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Identification of Induced Chemical Mutants for Improving Yield and its Attributing Traits in Wheat (*Triticum aestivum* L.) against Changing Climate

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ABSTRACT

Background: The enhancement of genetic traits in any crop relies on the existing variability within that particular crop. Through hybridization and traditional breeding methods, the variability within wheat crops has been nearly exhausted. Consequently, mutation breeding emerges as the sole viable and promising approach to introduce new variability for further improvements.

Methods: An experiment was conducted during the *Rabi* seasons of 2020-21 (M1 generation) and 2021-22 (M2 generation) at the Crop Research Centre, Department of Genetics and Plant Breeding, ITM University Gwalior. The experiment assessed the impact of five different concentrations of Sodium Azide (0.02%, 0.03%, 0.04%, 0.05%, and 0.1%) and Hydroxyl Amine (0.2%, 0.3%, 0.4%, 0.5% and 0.6%), in addition to a control, on the wheat varieties MP-3382 and RVW-4106. It employed a randomized block design with three replications.

Results: The findings revealed that Sodium Azide and Hydroxyl Amine, the most effective dosages were observed to be 0.1per cent and 0.4 per cent, respectively, in inducing genetic and phenotypic changes in seed quality parameters. Specifically, for the flag leaf area characteristic, the most effective concentrations were 0.03 per cent Sodium Azide and 0.4 percent Hydroxyl Amine. Plant height exhibited the greatest variability at 0.03 per cent Sodium Azide, 0.5 per cent Hydroxyl Amine and 0.1 percent Sodium Azide. A dosage of 0.5 per cent Hydroxyl Amine was found to be most effective in altering spike length. In terms of days to 50 per cent flowering, 0.05 per cent Sodium Azide was identified as the most effective dosage. From the study of traits in both the M1 and M2 generations, 12 mutants were confirmed, with seven validated for variety MP-3382 and five for variety RVW-4106.

Key words: Genetic, Hydroxyl Amine, Mutagen, Phenotypic, Sodium Azide, Variability.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a widely cultivated, consumed and traded cereal crop, playing a pivotal role in global agriculture. The versatile grain contains substantial levels of various nutrients, with carbohydrates comprising 78.1 per cent, protein 14.7 per cent, fat 2.1 per cent and minerals 2.1 per cent of its composition (Pawan and Ortloff, 2011). Wheat cultivation is a global endeavor, with a production volume reaching approximately 766 mt. It is grown across an expansive land area of nearly 216 mha, spanning over 125 countries (Sharma *et al*., 2021; Sharma *et al*., 2020).

Plant breeding hinges on the concept of genetic diversity, which can be achieved through either hybridization or mutations, followed by a selection process (Addisu and Shumet, 2015). Mutation, as a forward genetic technique, generates novel traits that can be harnessed in traditional breeding programs (Ahmar *et al.,* 2020). Mutation breeding offers the advantage of rapidly introducing specific desirable traits into otherwise acceptable plant varieties. This approach significantly reduces the time required for trait improvement by incorporating relevant genetic variants.

In 2019, the Mutant Variety Database (FAO/IAEA-MVD) data, managed by the Food and Agriculture Organization of the United Nations in collaboration with the International Atomic Energy Agency, documented 3,275 accessions originating from 225 different species. These accessions were created and shared with the public through the joint efforts of the FAO and IAEA. It improves cultivars in specific

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qualities by modifying existing germ plasm. In his classic study on mutations, De Vries (1904) studied mutations with small consequences, such as changes in the shape and size of leaves and pods.

Chemical mutagens are known for their ability to induce mutations that affect single nucleotide pairs, as evidenced by various studies (Kurowska *et al*., 2012; Ahmed *et al*., 2020). Powerful chemical mutagen is Sodium Azide (NaN_3), particularly noteworthy for its potency in inducing mutations in crop plants. The mutagenic potential of Sodium Azide has been well-documented in various screening tests.

