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Mutagenic Effects of Sodium Azide on the Growth and Yield Characteristics in Wheat (*Triticum aestivum* L. em. Thell.)

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Abstract: Sodium azide induced polygenic variably was studied on promising wheat variety HD-2733 in two subsequent cropping seasons during 2007-2009. For chemical treatment, 100 genetically pure seeds were soaked in distilled water for 6 h, blotted dry and treated with freshly prepared mutagenic solution of 0.02, 0.04% and 0.06% concentration In laboratory germination, root length and shoot length was observed. Among the different concentration of sodium azide, the highest germination was recorded at control (99.55%) followed by 0.02% concentration (97.11%), 0.04% concentration (95.55%) and lowest at 0.06% concentration (85.77%). Higher concentration of sodium azide reduces the germination percentage, root length and shoot length; however, at low concentration it was at par with control. The magnitude of genotypic and phenotypic variability, heritability and genetic gain for various polygenic traits were also decreases with the increases in concentration of sodium azide. However, yield attributing characters showed both positive and negative shift in mean than those of control. Some of the mutant lines (eight progeny for earliness, one for plant height, three for spike length and grain yield each, two for tillering and four for test weight) were found desirable. These lines were either comparable to or better than control for yield and its components. It is concluded that sodium azide with 0.02% concentration appear to be the most effective mutagenic treatment for induction of micro-mutation in yield component traits and selection in M_2 populations of these treatment would be effective in rectification of simply inherited morphological deficiencies and bringing out lines with yield improvement.

Key words: Mutation, sodium azide, agro-morphometric traits, crop improvement

INTRODUCTION

The prime strategy in mutation breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits which limit their productivity or enhance their quality. Wheat is an important food crop of the world. One way of creating variability in such a self pollinated crop is attempting crosses between two genotypes complementing the characters of each other but due to autogamous nature of the crop, hybridization at appropriate time is a difficult process. The only alternative left with breeders to create variability is mutation breeding. This method can be used as a potential source of creating variability (Novak and Brunner, 1992).

Mutations have played a great role in increasing world food security, since new food crop varieties embedded with various induced mutations have contributed to the significant increase of crop production (Kharkwal and Shu, 2009). Mutation induction offers the possibility of inducing desired attributes that either cannot be found in nature or have been lost during evaluation. Treatment with mutagens alters genes or breaks chromosomes. Gene mutations occur naturally as errors in DNA replication. Most of these errors are repaired but some may pass to the next cell division to become established in the plant offspring as spontaneous mutations. Gene mutations without phenotypic expressions are usually not recognized. Consequently, genetic variation appears rather limited and breeders have to resort to mutation induction (Novak and Brunner, 1992).

Chemical mutagenesis is regarded as an effective and important tool in improving the yield and quality characters of crop plants. In general alkylating agents are very effective mutagens in higher plants. However, Sodium azide has also proved its worth as chemical mutagens to induce genetic variability. Thus, this

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chemical mutagen has become important tool to enhance agronomic traits of crop plants. The role of mutation breeding in increasing the genetic variability for quantitative traits in various crop plants have been proved beyond doubt by a number of scientists (Khan *et al*., 1998, 1999; Das and Chakraborty, 1998; Rachovska and Dimova, 2000; Baloch *et al*., 2002; Kumar and Mishra, 2004; Erdem and Oldacay, 2004; Ilbas *et al*., 2005; Khan and Wani, 2006; Singh *et al*., 2006; Wani and Khan, 2006; Tah, 2006; Addai and Kantanka, 2006; Bhat *et al*., 2007; Seneviratne and Wijesundara, 2007; Tai *et al*., 2007; Adamu and Aliyu, 2007; Khan and Goyal, 2009; Kozgar *et al*., 2011; Mostafa, 2011).

The mutagenesis work in wheat has been reviewed and reported by Subudhi *et al*. (1991), Reddy (1992) and Rachovska (1995 and 1998). Bread wheat, *Triticum aestivum* L. (2n = 6x = 42 = AABBDD) being a polyploid, offers many opportunities exploitation of mutations, recombination and of increasing genetic variability in quantitatively inherited characters (Siddiqui *et al*., 2007). The presence of many triplicated and duplicated loci in wheat allows a large number of induced changes to be preserved and transmitted to the next generation. Also, with the advent of so called green revolution interest in the induction of directed changes has considerably increased for redesigning ideotypes suitable for various agricultural environments. Induced mutations are also useful when it is desired to improve one or two easily identifiable characters in an otherwise well adapted variety.

Sodium azide (NaN_3) is a chemical mutagen and has been one of the most powerful mutagens in crop plants. The mutagenicity is mediated through the production of an organic metabolite of azide compound. This metabolite enters into the nucleus, interacts to DNA and creates point mutation in the genome. Several factors such as properties of mutagens, duration of treatment, pH, pre and post treatment, temperature and oxygen concentrations etc. influence the effect of mutagens. The dose of a mutagen applied is an important consideration in any mutagenesis programme. Generally, it was observed that higher the concentrations of the mutagen greater the biological damage. Azide is perhaps the least dangerous and the most efficient mutagen in that high yields of mutations are achieved at moderate M_1 sterility rates. Although, in some cases it has been reported that treatments with sodium azide, the physiological effects of azide are weak, few chromosomal aberrations are induced and it delays germination and growth. However, Azide-treated seeds show complete apparently normal growth in M_1 except for M_1 sterility and a high frequency of M_1 chlorophyll chimaeras. To enhance the mutagenic effectiveness and efficiency of sodium azide and especially the metabolite, more knowledge about the effect of time, pH value, temperature, seed soaking and various concentrations are required (Khan *et al*., 2009).

The present studies have provided evidence on the induction of genetic variability connected with yield and yield components in wheat crop. Thus, induced genetic variability can effectively be exploited for evolving mutant strains possessing desirable attributes and for rectification of simply inherited morphological deficiencies.

