$9.12^{a}\pm0.73$	$6.42^{b}\pm0.32$	$6.12^{de}\pm0.21$	$3.38^{a}\pm0.25$	$4.71^{d}\pm0.07$	$3.38^{f}\pm0.07$	$1.89^{cde}\pm0.06$	$3.01^{a}\pm0.11$	$2.89^{a}\pm0.02$	$3.32^{a}\pm0.02$	$15.92^{d}\pm0.12$	$17.44^{a}\pm0.07$	mean
		1.23	0.89	1.45	1.44	1.11	0.79	0.73	1.14	1.22	1.32	0.86	1.03	mt/mc
6	1.5	$8.11^{bc}\pm0.10$	$6.66^{ab}\pm0.32$	$4.40^{f}\pm0.27$	$3.50^{cde}\pm0.21$	$4.59^{b}\pm0.07$	$3.50^{f}\pm0.20$	$2.06^{cd}\pm0.01$	$1.23^{e}\pm0.04$	$2.53^{b}\pm0.06$	$2.70^{d}\pm0.02$	$18.50^{a}\pm0.03$	$17.26^{a}\pm0.11$	mean
		0.11	0.98	1.04	1.07	1.10	0.81	0.79	0.47	1.06	1.06	0.9	1.02	mt/mc
6	3.0	$8.13^{bc}\pm0.18$	$6.29^{b}\pm0.13$	$3.83^{abc}\pm0.28$	$4.62^{e}\pm0.32$	$3.53^{d}\pm0.06$	$4.62^{cd}\pm0.07$	$1.78^{de}\pm0.04$	$2.77^{b}\pm0.08$	$2.60^{b}\pm0.05$	$2.12^{f}\pm0.02$	$16.32^{c}\pm0.02$	$15.78^{c}\pm0.03$	mean
		1.09	0.87	0.91	0.90	0.83	1.07	0.69	1.05	1.09	0.83	0.79	0.93	mt/mc
6	4.5	$9.04^{ab}\pm0.13$	$6.3^{b}\pm0.10$	$5.^{38de}\pm0.20$	$5.34^{ab}\pm0.27$	$5.23^{a}\pm0.08$	$5.34^{a}\pm0.08$	$1.75^{e}\pm0.02$	$1.90^{c}\pm0.06$	$2.10^{de}\pm0.04$	$2.52^{c}\pm0.03$	$13.17^{g}\pm0.04$	$13.69^{g}\pm0.04$	mean
		1.22	0.88	1.28	1.31	1.23	1.24	0.67	0.72	0.89	0.99	0.74	0.81	mt/mc
8	1.5	$8.06^{bc}\pm0.10$	$6.28^{b}\pm0.11$	$4.13d^{e}\pm0.46$	$3.61^{de}\pm0.30$	$4.11^{c}\pm0.02$	$3.61^{f}\pm0.11$	$1.91^{ce}\pm0.01$	$1.62^{d}\pm0.05$	$2.35^{c}\pm0.02$	$2.70^{b}\pm0.01$	$15.88^{d}\pm0.05$	$16.00^{c}\pm0.95$	mean
		1.09	0.84	0.98	0.99	0.97	0.84	0.74	0.61	0.99	1.06	0.88	0.86	mt/mc
8	3.0	$8.75^{ab}\pm0.15$	$6.0^{b}\pm0.06$	$3.89^{e}\pm0.26$	$4.09^{e}\pm0.32$	$3.60^{d}\pm0.12$	$4.09^{e}\pm0.09$	$1.94^{cd}\pm0.06$	$1.70^{cd}\pm0.03$	$2.07^{e}\pm0.03$	$2.20^{e}\pm0.01$	$14.06^{f}\pm0.04$	$13.48^{g}\pm0.80$	mean
		1.18	0.84	0.92	0.93	0.84	0.95	0.75	0.64	0.87	0.86	0.67	0.70	mt/mc
8	4.5	$9.01^{ab}\pm0.05$	$6.20^{b}\pm0.03$	$5.64^{ab}\pm0.17$	$5.13^{bcd}\pm0.32$	$4.6^{b}\pm0.06$	$5.13^{ab}\pm0.24$	$7.7^{cd}\pm0.03$	$1.33^{e}\pm0.03$	$1.41^{f}\pm0.02$	$1.37^{g}\pm0.01$	$9.92^{i}\pm0.03$	$9.01^{h}\pm0.07$	mean
		1.22	0.86	1.34	1.14	1.08	1.19	0.75	0.51	0.59	0.54	0.74	0.53	mt/mc