MATERIALS AND METHOD

The research was carried out in the *Rabi* seasons of 2020-21 and 2021-2022. This research was conducted at the Crop Research Centre within the Department of Genetics and Plant Breeding, School of Agriculture, ITM University, Gwalior, Madhya Pradesh. The wheat genotype selected for the mutagenic treatment was MP-3382 and RVW-4106, which are considered promising and leading wheat varieties. These genotypes were sourced from KVK Gwalior. Chemical mutagen that is Hydroxyl Amine (HA) and sodium Azide (SA) was used as mutagen for the present experiment, seeds were exposed to different doses of Hydroxyl Amine (HA), and Sodium Azide (SA) making 22 treatments including control tabulated in Table 2. To begin with experiment, it was conducted to determine the suitable concentrations of HA and SA as per Table 1. The research involved subjecting the seeds to a range of concentrations of Hydroxyl Amine (0.2%, 0.3%, 0.4%, 0.5%, and 0.6%), as well as Sodium Azide (SA) at concentrations of 0.02%, 0.03%, 0.04%, and 0.1%.

Table 1: Chemical mutagen and it's different treatment concentrations.

Mutagen	Treatment concentration (%)
Hydroxyl Amine (HA)	0.2, 0.3, 0.4, 0.5 and 0.6
Sodium Azide (SA)	0.02 ,0.03 ,0.04, 0.05 and 0.1

Table 2: Treatments details under investigation.

Treatment used	Chemical mutagen with concentration
T_1V_1	Control and untreated
T_2V_1	Sodium azide 0.02%
T_3V_1	Sodium azide 0.03%
T_4V_1	Sodium azide 0.04%
T_5V_1	Sodium azide 0.05%
T_6V_1	Sodium azide 0.1%
T_7V_1	Hydroxyl amine 0.2%
T_8V_1	Hydroxyl amine 0.3%
T_9V_1	Hydroxyl amine 0.4%
$T_{10}V_1$	Hydroxyl amine 0.5%
$T_{11}V_1$	Hydroxyl amine 0.6%
$T_{12}V_2$	Control and untreated
$T_{13}V_2$	Sodium azide 0.02%
$T_{14}V_2$	Sodium azide 0.03%
$T_{15}V_2$	Sodium azide 0.04%
$T_{16}V_2$	Sodium azide 0.05%
$T_{17}V_2$	Sodium azide 0.1%
$T_{18}V_2$	Hydroxyl amine 0.2%
$T_{19}V_2$	Hydroxyl amine 0.3%
$T_{20}V_2$	Hydroxyl amine 0.4%
$T_{21}V_2$	Hydroxyl amine 0.5%
$T_{22}V_2$	Hydroxyl amine 0.6%

M_1 generation

In the M_1 generation, we recorded observations related to germination percentage, flowering, and various other characteristics. Subsequently, after the crop was harvested, we harvested selected plants separately from each treatment. These chosen plants from different treatments were specifically labeled as M_1P_1, M_1P_2, M_1P_5, and so forth. To initiate the next phase of the experiment, the treated seeds, as well as the control group, were sown on October 25, 2020.

M_2 generation

The seeds obtained from the selected plants were harvested separately for each treatment and subsequently sown during the *Rabi* season in 2021. The crop planting took place on October 28, 2021 in three replication.

To evaluate seedling vigor, the Seedling Vigor Index (SVI-I) was determined using the formula established by Abdul Baki and Anderson (1973).

SVI =

Germination percentage × (Root length + Shoot length)

RESULTS AND DISCUSSION

Analysis of Variance

Analysis of Variance for MP-3382 and RWL-4106 for field and Laboratory characters as shown in Table 3 and 4 respectively indicated that there were significant differences among treatments for various characters. These differences were highly significant for field traits such as days to 50 percent flowering, plant height, number of tillers per plant, flag leaf length, flag leaf width, spike length, spikelets per spike, grain yield per plant, days to maturity, test weight and laboratory traits *viz.* Germination (%), root length, shoot length and seed vigour index-I.

