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Assessment of Quality Parameters of Chemically Mutagenized Wheat Seeds

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ABSTRACT: Wheat being one of the most important staple foods globally plays an important role for food security in terms of cereal source of nutrients. Presently, with global population booming up and billions of people suffer micronutrient malnutrition and therefore improving seed quality traits through genomic assisted breeding or mutation breeding to create genetic variations of utmost importance. The present study was undertaken to evaluate the effect of mutagens Hydroxyl Amine (HA) and Sodium Azide (SA) on different seed quality parameters in two wheat genotypes *i.e.,* MP-3382 and RVW-4106. An experiment was conducted to evaluate wheat varieties MP-3382 and RVW- 4106. Seeds were pre-soaked in distilled water for six hours and later treated with five different doses of Sodium Azide (0.2 % ,0.3% ,0.4% ,0.5% and 0.1%) and five different doses of Hydroxyl Amine (0.02%, 0.03%, 0.04%, 0.05% and 0.1%) including control during *Rabi* 2021- 22 (M1 generation) at Genetics and Plant Breeding Laboratory, ITM University Gwalior. The experiment was conducted in Completely Randomized Design with 4 replications and to determine mutagen sensitivity with regard to germination (%), root-shoot length (cm), seed vigorindex -I, dry weight and seed vigor index-II under laboratory conditions on mutagenized seeds. Itwas observed that both the mutagens were significantly affected all the seed quality parameters and induced genetic variability.

Keywords: Hydroxyl Amine, Mutagen sensitivity, Sodium Azide, Variability, Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the major staple food crop feeding about 35 % of the world population. The global production of wheat is about 766 million tons and it is cultivated across nearly 216 million hectares in more than 125 countries (Sharma *et al.,* 2021). The crop is self-pollinated and allohexaploid with chromosome number 2n =6x = 42 (Bonjean *et al.,* 2001). Wheat is a rich source of complex carbohydrates (74-77%) and protein (11-15%) and poor source of sodium, amino acids and total fat (Dziki *et al.,* 2021). The existing and projected high population growth rates would worsen the gap between wheat output and demand, adding to the country's limited food supplies. This situation emphasizes the importance of wheat breeding in increasing current productivity. Plant breeding is based on the notion of genetic variability. It could be developed by hybridization or mutations followed by selection (Addisu and Shumet 2015). Hybridization is tedious, time consuming, labour intensive and expensive nature of manual emasculation and pollination (Yahaya *et al.,* 2020). Mutation breeding provides the advantage of being able to change specific qualities in otherwise acceptable varieties in a shorter amount of time by inserting some relevant variant. The Food and Agriculture Organization of the United Nations/International Atomic Energy Agency's Mutant Variety Database (FAO/IAEA-MVD data 2019) reports on 3,275 accessions from 225 species generated and freely disseminated by the FAO/IAEA. In crops, changes in the gene structure and sequence can be randomly caused by rupturing the DNA through physical and chemical mutagens (Viana *et al.,* 2019). Nucleotide substitution causes random point mutation in plant material. The use of Sodium Azide and Hydroxyl Amine to induce mutants has been reported by number of workers (Weldemichael *et al.,* 2021), (Ibukun *et al.,* 2019) and (Kirtane *et al.,* 2018). Keeping this in view, the present investigation was carried out to determine the effect of SA and HA on seed characteristics *viz.,* germination percentage, shoot length, root length, seed vigour index-I, dry weight and seed vigour index-II. Different doses of HA and SA were also evaluated for vitality of the seeds.

MATERIALS AND METHODS

The study was conducted during *Rabi* 2021-2022 at Genetics and Plant Breeding Laboratory, School of Agriculture, ITM University, Gwalior (M.P.).

Experimental material: "MP-3382" Matures in 119 days, high yielding (5975 kg/ ha), bold seeded, multiple resistances and heat tolerant. Recommended for irrigated, timely sown conditions of M.P.

"RVW-4106": It matures in 115-120 days. Resistant to black and brown rust disease. Average yield is 5035 kg/ha. Recommended for late sown irrigated conditions of M.P.