Means with the same letters within the same columns are non significant

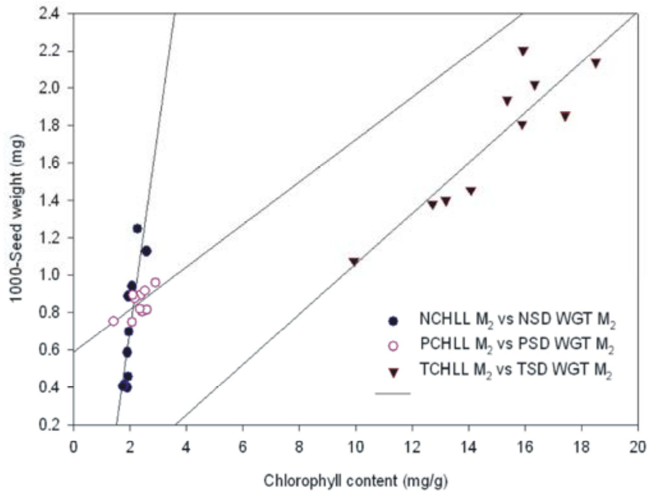

Fig. 2: Correlation graph between 100 seed weight (M_2) and chlorophyll content (M_2) rn=0.838; rp=0.644; rt=0.902

Table 3: Mean square estimation of the effects of sodium azide on three genera

Source of variation	Df	Seedling height M_1	Seedling height M_2	No of branches M_1	No of branches M_2	Wet/dry weight M_1	Wet/dry weight M_2	Seed weightM1	Seed weight M_2
Treatment	9	0.099^{ns}	1.205***	0.299***	1.751***	0.280***	0.368***	0.248***	0.172*
Error	80	0.019	0.031	0.026	0.034	0.024	0.022	0.019	0.038

*** significant at. 001

** significant at. 05

Table 4: Effects of sodium azide on three genera

Source of variation	Seedling height M_1	Seedling height M_2	No. of branches M_1	No. of branches M_2	Wet/Dry weight M_1	Wet/Dry weight M_2	Seed weight M_1	Seed weight M_2
Control	1^a	1^a	1^a	1^a	1^b	1^a	1^a	1^a
1.5 mM	0.8939^b	1.0481^a	0.8977^a	0.7976^b	1.0898^b	0.9877^a	0.9007^b	0.8881^{ab}
3.0 mM	$0.8064b^c$	1.1069^a	0.7769^b	0.6905^b	0.9985^b	0.9744^a	0.8646^{bc}	0.8816^{ab}
4.5 mM	0.7448^c	0.9765^a	0.7711^b	0.6774^b	1.2635^a	1.0883^a	0.7878^c	0.8466^b

Means with the same letters within the same columns are non significant.

shown that as the time period for treatment is increased the lower concentrations (i.e. 1.5 and 3.0 mM) bring about the effect of higher dose (i.e. 3.0 and 4.5 mM). This behavior is more prominent in *Plantago* and *Trigonella*, whereas in *Nigella* a general decreasing pattern was encountered in case of seedling height. This can be attributed to the fact that NaN_3 is converted into real mutagen in plant cells [14,15]. In *Nigella, Plantago* and *Trigonella*, the effect of NaN_3 is multiplied with time as reported by Hasegawa and Inoue [16].

Relative analysis between 1000-seed weight and chlorophyll content in M_2 generation plants presented as correlation graph in Fig. 2. showed positive correlation in all the 3 plants. Correlation coefficient varies from 0.90 to 0.64 not reaching to 0.99 [17]. The reason might be that the previous reports are on EMS which does not affect chlorophyll synthetic machinery, whereas azide inhibits photosynthesis and thus has direct effect on photo-assimilation. Since azide is converted into effective mutagen (HN_3) in plant cells and its converting potential is dose and pH dependent [8] different doses of HN_3 contribute differentially to chlorophyll content and so to the seed weight and wet to dry weight ratio. M_1 and M_2 generations showed highly significant difference (P<0.05) for all the traits studied in *Nigella*, *Plantago* and *Trigonella* species exposed to sodium azide (Table 3). The results indicated that the mutagen used is effective for all the three crops, showing significant variation in nearly all the quantitative traits studied. There were highly significant differences (P<0.01) in almost all the traits studied i.e., seedling height, wet to dry weight ratio, 1000-seed weight in M_1 and the number of branches per plant in both M_1 and M_2 generation (Table 4) with 4.5 mM sodium azide treatment, indicating the said dose to be the most effective mutagenic dose for all the three genera, investigated.