M_1 generation

The inferences drawn for the results are based on mean performance where, effect of SA and HA on MP- 3382 and RVW-4106 for various characters and different treatments in M_1 generation tabulated in Table 5 and Table 6 respectively.

Germination percentage and root length

In the both variety MP 3382 and RVW 4106, SA significantly reduced but HA significantly increased the germination percentage and root length as compared to control as shown in Table 6. Ibukun *et al*. (2019) have also reported these results. The mutagen HA acted in a reverse trend with that of HA. The increase may be due to high rate of mitotic cell division and hence increased embryonic growth.

Table 3: Analysis of variance for MP-3382 and RWL-4106 for field characters.

Characters	MP-3382			RWL-4106		
	Mean sum of squares			Mean sum of squares		
	Replication	Treatment	Error	Replication	Treatment	Error
Days to 50 %flowering	4.73^{NS}	31.42**	2.73	12.64*	25.36**	2.24
Plant height (cm)	5.54**	90.80**	0.77	0.96^{NS}	141.14**	2.08
No of tillers per plant	0.09^{NS}	9.82**	0.69	0.64^{NS}	6.16**	0.94
Flag leaf length (cm)	0.57^{NS}	38.00**	1.07	0.11^{NS}	75.82**	0.97
Flag leaf width (cm)	0.01^{NS}	0.19**	0.01	0.02^{NS}	0.19**	0.01
Spike length (cm)	0.31^{NS}	6.87**	0.43	0.00^{NS}	3.08**	0.16
Spike lets per Spike	4.73^{NS}	99.22**	2.13	17.18**	35.29**	0.582
Grain yield per plant (g)	4.33^{NS}	110.28**	3.46	11.65^{NS}	36.04**	3.39
Days to maturity	22.45*	31.37**	4.95	9.36^{NS}	14.67*	4.66
Test weight (g)	0.01^{NS}	28.10**	0.53	0.144^{NS}	85.79**	0.81

Level of significance at 5 %.

Table 4: Analysis of variance for MP-3382 and RWL-4106 for laboratory characters.

Characters	MP-3382		RVW-4106	
	Mean sum of squares		Mean sum of squares	
	Treatment	Error	Treatment	Error
Germination percentage	41.62**	1.56	39**	2.09
Root length	5.98**	0.56	15.38**	0.18
Shoot length	18.63**	0.25	11.50**	0.13
Seed vigour index-I	520773.74**	6906.69	525784.76**	4235.99

Level of significance at 5 %.

Shoot length (cm)

In the variety MP 3382, the effect of mutagen SA on shoot length of M1 generation revealed that control recorded maximum shoot length 9.70 cm. After the treatment there was significantly decreased in shoot length up to 3.70 cm (T6) with increasing dose of mutagen. For HA, the data revealed that T9 recorded maximum shoot length 11.20 cm followed by T8 (10.40 cm) and shortest shoot length was found in T11 (9.52 cm).

In the variety RVW 4106 both the mutagens act in similar way as they were performed in variety MP 3382 for the character shoot length. The mean shoot length for the mutagens SA and HA was recorded to be 6.10 cm and 10.12 cm respectively for the variety MP 3382.

Seed vigour index (SVI)

The effect of SA on seed vigour index of M_1 generation revealed that SA significantly decreased the SVI for both of the varieties The mutagen HA showed maximum SVI in concentration 0.4 per cent HA followed by 0.3 per cent HA and 0.2 percent HA for both of the varieties.

The results of root length, shoot length and seed vigor index showed a pattern of growth for the mutagen SA *i.e.,* with increase in dose there was adverse effect on growth on wheat in laboratory conditions. Mutagen HA enhanced seedling growth and SVI. This may be due to Gushing of water inside the cell (cell elongation) or increased somatic cell division due to higher rate of respiration. Sharma *et al*. (2015) also recorded this similar pattern of observation.