Chemical Treatment: To begin with experiment, selected seeds were soaked in distilled water for 6 hours and the wet seeds were treated with different concentrations of HA and SA for six hours (Jeberson *et al.,* 2020). The seeds were treated with 0.2%, 0.3%, 0.4%, 0.5% and 0.6% HA, whereas, 0.02%, 0.03%, 0.04%, 0.05% and 0.1% SA. The untreated seeds served as control.

The treated seeds were washed thoroughly in running water for two hours to terminate the reaction of chemical mutagen and to leach out the residual chemicals. We have total 11 treatments including control for each variety. The treatment details are given in table 1.

Table 1: Treatment details.

Treatment	Chemical Mutagen	Concentration
T1	Control	Untreated
T2	Sodium Azide	0.02%
T3	Sodium Azide	0.03%
T4	Sodium Azide	0.04%
T5	Sodium Azide	0.05%
T6	Sodium Azide	0.1%
T7	Hydroxyl Amine	0.2%
T8	Hydroxyl Amine	0.3%
T9	Hydroxyl Amine	0.4%
T10	Hydroxyl Amine	0.5%
T11	Hydroxyl Amine	0.6%

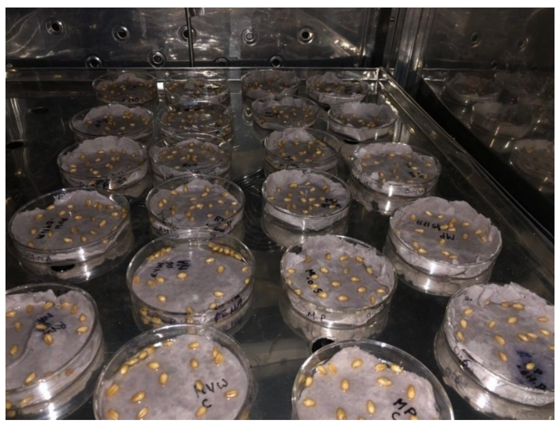

Fig. 1. The petridish were kept in seed germinator.

Observations to be recorded: For the assessment of seed quality parameters, 100 seeds of each treatments and replication including control were sown in petridish cover by blotting paper and half filled with water. Each of the treatments were replicated four times. The petridish were kept in seed germinator at 25±1ºC temperature and relative humidity was 85 ± 1 %. Observations were recorded for six characters *i.e.,* germination percentage, root length, shoot length, seed vigour index – I, dry weight and seed vigour index – II.

Statistical analysis: Data were collected and subjected to Analysis of Variance by using OP STAT (O.P. Sheoran Programmer, Computer Section, CCS HAU and Hisar) and significant mean was separated using t-test (One factor analysis).

RESULTS AND DISCUSSION

Analysis of Variance (ANOVA) revealed highly significant variation among the treatments for both the varieties for all six characters under study as shown in Table 2 and 3.

The observation on six characters *viz.,* germination percentage, root length, shoot length, seed vigor index - 1, dry weight and seed vigor index –II on two different varieties; MP-3382 and RVW-4106 has been analyzed for the inference of result discussed as under. The effect of different doses of mutagens SA and HA is depicted through Fig 2. The graph represents the effect of the mutagens on two varieties with four characters.

For the character Germination Percentage, maximum germination was found in MP-3382 for 0.4% HA (98%) which is more than control (95%). Least value of germination (83%) was found in RVW-4106 for 0.1% SA. The treatment V1T9 was observed to display maximum Root and Shoot length (11.31 cm and 11.20 cm) respectively. Least values of root and shoot length (3.26 cm and 2.85 cm) was observed in V2T6. Maximum Seed Vigor Index-I was again observed in same treatment V1T9 (2205.98) because the germination percentage as well as both root and shoot length was highest in this treatment as compared to control (1899.10) . The SVI of V1T9 was evaluated to be higher than 306 degrees. The character Dry Weight depends upon seedling length as more the seedling length more will be the weight therefore maximum dry weight was obtained in V1T9 because of maximum seedling length. The character SVI-II depends upon germination percentage and dry weight. Both the parameters displayed maximum magnitude for the treatment V1T9. Therefore, it is obvious to have maximum SVI-II (37.89) for the treatment T9.

As per the Table 4, it is clearly evident that the different doses of mutagens created a significant variability for the characters still no such dose is validated which may act as LD50. All the treatments were found to be sub vital in survivability.