ACKNOWLEDGEMENTS

Financial assistance provided by UGC, BHU fellowship, to the authors is gratefully acknowledged.

Aberration: rn= Correlation coefficient of *Nigella sativa*; rp=Correlation coefficient of *Plantago ovata*; rt= Correlation coefficient of *Trigonella foenum graecum:* NCHLL=Chlorophyll content of *Nigella sativa*; NSD WGT=1000-seed weight of *Nigella sativa*; PCHLL= Chlorophyll content of *Plantago ovata*; PSD WGT=100-seed weight of *Plantago ovata*; TCHLL=Chlorophyll content of *Trigonella foenum graecum*; TSD WGT=1000-seed weight of *Trigonella foenum graecum:* Mt/mc=Mean of treatment/mean of control

REFERENCES

1. Scossiroli, R.E., 1970. Mutations in characters with continuous variation. In: FAO/IAEA. Manual on Mutation Breeding (IAEA, ed.). Technical Report Series, Vienna, pp: 117-123.
2. Muller, H.J., 1927. Artificial transmutation of the gene. Science, 66: 84-87.
3. Stadler, L.J., 1928. Mutation in barley induced by X-rays and radium. Science, 68: 186-187.
4. Auerbach, C.H. and J.M. Robson, 1946. Chemical production of mutation. Nature, 157: 302.
5. Heslot, H., 1977. Review of main mutagenic compounds. In Manual on Mutational Breeding, 2nd Ed FAO/IAEA. Technical Report Series, 119: 51-59.
6. Vaidya, K.R. and M.M. Young, 1993. Ethyl mthanesulphonate induced variation in qualitative and quantitative characters of roselle (*Hibiscus sabdariffa* L.) (Malvaceae). Brazilian J. Genetics, 16(2): 381-291.
7. Khursheed, T., M.Y.K. Ansari and V. Shahab, 2009. Studies on the effect of caffeine on growth and yield parameters in *Helianthus annuus* L. variety Modern. Toxeminar, 1(2): 56-60.
8. Kleinhofs, A., Owais, W.M. and R.N. Nilan, 1978. Azide. Mutation research, 55: 165-195.
9. Awan, M.A., C.F. Konzak, J.N. Rutger and R.N. Nilan, 1980. Mutagenic effects of sodium azide in rice. Crop Science, 20: 663-668.
10. Sadiq, M.F. and W.M. Owais, 2000. Mutagenicity of sodium azide and its metabolite azidoalanine in *Drosophila melanogaster*. Mutation Research, 469: 253-257.
11. Gichner, T. and J. Veleminsky, 1972. Differential response of mutagenic effects to storage of barley seeds treated with propyl methanosulphonate and isopropyl methane-sulphonate. Mutation Research, 16: 35-40.
12. Arnon, D.L., 1949. A copper enzyme is isolated chloroplasts polyphenol oxidase in *Beta velgaris*. Plant Physiology, 24: 1-15.
13. Gaul, H., 1964. Mutation in plant breeding. Radiation Botany, 4: 155-232.
14. Owais, W.M., M.A. Zarowitz, R.A. Gunovich, A.L. Hodgdon and R.N. Nilan, 1978. A mutagenic *in vivo* metabolite of sodium azide. Mutation Research, 53: 355-358.
15. Veleminsky, J., L. Silhankova, V. Smiovska and T. Gichner, 1979. Mutagenesis of *Saccharomyces cerevisiae* by sodium azide activated in barley. Mutation Research, 61: 197-205.
16. Hasegawa, H. and M. Inoue, 1980. Mutagenic effect of sodium azide in barley. Japanese Journal of Breeding, 30: 20-26.
17. Larik, A.S. and Y.A. Al-Saheal, 1987. Regulatory effect of ethyl-methane sulphonate on chlorophyll metabolism in *Triticum aestivum* L. Cytologia, 52: 75-79.