MATERIALS AND METHODS

Data collection: The genotype used for mutagenic treatment was HD-2733, a promising and leading wheat variety which was obtained from Wheat Breeding Programme, Directorate of Research, Allahabad Agricultural Institute-Deemed University. It is a high yielding variety, suitable to grow in Allahabad Agro climatic conditions under timely and late sown condition. Three different concentrations of sodium azide (0.02, 0.04 and 0.06%) were freshly prepared for conducting the mutagenic treatments. For each chemical treatment, 100 seeds were taken which were soaked in distilled water for 6 h. After pre-soaking the seeds were blotted, dry and treated with freshly prepared chemical mutagen solution of sodium azide in different concentration of 0.02, 0.04 and 0.06%. The seeds were kept in the mutagenic solution for 6 h at room temperature 26±2°C with intermittent shaking for providing uniform treatment to the dipped seeds. An equal number of same genotypes were soaked in distilled water which served as control. After the treatment time is over, the seeds were thoroughly washed in running tap water for two hours and then blotted dry. Out of 100 seeds, 30 seeds were sown in petridishes for all the three concentrations and in control for laboratory experiment. In the laboratory observations were recorded germination percent, root length and shoot length. The laboratory experiment was followed by field experiments.

M_1 generation: The sowing of M_1 generation was done on 25th November, 2007 in four different plots keeping 25×5 cm spacing at Field Experimentation centre of the Department of Genetics and Plant Breeding, Allahabad Agricultural Institute-Deemed University, Allahabad. This experimental site is situated at 25.87°N latitude and 81.5°E latitude and 98 meter above the sea level. In M_1 generation the observations on germination, flowering, seedling survival and other characters were noted. The first spike of randomly selected plants was selfed by

using paper bags in treated population. After the crop was harvested 16 plants from each concentration were selected randomly for post harvest observations. Plants within the population of different concentrations were further designated as F_1P_1, F_1P_2...., F_1P_{16}. Similarly, in all others concentrations 16 random plants were selected and designated as above.

M_2 generation: The seeds of all randomly selected 16 plants were harvested from each concentration separately and were grown in augmented randomized compact family block design with two replications during next crop season i.e. winter 2008-09. Each entry was sown in two rows plot of 2 m length with a spacing of 30×5 cm. The all recommended agronomic packages and practices were done timely to raise good crop stand. The observations recorded in M_2 generation for eight polygenic traits were viz.; days to 50% flowering, days to 50% maturity, plant height (cm), spike length (cm), flag leaf width (cm), number of tillers per plant, 1000 seed weight (g) and seed yield per plant (g).

Statistical analysis: All obtained data were subjected to the analysis of variance according to the procedure outlined by Steel and Torrie (1982). The estimation of variability was done on family and population basis using the standard statistical procedure (Panse and Sukhatme, 1967). The observed F-value was compared against the corresponding table value given by Fisher and Yates (1957) for deciding the significance between progenies and within progenies component of variances. PCV and GCV were calculated by the formula given by Burton (1952), heritability in broad sense (h^2) by Burton and de Vane (1953) and expected genetic gain were calculated by using the procedure given by Johnson *et al.* (1955).

RESULTS

In M_1 generation the observations recorded on germination and growth of seedlings in laboratory. Out of 100 treated seeds, 30 seeds were sown in petridishes for 3 different concentrations (0.02, 0.04 and 0.06%) of sodium azide along with a control. The results indicated that sodium azide significantly influences the rate of growth and germination in wheat. The salient findings are summarized as following heads.

Germination percentage: The data recorded on seed germination at 7, 10 and 14th Days After Sowing (DAS) in three replications illustrated that among the different concentration of sodium azide, 97.11% germination was observed in 0.02% followed by 95.55% germination in 0.04% concentration and 85.77 in 0.06% concentration and 99.55% in control (Table 1). These results depicted that with the increase in concentration of mutagen there was a decrease in germination percentage of wheat seeds.

Root length (cm): The root length of the treated seeds was recorded at 7, 10 and 14th days after sowing. The observed data (Table 2) depicted that the highest root length was observed in control 7.53 cm followed by 6.36 cm in 0.02%, 6.21 cm in 0.04% and 5.93 cm in 0.06% concentration.

Shoot length (cm): A close perusal of data (Table 3) illustrated that the shoot length of the treated seeds was 6.58 cm in control, 6.15 cm in 0.02% concentration, 5.54 in 0.04% concentration and 5.01 in 0.06% concentration, when observations were made at 7, 10 and 14th days after sowing in three replications. Induced polygenic variability in respect of growth and yield contributing traits were

Table 1: Germination percent at 7th, 10th and 14th days after sowing

	R I			R II			R III			
Treatment	7	10	14	7	10	14	7	10	14	Mean (%)
Control	100	100	100	100	100	100	98	100	98	99.55
0.02%	98	97	97	98	97	97	96	98	96	97.11
0.04%	96	94	92	96	96	98	94	98	96	95.55
0.06%	85	90	90	90	84	84	82	83	84	85.77

R: Replication

Table 2: Root length (cm) at 7th, 10th and 14th days after sowing

	R I			R II			R III			
Treatment	7	10	14	7	10	14	7	10	14	Mean (cm)
Control	7.2	7.4	7.4	7.2	7.6	7.9	7.4	7.8	7.9	7.53
0.02%	6.4	6.6	6.7	6.2	6.4	6.5	5.9	6.2	6.4	6.36
0.04%	6.2	6.4	6.4	5.8	5.9	6.2	6.3	6.3	6.4	6.21
0.06%	5.4	5.5	5.5	5.2	5.3	5.4	5.5	5.6	5.5	5.93

R: Replication

studied in M_2 generation indicated that higher concentration of sodium azide reduces the germination percentage, root length and shoot length; however, at low concentration it was at par with control.