In total, it was analyzed two categories of effect of mutagens.Increase in the magnitude and Decrease in the magnitude.

An increase in the values of all the seed parameters taken under study can be explained due to increased embryonic growth coupled with high rate of mitotic cell division. Similar pattern of mutagenic effect has been recorded by Sharma *et al.*, (2015), Irfaq and Nawab (2001); Jain *et al.* (2015). The mutagen Sodium Azide displayed delayed or inhibition in all the characters which might be due to suppressed enzymatic activities, hormonal imbalance and slow down of physiological process necessary for seed germination .This result is in synchronization with Herwibawa *et al.* (2018) in chill pepper, Abu *et al.,* (2019); Ibukun *et al.* (2019); Akinyosoye (2020) in maize and Julia *et al.* (2022) in Indian Mustard.

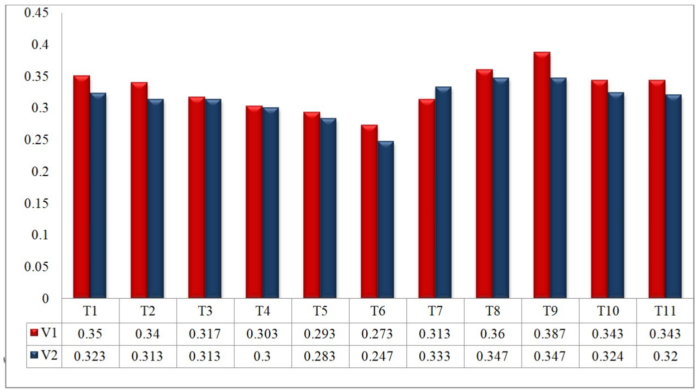

Fig. 2. The effect of mutagens on two varieties for dry weight.

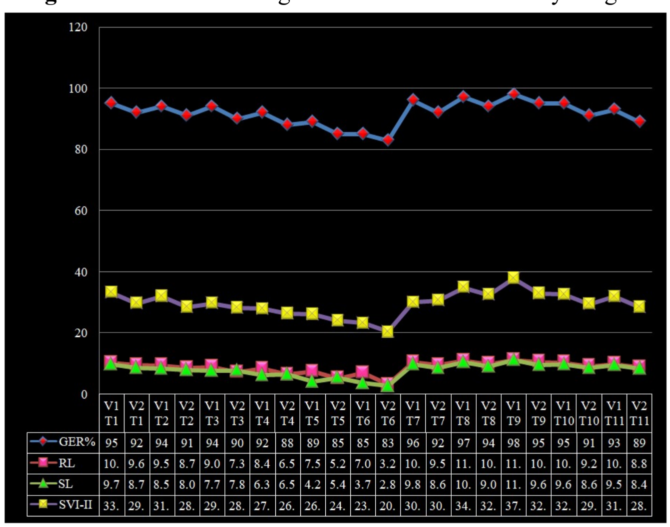

Fig. 3. The effect of mutagens on two varieties with four characters.

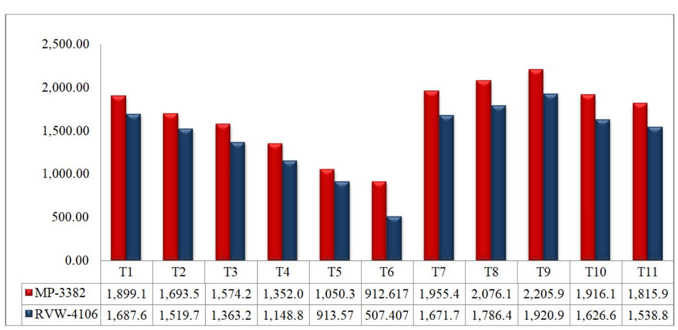

Fig. 4. The effect of mutagens on two varieties for seed vigour index – I.

Table 2: Analysis of variance of MP – 3382.

Sr. No.	Characters	Mean Sum of Squares	
		Treatment	Error
1.	Germination Percentage	41.62**	1.54
2.	Root Length	5.98**	0.56
3.	Shoot Length	18.63**	0.25
4.	Seed Vigour Index-I	520773.74**	6906.69
5.	Dry Weight	0.0032**	0.0004
6.	Seed Vigour Index-II	50.56**	3.13

Level of significance at 5%

Table 3: Analysis of Variance of RVW -4106.