Days to 50 per cent flowering and Days to maturity

In the variety MP 3382, the effect of SA and HA on the days to 50 percent flowering in M_1 generation revealed that there is early emergence for control (70 days) as compare to all the treatments. Mutagen SA and HA delayed the days to 50 percent flowering by five and ten respectively. In the variety RVW 4106, control (68 days) recorded early emergence for all the treatments. The mean days to 50 percent flowering for SA and HA was 73.20 and 76.60 days respectively for the variety MP 3382.

For the variety MP 3382**,** both the mutagens delayed the days to maturity. For the variety RVW 4106, both the mutagens delayed the days to maturity. Control recorded the earliest maturity (118 days).

This delay might be due to profuse and persistent vegetative growth which might have consume more time for mitotic arrest in flowering primordial or this character might be primarily controlled by the major genes whose expressions would have affected either by modifies or complementation and hence delayed the days to 50 percent flowering. Odeje *et al*. (2016), have reported similar results.

Plant height (cm)

In the variety MP 3382, both the mutagen decreased plant height but SA decreased more height as compared to HA. SA decreased height up to T3 (82.50 cm) then increased in T4 (88.30 cm) but less than control (97.50 cm) then afterwards decreased with increased dose of mutagen. In the variety RVW 4106, data revealed that plant height

Table 5: Effect of SA and HA on MP- 3382 for various characters and different treatments in M_1 generation.

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seed vigour index (SVI)	Days to 50% flowering	Plant height (cm)	Number of tillers per plant	Flag leaf Length (cm)	Flag leaf Width (cm)	Spike length (cm)	Spikelets per spike	Grain yield per plant (g)	Days to maturity	Test weight (g)
Control	95.00	10.30	9.70	1899.10	70.00	97.50	8.00	20.32	1.42	11.50	50.00	19.94	117.00	49.80
Sodium azide 0.02%	94.00	9.51	8.50	1693.56	72.00	84.80	7.00	22.00	1.26	7.80	39.00	13.01	119.00	47.82
Sodium azide 0.03%	94.00	9.00	7.274	1574.22	74.00	82.50	6.00	25.70	2.00	7.50	36.00	9.59	124.00	44.40
Sodium azide 0.04%	92.00	8.40	6.30	1352.03	73.00	88.30	6.00	23.00	1.82	11.00	47.00	13.65	125.00	48.49
Sodium azide 0.05%	89.00	7.53	4.27	1050.35	75.00	87.80	5.00	20.96	1.88	10.35	45.00	9.72	124.00	43.20
Sodium azide 0.1%	85.00	7.02	3.71	912.61	72.00	86.40	5.00	22.19	1.66	10.30	45.00	9.90	122.00	44.00
Hydroxyl amine 0.2%	96.00	10.55	9.81	1955.44	72.00	99.40	9.00	18.01	1.48	10.70	48.00	19.47	118.00	45.11
Hydroxyl amine 0.3%	97.00	11.00	10.40	2076.16	78.00	96.90	9.00	14.75	1.35	11.50	52.00	20.78	118.00	44.39
Hydroxyl amine 0.4%	98.00	11.31	11.20	2205.98	80.00	90.20	10.00	26.96	1.82	10.40	48.00	19.97	123.00	41.00
Hydroxyl amine 0.5%	95.00	10.47	9.69	1916.15	74.00	88.30	9.00	21.98	1.92	12.60	57.00	28.71	119.00	50.32
Hydroxyl amine 0.6%	93.00	10.00	9.52	1815.95	79.00	92.30	9.00	25.87	1.76	10.50	45.00	19.79	126.00	48.90
C.D.	2.12	1.27	0.85	141.63	2.83	1.50	1.43	1.77	0.12	1.12	2.50	3.19	3.82	1.25
SE(m)	0.72	0.43	0.29	47.98	0.95	0.51	0.48	0.60	0.04	0.38	0.84	1.07	1.28	0.42
SE(d)	1.01	0.61	0.41	67.85	1.35	0.71	0.68	0.84	0.06	0.53	1.12	1.52	1.82	0.60
C.V.	1.33	7.83	6.01	4.95	2.21	0.97	11.02	4.70	4.33	6.30	3.13	11.08	1.83	1.58

Table 6: Effect of SA and HA on RVW-4106 for various characters and different treatments in M_1 generation.