Significant variance was observed between families for days to maturity (53.4375**), plant height (218.894**), spike length (11.6169**), flag leaf width (0.0472*), number of tillers per plant (35.9670**), test weight (20.5072*) and grain yield per plant (1769.239**) whereas within progenies it was non-significant for most of the characters, indicating that variance within progeny was not different than the control except for spike length for 0.02% concentration and test weight for 0.06% concentration (Table 4).

Progeny means for different quantitative characters: A critical study of progeny mean (Table 5) reveals that high and/or at par progeny mean value was observed for all the quantitative traits studied in 0.02% concentration of sodium azide. Among them in family I (control) days to flowering ranged from 73 (F_1P_7 and F_1P_{13}) to 76 days (F_1P_{11} and F_1P_{14}) in; in family II (0.02% concentration) from 73 (F_2P_2 and F_2P_6) to 76 days (F_2P_{14} and F_2P_{16}); in family III (0.04% concentration) ranged from 73 (F_3P_9 and F_3P_{12}) to 76 days (F_3P_1 and F_3P_3) and in family IV (0.06% concentration) it was ranged from 72 (F_4P_{12}) to 76 days (F_4P_8).

Days to maturity in different families showed that it varied from 108.00 (F_1P_9) to 112.50 days (F_1P_{14}) in family I (control), from 106.50 (F_2P_{11}) to 114.50 days (F_2P_7) in family II (0.02% concentration), from 107.00 (F_3P_{13}) to 109.50 days (F_3P_3 and F_3P_6) in family III (0.04% concentration) and 105.00 (F_4P_{13}) to 109.00 days (F_4P_3) in family IV (0.06% concentration).

The estimated value of plant height ranged from 76.27 (F_1P_8) to 83.43 cm (F_1P_6) in family I (control), from 69.31 (F_2P_6) to 81.52 cm (F_2P_3) in family II (0.02% concentration) from 57.08 (F_3P_6) to 81.25 cm (F_3P_4) in family III (0.04% concentration) and from 69.31 (F_2P_6) to 81.52 cm (F_2P_3) in family IV (0.06% concentration).

Variation in length of the spike ranged from 9.37 (F_1P_{11}) to 10.18 cm (F_1P_1) in family I (control), from 7.87 (F_2P_{10}) to 10.50 cm (F_2P_7) in family II (0.02% concentration), from 7.42 (F_3P_1) to 11.12 cm (F_3P_2) in family III (0.04% concentration) and from 73.06 (F_4P_2) to 82.27 cm (F_4P_8) in family IV (0.06% concentration).

The observed data for of flag leaf width illustrated that it ranged from 1.34 (F_1P_{10}) to 1.52 cm (F_1P_1) in family I (control) from 1.24 (F_2P_2) to 1.43 cm (F_2P_3) in family II (0.02% concentration) and from 1.28 (F_3P_{11}) to 1.57 cm (F_3P_2) in family III (0.04% concentration) whereas in family IV the ranged varied from 1.32 (F_4P_{11}) to 1.54 cm (F_4P_{14}).

Highest number of tillers percent plant was observed in family I (control) 8.60 (F_1P_{11}) to 11.20 (F_1P_7) followed by 7.50 (F_2P_9) to 10.60 (F_2P_{12}) in family II (0.02% concentration), 7.01 (F_3P_6) to 11.70 (F_3P_{12}) in family III (0.04% concentration) and from 5.20 (F_4P_{10}) to 9.90 (F_4P_{15}) in family IV (0.06% concentration).

Test weight ranged from 24.55 (F_1P_{12}) to 34.85 g (F_1P_{15}), followed by 26.55 (F_2P_{12}) to 34.70 g (F_2P_{13}) in family II (0.02% concentration), 25.42 (F_3P_{11}) to 33.00 g (F_3P_2) in family III (0.04% concentration) and from 14.86 (F_4P_{16}) to 31.05 g (F_4P_7) in family IV (0.06% concentration).

Grain yield per plant ranged from 16.65 (F_2P_{12}) to 24.60 g (F_2P_{13}) in family II (0.02% concentration) from 17.95 (F_3P_7) to 22.70 g (F_3P_9) in family III (0.04% concentration) and from 16.85 (F_4P_9) to 21.05 g (F_4P_7) in family IV (0.06% concentration). However, in control, it ranged from 14.55 (F_1P_{13}) to 24.8 g (F_1P_{15}).

Table 3: Shoot length at 7th, 10th and 14th days after sowing

	R I			R II			R III			
Treatment	7	10	14	7	10	14	7	10	14	Mean (cm)
Control	6.6	6.8	6.9	6.4	6.6	6.8	6.2	6.4	6.6	6.58
0.02%	6.2	6.4	6.4	6.0	6.1	6.2	5.9	6.0	6.2	6.15
0.04%	5.5	5.6	5.7	5.2	5.4	5.6	5.4	5.5	6.0	5.54
0.06%	4.8	5.0	5.2	5.0	5.2	5.3	4.6	4.8	5.2	5.01

R: Replication

Table 4: Analysis of variance for different characters in M_2 generation

		Mean sum of squares							
Source of variation	d.f	Days to 50% flowering	Days to maturity	Plant height	Spike length	Flag leaf width	No. of tillers per plant	Test weight	Grain yield/ plant
Bet/Family	63	1.7188	53.4375**	218.894**	11.6169**	0.0472*	35.9670**	20.5072*	1769.239**
Rep Family	15	2.7031	3.8281	61.626	2.7643**	0.4503**	15.2922**	116.2675**	7.7278
WF_1	15	2.2313	3.7979*	11.6096	0.1068	0.0079	2.0546	17.3252**	8.1628
WF_2	15	1.9333	5.9479**	19.7167	1.0867**	0.0050	1.5947	8.1792	9.4351
WF_3	15	1.6583	1.0583	71.3240*	0.2264	0.0134	2.8522	7.6181	5.1807
WF_4	15	1.6979	2.9000	18.4346	0.4972	0.0089	3.5653	15.1466*	9.2376
Error	3	1.5531	1.6781	31.7884	0.3071	0.0135	2.6463	6.6086	5.5055