Sr. No.	Characters	Mean Sum of Squares	
		Treatment	Error
1.	Germination Percentage	39**	2.090909
2.	Root Length	15.384**	0.182
3.	Shoot Length	11.502**	0.134
5.	Seed Vigour Index-I	525784.761**	4235.988
6.	Dry Weight	0.0025**	0.0002
7.	Seed Vigour Index-II	39.536**	1.806

Level of significance at 5%

Table 4: Vitality status of mutagenized seeds.

Treatments	Variety	Mutagen	Dose	Germination %	Survivability
T1	MP-3382	Control	Untreated	95	Sub Vital
T2	MP-3382	SA	0.02	94	Sub Vital
T3	MP-3382	SA	0.03	94	Sub Vital
T4	MP-3382	SA	0.04	92	Sub Vital
T5	MP-3382	SA	0.05	89	Sub Vital
T6	MP-3382	SA	0.1	85	Sub Vital
T7	MP-3382	HA	0.2	96	Sub Vital
T8	MP-3382	HA	0.3	97	Sub Vital
T9	MP-3382	HA	0.4	98	Sub Vital
T10	MP-3382	HA	0.5	95	Sub Vital
T11	MP-3382	HA	0.6	93	Sub Vital
T1	RVW-4106	Control	Untreated	92	Sub Vital
T2	RVW-4106	SA	0.02	91	Sub Vital
T3	RVW-4106	SA	0.03	90	Sub Vital
T4	RVW-4106	SA	0.04	88	Sub Vital
T5	RVW-4106	SA	0.05	85	Sub Vital
T6	RVW-4106	SA	0.1	83	Sub Vital
T7	RVW-4106	HA	0.2	92	Sub Vital
T8	RVW-4106	HA	0.3	94	Sub Vital
T9	RVW-4106	HA	0.4	95	Sub Vital
T10	RVW-4106	HA	0.5	91	Sub Vital
T11	RVW-4106	HA	0.6	89	Sub Vital

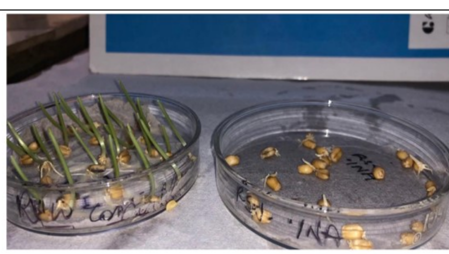

Fig. 5. Inhibitory effect at 0.1% sodium azide.

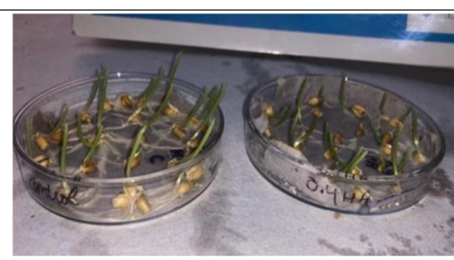

Fig. 6. Enhancing effect at 0.4% hydroxyl amine.

CONCLUSION

A general trend has been observed that mutagen HA is more effective than SA as there is trend of reduction in the magnitude of characters with increase in dose of SA, whereas, a bell shaped curve is clearly evident in HA where increase in dose from 0.2% increased the magnitude of character up to 0.4%. Further increase in the dose leads to decline in the magnitude of the characters. Hence, the optimum dose for HA which is most effective was found to be 0.4 %.

FUTURE SCOPE

Wheat is the most extensively grown cereal crop in the world and third most important crop in terms of global production. The existing high population growth rates would worsen the gap between wheat production and country's demand. This situation emphasis the need of wheat breeding for increasing productivity. Plant breeding is based on notion of genetic variability. It may be developed by hybridization or mutation breeding but hybridization is much labour intensive, time consuming and results in low genetic change in self pollinated crops as compared to mutation. As wheat is a self-pollinated crop, hence the variability is very less in wheat as compared to cross-pollinated crops. Mutation is random and provides the advantage of change specific qualities in acceptable varieties in shorter amount of time by alteration of nucleotide pairs as mutation is the ultimate source of all the variation. Hence, through the mutation breeding or in association with latest technologies of genetic engineering surely will be a major impact on future crop improvement programmes.

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