Treatments	Germination %	Root length (cm)	Shoot length (cm)	Seed Vigour index (SVI)	Days to 50 % Flowering	Plant height (cm)	Number of tillers per plant	Flag Leaf length (cm)	Flag Leaf width (cm)	Spike length (cm)	Spike lets per spike	Grain yield per plant (g)	Days to maturity	Test weight (g)
Control	92.00	9.60	8.73	1687.68	68.00	103.00	10.00	24.05	1.43	11.30	52.00	23.43	118.00	45.03
Sodium azide 0.02%	91.00	8.70	8.00	1519.70	70.00	99.20	9.00	27.09	1.38	9.60	45.00	18.30	118.00	45.16
Sodium azide 0.03%	90.00	7.31	7.82	1363.287	78.00	96.80	9.00	28.82	1.88	9.20	44.00	14.15	119.00	35.75
Sodium azide 0.04%	88.00	6.50	6.54	1148.83	68.00	98.80	8.00	26.32	1.56	9.90	48.00	15.12	119.00	39.45
Sodium azide 0.05%	85.00	5.26	5.48	913.57	72.00	88.60	8.00	17.29	1.42	9.50	45.00	16.20	120.00	45.03
Sodium azide 0.1%	83.00	3.26	2.85	507.40	70.00	82.80	7.00	20.18	1.54	8.50	42.00	14.33	122.00	48.83
Hydroxyl amine 0.2%	92.00	9.55	8.61	1671.76	72.00	87.80	10.00	13.95	1.04	8.20	44.00	21.37	122.00	48.40
Hydroxyl amine 0.3%	94.00	10.00	9.00	1786.4	72.00	87.96	10.00	21.63	1.80	9.90	47.00	15.03	119.00	31.98
Hydroxyl amine 0.4%	95.00	10.61	9.61	1920.91	75.00	87.40	12.00	28.12	1.90	9.70	45.00	20.19	125.00	40.76
Hydroxyl amine 0.5%	91.00	9.27	8.60	1626.59	72.00	86.50	11.00	19.93	1.62	8.90	42.00	21.73	119.00	47.04
Hydroxyl amine 0.6%	89.00	8.90	8.40	1538.87	71.00	84.70	9.00	16.93	1.57	7.50	39.00	14.38	118.00	41.03
C.D.	2.46	0.73	0.62	110.92	2.56	2.48	1.66	1.69	0.13	0.70	1.31	3.16	3.70	1.54
SE(m)	0.83	0.25	0.21	37.58	0.87	0.83	0.56	0.57	0.04	0.23	0.44	1.06	1.25	0.52
SE(d)	1.18	0.35	0.30	53.14	1.22	1.18	0.80	0.80	0.06	0.33	0.62	1.50	1.76	0.73
C.V.	1.61	5.27	4.81	4.56	2.01	1.58	10.33	4.43	4.89	4.36	1.70	10.43	1.80	2.11

decreased significantly with increased dose of both mutagens.

The result here may be due to chemical mutagens disrupting cell division within the meristematic tissues responsible for regulating plant height. Mutations have the potential to hinder proper cell division and growth in these regions. These findings were in conformity of Weldemichael *et al.* (2021) and Omeke *et al.,* (2021).

Number of tillers per plant and flag leaf length (cm)

In the variety MP 3382, the mutagen SA significantly reduced the tillers number up to 5 in T6 as compared to control (8). Mutagen HA, revealed the increase in tillers number and maximum was found in T9 (10). In the variety RVW 4106, same trend was observed for both the mutagens.