*Significant at 5% and ** significant at 1% level

Table 5: Progeny means for different quantitative characters in M_2 generation

Treatment	Days to 50% flowering	Days to maturity	Plant height (cm)	Spike length	Flag leaf width	Number tillers per plant	Test weight	Seed yield /plant
$F_1 P_1$	75.0	110.0	81.97	10.18	1.52	10.00	34.60	24.60
$F_1 P_2$	74.0	111.0	82.76	9.88	1.49	10.80	34.53	24.53
$F_1 P_3$	74.5	108.5	82.74	9.81	1.40	8.90	31.00	21.00
$F_1 P_4$	73.5	109.0	82.01	10.10	1.44	9.40	27.90	17.90
$F_1 P_5$	75.5	108.5	81.00	9.87	1.56	9.10	31.90	21.90
$F_1 P_6$	74.0	110.5	83.43	10.04	1.48	10.20	32.55	22.55
$F_1 P_7$	73.0	108.5	76.83	9.58	1.37	11.20	30.05	20.05
$F_1 P_8$	73.0	108.5	76.27	9.76	1.45	9.90	33.75	23.75
$F_1 P_9$	75.0	108.0	77.32	10.06	1.37	10.03	32.60	22.60
$F_1 P_{10}$	75.5	111.0	76.38	9.37	1.34	10.00	32.25	22.25
$F_1 P_{11}$	76.0	109.0	78.90	9.70	1.46	8.60	28.95	18.95
$F_1 P_{12}$	75.0	111.0	79.07	9.51	1.34	8.90	24.55	14.55
$F_1 P_{13}$	73.0	111.5	78.56	9.79	1.42	9.75	27.55	17.55
$F_1 P_{14}$	76.0	112.5	79.78	9.65	1.43	11.20	31.85	21.85
$F_1 P_{15}$	75.5	108.5	81.69	9.61	1.39	8.80	34.85	24.85
$F_1 P_{16}$	74.0	110.5	79.64	9.58	1.40	9.10	33.95	23.95
$F_2 P_1$	74.0	108.5	76.64	9.84	1.38	9.70	29.30	19.20
$F_2 P_2$	73.0	110.5	77.85	10.47	1.24	8.50	29.75	19.65
$F_2 P_3$	73.0	108.5	81.52	10.02	1.43	9.30	27.90	17.80
$F_2 P_4$	74.0	109.0	70.79	9.57	1.43	8.40	29.90	19.90
$F_2 P_5$	75.0	109.0	77.62	10.16	1.28	9.80	30.95	20.95
$F_2 P_6$	73.0	108.5	69.31	9.01	1.33	9.70	30.40	20.20
$F_2 P_7$	74.0	114.5	74.91	10.50	1.33	10.00	31.05	21.05
$F_2 P_8$	73.5	109.0	77.86	9.73	1.37	9.80	29.60	20.60
$F_2 P_9$	74.0	108.5	74.13	8.42	1.34	7.50	31.90	21.90
$F_2 P_{10}$	74.0	110.0	71.12	7.87	1.32	10.20	31.25	21.25
$F_2 P_{11}$	74.0	106.5	73.42	8.68	1.34	9.10	29.35	19.35
$F_2 P_{12}$	74.0	109.0	76.40	9.26	1.35	10.60	26.55	16.65
$F_2 P_{13}$	74.0	111.0	73.93	9.15	1.30	8.40	34.70	24.60
$F_2 P_{14}$	76.0	108.5	72.89	9.73	1.34	9.30	26.65	18.65
$F_2 P_{15}$	76.0	109.0	73.52	8.98	1.39	8.70	28.55	18.55
$F_2 P_{16}$	76.0	110.5	73.34	9.51	1.32	8.60	31.10	21.10
$F_3 P_1$	76.0	108.5	69.40	7.42	1.31	10.20	32.45	22.45
$F_3 P_2$	75.0	110.0	70.90	11.12	1.57	9.30	33.00	22.01
$F_3 P_3$	76.0	109.5	81.18	8.51	1.43	7.90	29.55	19.55
$F_3 P_4$	76.0	108.5	81.25	8.27	1.44	8.50	29.20	20.10
$F_3 P_5$	74.0	108.0	76.94	9.03	1.36	10.00	31.35	21.35
$F_3 P_6$	75.0	109.5	57.08	8.64	1.45	7.01	32.45	22.45
$F_3 P_7$	75.0	109.0	74.75	8.77	1.36	8.40	29.92	17.95
$F_3 P_8$	75.0	108.5	79.29	8.48	1.41	9.10	28.50	20.50
$F_3 P_9$	73.0	108.5	77.04	8.41	1.36	8.30	32.70	22.70
$F_3 P_{10}$	74.0	109.5	74.92	7.68	1.32	7.90	32.40	22.40
$F_3 P_{11}$	75.0	108.5	68.97	8.67	1.28	7.40	25.42	15.07
$F_3 P_{12}$	73.0	109.0	75.87	7.97	1.48	11.70	28.20	18.40
$F_3 P_{13}$	75.0	107.0	70.44	8.90	1.36	9.20	28.20	18.20
$F_3 P_{14}$	75.0	108.5	77.97	7.54	1.34	9.10	32.45	22.45
$F_3 P_{15}$	75.0	109.5	76.53	8.28	1.47	9.00	32.45	22.48
$F_3 P_{16}$	75.0	109.0	72.30	8.79	1.53	7.50	29.21	18.95
$F_4 P_1$	75.0	106.0	75.55	9.29	1.48	6.80	29.75	19.75
$F_4 P_2$	75.5	106.5	73.06	8.05	1.38	9.10	29.72	19.92
$F_4 P_3$	73.0	109.0	74.76	7.79	1.39	7.20	28.26	19.02
$F_4 P_4$	75.0	108.5	75.54	7.62	1.43	7.60	30.42	20.08
$F_4 P_5$	75.0	106.0	77.72	8.61	1.49	7.60	27.72	17.81
$F_4 P_6$	75.0	108.5	75.94	8.55	1.33	5.70	28.42	18.26
$F_4 P_7$	75.0	107.0	77.38	8.13	1.33	7.60	31.05	21.05
$F_4 P_8$	76.0	106.0	82.27	8.29	1.45	9.90	28.42	19.18
$F_4 P_9$	74.0	106.0	76.28	7.70	1.37	6.20	27.63	16.85
$F_4 P_{10}$	73.5	106.0	80.11	8.76	1.46	5.20	28.04	17.74
$F_4 P_{11}$	74.5	108.0	76.21	8.35	1.32	6.80	26.95	18.95
$F_4 P_{12}$	72.5	106.0	76.69	7.94	1.39	6.80	29.42	18.03
$F_4 P_{13}$	75.0	105.0	74.89	8.60	1.39	6.90	27.82	18.42
$F_4 P_{14}$	74.0	106.0	80.09	8.71	1.54	6.70	28.20	19.25
$F_4 P_{15}$	74.5	107.5	73.96	7.73	1.51	9.90	29.50	20.50
$F_4 P_{16}$	74.0	108.0	69.31	9.00	1.44	6.80	14.86	16.05
Gm	74.52	108.72	76.22	9.25	1.40	8.56	31.09	20.03
SE	0.88	0.92	3.99	0.39	0.08	1.15	1.82	1.66
CD 5%	2.49	2.59	11.28	1.11	0.23	3.25	5.14	4.69
CD 1%	3.32	3.45	15.00	1.47	0.31	4.33	6.84	6.24
No. of desired progenies	Nil	8.00	1.50	3.00	Nil	2.00	4.00	3.00