In the variety MP 3382, the data for flag leaf length revealed that mutant SA significantly increased the length as compared to control (20.32 cm). Mutagen HA reduced the length up to the concentration 0.3 per cent (14.75 cm) compared to control (20.32 cm). After that length was increased to 26.96 in T9, followed by T11 (25.87 cm).

In the variety RVW 4106, mutagen SA data revealed that length was more up to the concentration 0.04 percent (26.32 cm) compared to the control (24.05 cm). Maximum length was observed at concentration 0.03 per cent (28.82 cm). After 0.04 per cent SA, length was reduced and less than control.

Flag leaf width (cm)

In the variety MP 3382, data revealed that mutagen SA increased the width compared to control (1.42 cm) except in T2 (1.26 cm). Mutagen HA increased the width in all the treatments except in T8 (1.35 cm) compared to control. In the variety RVW 4106, mutagen SA followed the same trend in this variety as followed in MP- 3382. Mutagen SA and higher doses of HA tends to increases the flag leaf area 0.03 per cent SA and 0.4 per cent.

HA is the most effective dose observed for both of the varieties for increasing flag leaf area due to proper adaptability of the variety to the respective mutagenic treatments compared to other treatments. Abaza *et al.*, (2020), reported this similar observation of increase in flag leaf area by chemical mutagens at particular concentrations.

Spike length (cm) and Spike lets per spike

In the variety MP 3382, both the mutagens had significant effect on spike length (Plate 1). Mutagen SA and HA reduced the spike length compared to control except in two treatments where length was more than control (Plate 2). In the variety RVW 4106, both the mutagens decreased the spike length. At 0.5 percent HA, longest spike length and highest spike lets per spike was observed in variety MP-3382.

For the variety MP 3382, control contains 50.00 spike lets. Among all the treatments, maximum number of spikes were found i.e. 57.00 and 52.00 in T10 and T8 respectively and lowest was found in T3 (36.00). In the variety RVW 4106 maximum number of spikes were found in control (52.00) followed by T15 (48.00), T19 (47.00) and lowest was found in T22 (39.00) among all the treatments.

This may be due to proper adaptability of the variety to that particular treatment or due to physiological alteration by hydroxylamine or enhanced enzymatic activities. Abaza *et al*. (2020) also observed increased spike morphology by chemical mutagen in bread wheat and Rahman *et al*. (2021) in bread wheat.

Grain yield per plant (g)

For the variety MP 3382, after treated with SA, maximum yield was recorded in control (19.94 g) among all the treatments. Mutagen HA increased the yield compared to control. Maximum yield was found in T10 (28.71 g) followed by T8 (20.78 g), T9 (19.97 g) and least was found in T7 (19.47 g).

In the variety RVW 4106, mutagen SA significantly decreased the yield per plant. Mutagen HA also reduced the yield but less than sodium SA. Here also control recorded the maximum yield followed by T21 (21.73), T18 (21.37) and least yield found in T22 (14.33).

Increase in grain yield with HA in MP 3382 may be due to mutagen HA could promote seed production. Sharma *et al*.,

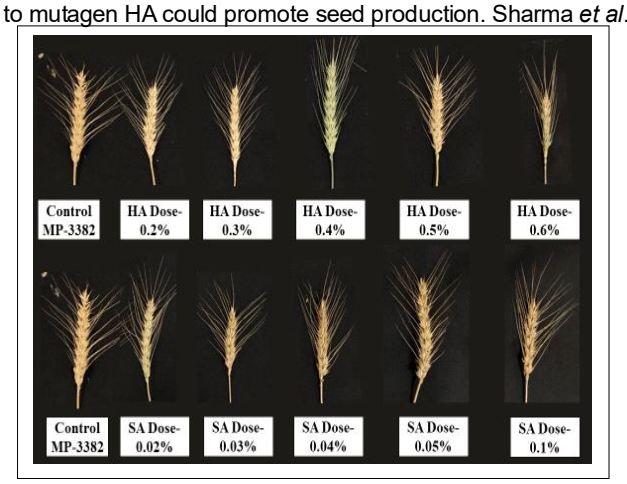

Plate 1: Variation in spike length with different treatments on MP-3382 with HA and SA.