Family means for different quantitative characters: The data on family mean (Table 6) reveals that high and/or at par family mean value was observed for all the quantitative traits studied in 0.02% concentration sodium azide and control. Minimum value for days to 50% flowering (74.25 days) was observed in family II followed by family IV and I (74.47 and 74.53 days) and in family III (74.81 days). However, for maturity it was minimum in family IV (106.88 days), followed by 108.81 days in family III, 109.41 days in family II and 109.78 days in family I.

Minimum plant height 74.05 cm was observed in family III (0.04%), followed by 74.70 cm in family II and 76.24 in family IV and 79.90 in family I. Maximum spike length was observed in family I (9.78 cm) and minimum spike length was observed in family IV (8.03 cm) whereas, family II (9.40 cm) and family III (8.53 cm) showed intermediate values for spike length.

Maximum flag leaf width was observed in control (1.43 cm) and the minimum width was observed in family II (1.34 cm) where family III (1.40 cm) and family IV (1.42 cm) showed intermediate values for this trait. Highest tillering was observed in family I (9.73), followed by family II (9.22), family III (8.78) and family IV (8.32). However, for test weight the highest estimates was recorded in family I (31.43 g) followed by family III (30.46 g) family II (29.93 g) and family IV (27.88 g). For grain yield highest family mean was exhibited by family I (21.42 g) followed by family III (20.43 g) family II (20.08 g) and family IV (18.80 g).

Estimation of variability parameters for various quantitative traits: In crop improvement studies, information on genetic parameters like range, mean, genotypic variance, phenotypic variance, genotypic and phenotypic coefficient of variation, heritability and genetic advance for different traits gives indication in the improvement of trait through selection. Thus, genetic parameters of eight traits were studied in different mutagenic families and presented in Table 7 to get an idea about scope of improvement through selection. It can be described characterwise as follows:

Days to 50% flowering: Estimates for phenotypic and genotypic variance revealed that it was the highest in family III (1.87 and 0.32) while the lowest in family I (1.51 and 0.58). Phenotypic and genotypic coefficient of variation (PCV, GCV) the was highest in family III (1.82 and 0.76) and the lowest by control (1.65 and 1.02). Highest broad sense heritability for days to 50% flowering was exhibited by family I (38.39%) and lowest by family II (8.61%). Family III exhibited maximum genetic gain (6.40) followed by family I (1.30) and family II (0.31) while lowest genetic gain was shown by family IV (0.03).

Days to maturity: Highest estimates for phenotypic and genotypic variance was recorded in family II (4.10 and 1.47) followed by family III (2.83 and 0.17), family I (2.43 and 1.13) and family IV (2.42 and 0.30). However, phenotypic and genotypic coefficient of variation (PCV, GCV) was highest in family II (1.85 and 1.11) and lowest in family I (1.42 and 0.97). Heritability (bs) estimates showed that highest heritability for days to maturity was exhibited by family I (46.57%) followed by family II (35.88%) and family III (20.23%) and lowest estimates of heritability for this trait was exhibited by family IV (14.80%). Maximum genetic gain for days to maturity was observed in family II (1.37) and minimum in family III (0.35).

Plant height (cm): Estimates of phenotypic and genotypic variance revealed that it was the highest in family III (72.32 and 5.45) followed by family II (22.65 and 4.17) and family IV (16.52 and 0.76) and family I (8.84 and 4.04). Phenotypic and genotypic coefficient of variation (PCV, GCV) showed that highest estimates were depicted by family III (11.40 and 3.16) and lowest by family I (3.72 and 1.79). Highest heritability (bs) observed in family III (27.53%) while family IV had lowest estimates (14.80). However, for genetic gain family II had maximum value (13.64 cm) while family IV had lowest estimates (0.51 cm).

Spike length (cm): Phenotypic and genotypic variance was the highest in family II (0.60 and 0.42) and lowest for family I (0.13 and 0.03). Phenotypic and genotypic coefficient of variation was highest in family II (8.43 and

Table 6: Family means for different quantitative characters in M_2 generation

Family	Days to 50% flowering	Days to maturity	Plant height (cm)	Spike length	Flag leaf width	Number of tillers per plant	Test weight	Seedyield /plant
F1	74.53	109.78	79.90	9.78	1.43	9.73	31.43	21.42
F2	74.25	109.41	74.70	9.40	1.34	9.22	29.93	20.08
F3	74.81	108.81	74.05	8.53	1.40	8.78	30.46	20.43
F4	74.47	106.88	76.24	8.32	1.42	8.32	27.88	18.80
Gm	74.52	108.72	76.22	9.25	1.40	9.01	29.92	20.18
SE	0.220	0.229	0.997	0.098	0.021	0.288	0.454	0.415
CD 5%	0.623	0.648	2.819	0.277	0.058	0.813	1.286	1.173
CD 1%	0.829	0.862	3.751	0.369	0.077	1.082	1.710	1.561
CV	1.672	1.192	7.397	6.113	8.300	19.012	8.269	11.675

Table 7: Estimates of genetic parameters for different quantitative characters in different families

Character	Family	VG	VP	GCV	PCV	h2	GG
Days to 50% flowering	1	0.58	1.51	1.02	1.65	38.39	1.30
	2	0.14	1.67	0.51	1.74	8.61	0.31
	3	0.32	1.87	0.76	1.82	17.11	6.40
	4	0.01	1.58	0.16	1.69	14.86	0.03
Days to maturity	1	1.13	2.43	0.97	1.42	46.57	1.36
	2	1.47	4.10	1.11	1.85	35.88	1.37
	3	0.17	2.83	0.38	1.83	20.23	0.35
	4	0.30	2.42	0.51	1.46	12.26	0.37
Plant height	1	4.04	8.84	1.79	3.72	23.05	1.77
	2	4.17	22.65	2.73	6.37	18.91	13.64
	3	5.45	72.32	3.16	11.40	27.53	6.45
	4	0.76	16.52	1.15	5.33	14.80	0.51
Spike length	1	0.03	0.13	1.77	3.68	23.00	9.44
	2	0.42	0.60	7.05	8.43	69.97	12.15
	3	0.09	0.31	3.26	6.06	29.00	3.65
	4	0.01	0.47	1.20	8.23	32.12	5.40
Flag leaf width	1	0.02	0.03	2.39	5.54	28.60	2.12
	2	0.05	0.06	7.46	10.50	50.00	10.76
	3	0.03	0.05	1.57	8.62	33.33	5.98
	4	0.05	1.50	1.58	7.04	51.06	7.50
Number of tillers per plant	1	0.26	1.67	6.09	15.58	15.29	4.91
	2	0.57	2.06	7.66	14.50	27.66	8.27
	3	0.02	2.65	1.75	18.54	18.90	0.34
	4	0.29	3.63	7.66	19.30	79.88	42.82
Test weight	1	6.90	9.34	8.36	9.72	73.92	14.81
	2	1.15	8.81	3.65	10.06	68.21	13.89
	3	0.48	6.66	2.22	8.26	67.24	1.23
	4	3.17	11.03	5.61	10.46	38.74	6.19
Seed yield/plant	1	1.77	5.88	4.26	15.77	30.04	9.81
	2	0.07	8.91	8.32	16.82	20.78	8.47
	3	0.19	4.66	2.60	12.71	14.18	4.89
	4	2.10	6.56	1.68	7.77	12.02	1.09

VG: Genotypic variance, VP: Phenotypic variance, GCV and PCV; Genotypic and phenotypic coefficient of variation, h^2: Heritability in broad sense, GG: Genetic advance

7.05) while the lowest estimates were depicted by family I (3.63 and 1.77). Highest heritability in spike length was exhibited by family II (69.97%) followed by family IV (32.12 %), family III (29.00%) and lowest by family I (23.00%). For genetic gain family II exhibited maximum value (12.15) followed by family I (9.44) and family IV (5.40) while family III showed minimum estimates (3.65).

Flag leaf width (cm): Highest phenotypic and genotypic variance was recorded in family IV (1.50 and 0.05) and lowest in family I (0.03 and 0.02). Phenotypic and genotypic coefficient of variation showed that highest estimates were depicted by family II (10.50 and 7.46) and family I (5.54 and 2.39) had lowest value. Heritability (bs) was highest in family IV (51.06%) followed by family II (50.00%) and family III (33.33%) and lowest in family I (28.60%). Family II exhibited maximum genetic gain (10.76) while family I (2.12) had minimum genetic gain.

Number of tiller per plant: Phenotypic and genetic variance was the highest in family IV (3.63 and 0.29) and the lowest in family I (1.67 and 0.26). Highest estimates of phenotypic and genotypic coefficient of variation were depicted by family IV (19.30 and 7.66) and lowest by family II (14.50 and 7.66). Maximum heritability was observed in family IV (79.88%) followed by family II (27.66%) and family III (18.90%) and lowest estimates of heritability for this trait was exhibited by family I (15.29%). Estimates for genetic gain revealed that family III exhibited maximum genetic gain for number of tillers per plant (42.82) followed by family II (8.27) and family I (4.91) while the lowest genetic gain was exhibited by family III (0.34).