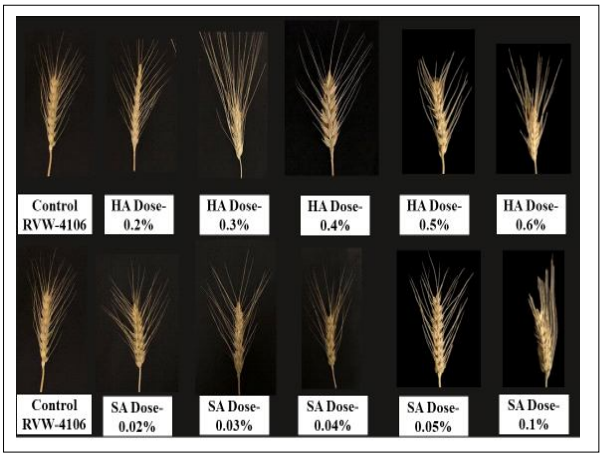

Plate 2: Variation in spike length with different treatments on RVW-4106 with HA and SA.

(2015), have reported a similar trend after treatment by chemical mutagen in wheat and Dahiru *et al*., (2021) in maize. Wheat varieties exhibit differential sensitivity to mutagens like HA, potentially influenced by variations in DNA repair mechanisms or the presence of genes that mitigate mutagenic effects (Chatterjee *et al.,* 2017).

Test weight (g)

For the variety MP 3382**,** data displayed that both mutagen SA and HA significantly reduced the test weight as compared to control. However, for the variety RVW 4106, data revealed that mutagen SA reduced the test weight in all the treatments compared to control (45.03 g) except in T17 (48.82 g). Mutagen HA data revealed that maximum test weight was present in T18 (48.40 g) and least weight was found in T19 (31.98 g).

Generally, both mutagens reduced the test weight except 0.5% HA in MP-3382 and 0.1% SA and 0.2% HA in RVW-4106 where test weight was observed to be increased. Data revealed that generally all the doses adversely affected test weight. Present study was contradicted with the findings of Aggarwal *et al.* (2022)**.**

M_2 generation

Total 86 mutants were isolated in the central focus of this mutation analysis, with their characterization based on morphological plant type. Notably, the mutagenic agents Sodium Azide (SA) and Hydroxyl Amine (HA) yielded a substantial number of mutants. The frequency of mutants displaying desirable and variable morphological traits is presented in Table 7.

Germination percent

The dose of SA with concentration of (0.1%) was found to be most effective in the variety MP-3382 and RVW-4106 on contrary to the control. The M_1 and M_2 generation was observed with significant reduction in germination percentage *i.e.,* (85) and (87) respectively for MP-3382 and (83) and (85) for RVW-4106 respectively. The drastic reduction in the germination with increase in the concentration of sodium azide may be due to the induction of chromosomal structure abnormalities in the genes of vitality. This result is in synchronization with Abu *et al.* (2020) and Ibukun *et al.* (2019)**.**

The dose of HA with concentration of (0.4%) was found to be most effective in the variety RVW- 4106 on contrary to the control (92). The M_1 & M_2 generation was investigated with significant induction in germination percentage i.e. (95) and (94) respectively for RVW-4106. This increase may be due to high rate of mitotic cell division and hence increased embryonic growth. Sharma *et al.* (2015) also recorded the similar pattern of observation.

Plant height (cm)

Significant variation in plant height, the control of both the varieties MP-3382 and RVW-4106 displayed the values (97.50) and (103.00) respectively. M1 and M2 data revealed that SA with concentration 0.03% (82.50 and 84.50) respectively and 0.5% HA (88.30 and 90.00) respectively in the variety MP-3382 and 0.1 % SA (82.80 and 84.00) respectively in the variety RVW-4106 was found to be most effective dose. There is significant reduction in plant height at these particular doses. These finding were in conformity of Sharma *et al.,* (2015) in wheat and Weldemichael *et al.,* (2021).