Test weight (g): Estimates for phenotypic and genotypic variance revealed that it was the highest in family IV (11.03 and 3.17) and the lowest in family III (6.66 and 0.48). Phenotypic and genotypic coefficient of variation was highest in family IV (10.46 and 5.61) the lowest in family III (8.26 and 2.22). Highest heritability was exhibited by family I (73.92%) and the lowest estimates of heritability for this trait was exhibited by family IV (38.74%). Estimates of genetic gain revealed that family I exhibited maximum genetic gain (14.81) while the lowest genetic gain was shown by family III (1.23).

Grain yield per plant (g): Phenotypic and genotypic variance was highest in family II (8.91 and 0.07) while the lowest value recorded in family III (4.46 and 0.19). Highest

estimates for phenotypic and genotypic coefficient of variation (PCV, GCV) was depicted by family II (16.82 and 8.32) and the lowest by family IV (7.77 and 1.68). Heritability estimates showed that highest heritability for grain yield per plant was exhibited by family I (30.04%) while family IV (12.02%) had the lowest estimates. Maximum genetic gain was observed in Family I (9.81) while minimum genetic gain was shown by family IV (1.09).

DISCUSSION

The use of means and variances has been appointed as a potential technique for detecting the occurrence of variation when using mutagenic treatments (Scossiroli, 1977). Frequency distributions have also been used to characterize the presence of genotypic variability and to show the variation occurred when using mutagenical products, as to evaluate the performance in relation to the control genotype (Borojevic and Borojevic, 1972). Induction of sodium azide showed the possibility of identifying the changes that to occurred various quantitative traits. This was shown by the frequency distributions and by the changes in means and variances of treated populations. Present Results in this work showed sodium azide contributed significantly to obtain increase in variability of days to 50% flowering, days to maturity, plant height, width of flag leaf, number of tillers per plant, test weight and grain yield per plant. Significant variability at different concentrations of sodium azide for various quantitative characters in wheat were also reported by (Veleminsky and Angelis, 1987; Liang and Gao, 1986).

The application of sodium azide on crop is easy and inexpensive for improvement of agronomic traits. The mutagenic effects of sodium azide appear soon after sowing the seeds and can be observed by naked eyes. However, sodium azide has been being used in various crops to improve their yield and quality traits and create resistance to them against biotic and abiotic stresses. In present experiment it was observed that increase in concentration of mutagen (sodium azide) there was a decrease in germination percentage. Similar findings of mutagenic sensitivity induced by gamma rays and sodium azide in early generation of black gram was also studied by Lal *et al.* (2009) and they observed that an increase in azide concentrations resulted in decrease in germination; plant survival was also affected and depressive effect on seedling growth.

This reduction in seed germination in mutagenic treatments has been explained due to delayed or inhibition in physiological and biological processes necessary for seed germination which include enzyme activity (Chrispeels and Varner, 1967), hormonal imbalance (Ananthaswamy *et al.*, 1971) and inhibition of mitotic process (Sato and Gaul, 1967). Azide ion plays an important role in causing of mutation by interacting with enzymes and DNA in the cell. These azide anions are strong inhibitors of cytochrome oxidase which in turn inhibits oxidative phosphorylation process. In addition, it is a potent inhibitor of the proton pump (Kleinhofs *et al.*, 1978) and alters the mitochondrial membrane potential (Zhang, 2000). These effects caused by NaN_3 together may hamper ATP biosynthesis resulting in decreased availability of ATP molecule which may slow the germination rate and reduce the germination percentage. The another reason behind this is that seeds have probably developed tolerance to the inhibitory effect of NaN_3 on germination and had improved their physiological conditions on additional days with respect to seed germination. All living cells require energy in the form of ATP molecules to carry all biological reactions. At low energy level, the rate of biological reactions inside the cell decreases. Cheng and Gao (1988) treated barley seeds with sodium azide and found a significant decrease in the percentage germination. It is important to stress the fact that treatments with sodium azide, under the same conditions, produce a delay in the initiation of plant growth, as can be observed and mentioned by Pearson *et al.* (1975).

Root length and shoot length showed a similar pattern of growth i.e., with the increase of concentration of chemical mutagen there was adverse effect on the growth of the root length and shoot length of wheat seedlings in the laboratory conditions. The reduction in seedling survival is attributed to cytogenetic damage and physiological disturbances (Sato and Gaul, 1967). Thus, the probable reason of this may be the hindrance caused by the sodium azide on different metabolic pathway of the cells. Similar findings have also been reported by Rachovska and Dimova (2000) in wheat, Akhaury *et al.* (1996), Ilbas *et al.* (2005) in barley, Adamu and Aliyu (2007) in tomato, Khan *et al.* (2004) in mungbean, Al-Qurainy (2009) in *Eruca sativa* and Mostafa (2011) in sunflower.

The greater sensitivity at higher mutagenic level has been attributed to various factors such as changes in the metabolic activity of the cells, inhibitory effects of mutagens and to disturbance of balance between promoter and inhibitors of growth regulators (Krishna *et al.*, 1984). Adegoke (1984) reported that sodium azide induces chromosomal damages leading to

bridge formation during mitotic division and hence increased phenotypic aberration. It also plays important role in genetic sterility as shown in rice without changes in vigour (Mensah *et al*., 2005).

In M_2 generation variation is expected to be high for any character because of the segregation and a number of mutants for different quantitative characters can be identified in this generation. With this background an effort was made to screen for various mutants during present investigation. Similar to other mutation breeding experiments, the increase in variance was found to be associated with shift in mean during both the years in positive and negative directions. It became essential therefore, to focus attention on selected number of M_2 progenies. A progeny with a high or low mean (depending on the character under consideration) over the mean of control, with a high coefficient of variation is expected to yield desirable segregants in successive generations. At the same time families with a desirable shift in the mean were also considered desirable.

Larger spike length is desirable in wheat as it is an important yield attributing character, keeping this fact in mind 03 progenies (F_2P_2, F_2P_7 and F_3P_2) as well as desirable since their mean value indicating higher values than ground means, therefore, single plant selection can be made for this character to isolate desirable genotypes. Similar results in wheat were also reported by Rachovska and Dimova (2000) and Chowdhary and Das (2001).

The mean values indicated that sodium azide did not caused any significant changes in mean for days to maturity. However, in the present investigation, 8 progenies showed significantly lower days to maturity than grand mean. From these progenies desirable segregants can be obtained through selection. These findings are in accordance with the findings of Sharma and Bansal (1970) and Reddy *et al.* (1994) in wheat.

Semi dwarf plant height (85-90 cm) is desirable attribute in wheat as well as too tall plant is susceptible for lodging and height below the D_2 type causes significant reduction in straw weight which is also an essential by-product of wheat. In the present investigation, most of the progenies in the treated concentration and in control were not in the desirable plant height group which indicates that sodium azide did not cause any significant reduction in plant height. However, in higher concentration of sodium azide, progenies showed reduction in plant height, indicating their undesirability for selection. These findings were in conformity with the results of Sharma *et al.* (1989) in wheat, Sander *et al.* (1972), Conger (1973) and Konzak *et al*. (1975) in barley.

Spike length combining with more number of tillers per plant and test weight is desirable attributes of a genotype in order to obtain higher grain yield per plant. Based on overall mean performance for most of the yield contributing characters progenies F_2P_{13}, F_3P_9 and F_3P_{15} of 0.02 and 0.04% sodium azide concentrations of characters were identified to possess desirable combination of character with high mean. It is expected that true breeding genotypes with better performance would be expected in advanced generations and therefore these characters can be considered as a selection criteria for yield. These findings are in accordance with the findings of Singh and Singh (2001) and Singh *et al.* (2001).

An overall perusal of family mean revealed that with the increase in concentration of mutagen, there was a decrease in family mean of different progenies for different quantitative characters. It is evident from Table 6 that family means of control and smaller dose of sodium azide (0.02% concentration) were almost at par for different quantitative traits whereas all the yield attributing characters like spike length, number of tillers per plant and test weight showed marked decrease in family means consequently, low yield at higher dose of sodium azide. Similar findings of mutagenic treatment in wheat were also reported by Shukla *et al.* (1978), Sharma *et al.* (1989), Konzak *et al.* (1975), Micke *et al.* (1985), Reddy (1992) and Rachovska and Dimova (2000).

In crop improvement studies, information on genetic parameters like range, mean, genotypic variance, phenotypic variance, genotypic and phenotypic coefficient of variation, heritability and genetic advance for different traits gives indication in the improvement of trait through selection. In the present study, as described earlier, the mutagenic treatments have induced variability in M_2 generation for different traits due to induction of micro mutations. But the nature and magnitude of these induced variation in different traits varied with treatments. Thus, genetic parameters of eight traits were studied in different mutagenic families to get an idea about scope of improvement through selection.

In general, most of the mutagen treated M_2 families showed increased in variability in comparison to control. This induced phenotypic variability (PCV) is partly due to environmental effects (ECV) and partly due to genetic effects (GCV). These genetic effects can contributed to induced micro mutations in the trait. Further, most mutagenic treatments induced wider range of variation in treated families than control for all the 8 traits and magnitude of such variation in traits was either increased or decreased. The genetic component of induced variability (GCV) varied with mutagenic treatments and

characters studied. Similar differential GCV estimates for grain yield and its component in wheat has also been reported by Mendhulkar (2002).

It was observed that heritability estimates varied with the mutagen treatment as compared to control. High heritability was observed for characters like; test weight, number of tillers per plant, spike length, days to maturity and days to 50% flowering indicating that these characters are governed by additive gene action. Similar trends of heritability for different qualitative traits in mutagenic populations have been also reported by Sharma and Bansal (1970), Reddy *et al.* (1994), Chowdhary and Das (2001), Singh *et al.* (2001) and Mendhulkar (2002).

Genetic advance as percent of mean under selection in the M_2 populations varied with treatments and characters studied. The study revealed that selection in the treatment populations may lead to improvement up to 9.81 g in yield per plant, 14.81 g in test weight, 12.15 cm in spike length, 13.64 cm in plant height and 1.37 days for maturity. Genetic advance as percent of mean also increased in the treatments and it was relatively higher for different quantitative characters studied. Similar differential estimates of genetic advance in different mutagenic treatment populations for different traits have also been reported by Kalia *et al.* (2001).

Considering all genetic parameters for yield attributing traits in different mutagenic treated families, it can be inferred that the family in which sufficient genetic variability was induced and selection would be effective family IV (0.02%). Thus, in the present experiment, sodium azide with 0.02% concentration appear to be most effective mutagenic treatment for induction of micro-mutation in yield component traits and selection in M_2 populations of these treatment would be effective in bringing out lines with yield improvement.

CONCLUSION

The use of mutagens in crop improvement helps to understand the mechanism of mutation induction and to quantify the frequency as well as the pattern of changes in different selected plants by mutagens. The ability of these mutagens to enter the cell of living organisms to interact with the DNA produces the general toxic effects associated with their mutagenic properties. Thus, their effects are mainly due to the direct interaction between the mutagen and the DNA molecules. Sodium azide mutagenesis can not only generate diverse resistance but also provide an efficient method for breeding disease resistant varieties. By applying the technique to identify the desirable progenies for various characters in M_2 generation through high mean and low CV, eight progeny for earliness, one for plant height, three for spike length, two progeny for tillering, four for test weight and three progenies for grain yield was found desirable. These progenies may be rigorously tested in successive generations for further confirming their superiority. The other progenies which are still segregating need further selection for purification.

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