Days to 50% flowering

An increase in delayed in days to 50 percent flowering was evident in the variety MP-3382 in both M_1 and M_2 generation at 0.05 per cent SA as compared to control (70 days). Due to presence of suspected chromosomal aberrations, a delay in flowering was investigated. Abu *et al.* (2020) and Dahiru *et al*. (2021) also reported similar results in maize.

Spike length (cm)

The variety MP-3382 was most responsive toward the HA with concentration 0.5 per cent for spike length. An increased in spike length was observed in M_1 and M_2 generation that is 12.60 cm and 12.20 cm respectively as compared to control (11.50 cm). This change may be due to cell enlargement by gushing of water inside cell or tissue differentiation after treated with mutagen hydroxyl amine.

Table 7: Character wise mutant observed in M_2 generation.

Characters of Mutants	Mutagen	Dose (%)	Variety	Control	M_1	M_2
Germination (%)	SA	0.1	MP-3382	95.00	85.00	87.00
Plant Height (cm)	SA	0.03	MP-3382	97.50	82.50	84.50
Days to 50% flowering	SA	0.05	MP-3382	70.00	75.00	76.00
Flag Leaf area (cm^2)	SA	0.03	MP-3382	28.85	51.40	48.00
Spike length (cm)	HA	0.5	MP-3382	11.50	12.60	12.20
Plant height (cm)	HA	0.5	MP-3382	97.50	88.30	90.00
Flag leaf area (cm^2)	HA	0.4	MP-3382	28.85	49.06	47.50
Germination (%)	SA	0.1	RVW-4106	92.00	83.00	85.00
Plant height (cm)	SA	0.1	RVW-4106	103.00	82.80	84.00
Flag leaf area (cm^2)	SA	0.03	RVW-4106	34.15	54.10	52.60
Germination (%)	HA	0.4	RVW-4106	92.00	95.00	94.00
Flag leaf area (cm^2)	HA	0.4	RVW-4106	34.15	53.49	51.26

Abaza *et al*. (2020) and Rahman *et al*. (2021) also obtained the similar results after treatment with chemical mutagen in bread wheat.

Flag leaf area (cm^2)

The most effective dose for both the varieties was found to be 0.03 percent SA and 0.4 percent HA. The variety MP-3382 displayed the values for M_1 and M_2 generation are 51.40 cm^2 and 48.00 cm^2 respectively. Whereas, the variety RVW- 4106 displayed the values for M_1 and M_2 generation are 54.10 cm^2 and 52.60 cm^2 respectively at 0.03 percent SA and 53.49 cm^2. This similar observations of increase in flag leaf area by chemical mutagens at particular concentrations was reported by Odeje *et al*. (2016), Abaza *et al*. (2020) in bread wheat.

CONCLUSION

The optimum dose for mutagen Sodium Azide was found to be 0.02 % where germination, root length, shoot length and SVI was found at par with control and most effective dose for SA and HA was observed to be 0.1% and 0.4 % respectively to create genotypic and phenotypic changes in these parameters. For character flag leaf area 0.03% SA and 0.4% HA was found most effective for increasing flag leaf area. Most effective dose for plant height was observed to be 0.03% SA, 0.5% HA and 0.1% SA for creating variability and for spike length effective dose observed to be 0.5% HA. Regarding days to 50 percent flowering, 0.05% SA was found to be the most effective dose. Based on study of characteristics in M_1 and M_2 generation, 12 mutants were validated. Among the 12 mutants, seven were validated for variety MP-3382 and five for variety RVW-4106. Above mentioned doses were found to be the most effective dose of Sodium Azide and Hydroxyl Amine for creating mutations.

Conflict of Interest

No conflict of interest is there with this article.

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