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RESEARCH ARTICLE

Effect of chemical mutagens on wheat's morphology of two genotypes under normal and osmotic stress conditions

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Abstract

Drought caused by climate change results in water scarcity, reduced global wheat yields and unpredictable rainfall, impacting nearly 50 % of wheat production worldwide. India, the second-largest wheat producer after China, contributes about 12 % to global wheat output. Mutation breeding is a promising approach for improving drought tolerance in wheat, especially in drought-prone regions. This study evaluated the effects of varying EMS and SA concentrations on drought tolerance in wheat genotypes HD-3226 and HI-1620 under PEG-induced stress during 2020–2021 Rabi season at SVPUA&T, Meerut, India. *In-vitro* screening of EMS and SA induced mutants using PEG-6000 as a chemical drought agent proved to be an effective method for identifying drought-tolerant wheat lines. The results revealed that EMS treatments led to an increase in plant height and spike length as compared to control-wild types, whereas SA treatments caused a decline in these traits across both genotypes. Additionally, number of reproductive tillers per plant decreased in EMS treatments, while it increased in SA treatments relative to wild type. Further, phenotypic traits such as days to 50 % heading, anthesis and maturity were delayed in both EMS and SA treated plants, as well as in control-wild types. Yield-related attributes such as number of spikelets per spike and 1000-grain weight were significantly reduced under PEG treatment across all treatments. This study underscores the substantial influence of chemical mutagens on vital morphological and yield-related traits in wheat under drought stress. The differential response of EMS and SA treatments suggests their potential utility in generating genetic variability for drought tolerance. These findings support the role of mutation breeding as an efficient approach in developing resilient wheat cultivars suitable for water-limited environments.

Keywords: chemical mutagens; osmotic stress and morphological traits; water stress; wheat

Abbreviations: EMS - Ethyl Methane Sulphonate; SA - Sodium Azide; PEG-6000 - Polyethylene Glycol (6000)

Introduction

Wheat (*Triticum aestivum* L.) is a member of *Triticeae* tribe and Poaceae family. For the last 10000 years, tetraploid and hexaploid varieties of wheat have been domesticated. Hexaploid form is modern day bread wheat and fulfils dietary needs of global population. By 2050, global population is expected to reach 10 billion, which would require double of current global food production (1). Global climate change poses a significant threat to sustainable agricultural production. Major abiotic stresses like drought, heat, salinity and cold negatively influence wheat's survival, biomass growth and yield, posing a significant risk to global food security. Among these stresses, drought is the primary constraint on growth and yield in agriculture due to water deficits experienced by plants, both in cultivated fields and natural environments. Water stress results in toxicity, decreased photosynthesis, leaf bleaching, curling, wilting and ultimately plant death (2). Wheat crops are especially vulnerable to water shortages, which result in decreased yield and production. Insufficient water supply causes drought stress, inhibiting or disrupting normal physiological and metabolic processes, ultimately leading to plant death (3). The development of varieties that can efficiently withstand water stress is a difficult task for breeders. By exploiting the genetic diversity that exists, plant breeding is ongoing process of creating improved plant phenotypes that are more suited to human requirements. Breeders face a challenging task in developing varieties that can effectively tolerate drought stress. Over the past several decades, plant-breeding methods have played a crucial role in creating genetically improved crop varieties, thereby enhancing food security (4). Genetic variations can occur naturally or be induced through mutagenesis, a process that alters an

organism's genetic information in a stable way, resulting in mutations (5). Mutagenesis can occur naturally or be induced by exposure to mutagens, such as chemicals like sodium azide and ethyl methane sulphonate (EMS), or through radiation like X-rays and gamma rays. This process can result in mutant proteins with distinct characteristics or improved functions that may hold commercial significance (6). Both physical and chemical mutagens have been effectively utilized to develop new plant varieties with enhanced economic traits. EMS mutagenesis has been successfully applied to a variety of crops, including oat, barley, maize and wheat (7). On the other hand, sodium azide (NaN_3) is recognized as one of the most effective chemical mutagens for plants. An organic metabolite of the azide molecule is produced which mediates the mutagenicity, when compared to other mutagenesis treatments used to enhance plant characteristics; it generates chromosomal abnormalities at a relatively low rate (8). The efficiency of mutant generation is affected by several factors, including pH, soaking in water, temperature, azide concentration and treatment time (9). Induced mutations have led to the development of varieties with improved genotype and phenotype characteristics. These varieties are either introduced directly as new cultivars or employed in crossbreeding programs (10, 11).

Polyethylene glycol (PEG) is a chemical used to simulate drought conditions, often to evaluate drought tolerance in seedlings at an early stage under controlled laboratory conditions (12-14). It is often used to create water stress in crop plants while avoiding physiological damage, ensuring consistent water potential during the experiment (15). PEG 6000 molecules are small enough to have a negligible effect on osmotic potential, but large enough that they are not absorbed by plants or quickly penetrate intact plant tissues. Since polyethylene glycol does not enter the apoplast, it leads to water being removed from cells. As a result, PEG solutions more accurately simulate dry soil conditions than solutions with low molecular weight osmotica, which can enter cell walls and add solutes to the cells (16). Using PEG for screening has proven effective in assessing the effects of water stress on seed germination and seedling growth traits (17-20). This method is simple, cost-effective and enables efficient screening of large germplasm collections in a short time (18, 21). Numerous techniques exist for identifying drought-tolerant germplasm and PEG is widely regarded as a highly effective inducer of water stress (22). Aim of this research was development of drought resistant mutant wheat lines through chemical mutagenesis and see the effect of both chemical mutagens on morphological traits after osmotic stress induction in both wheat genotypes.

Materials and Methods

Plant materials

Mature dry seeds of latest released varieties- HD-3226 (susceptible) and HI-1620 (tolerance) were used for the present investigation conducted during Rabi season (2020-2021) in PG laboratory of the agricultural biotechnology department, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, 250110 (U.P.), India.

Experimental design

Seeds (M_0 seeds) of genotypes HD-3226 and HI-1620 were sterilized with 70 % ethanol for 3-5 min at room temperature and then pre-soaked for 12 hrs. Experiment was conducted during Rabi session 2020-2021 (M_1 generation) in PG laboratory of the agricultural biotechnology department, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, 250110 (U.P.), India. After overnight pre-soaking, one hundred fifty seeds (M_0) of each genotype were treated with four different concentrations of Ethyl Methane sulphonate (EMS)-0.25 %, 0.5 %, 0.75 % and 1.0 % (v/v) and three different concentrations of sodium azide (SA)-0.02 %, 0.04 % and 0.08 % (w/v) for 2 hrs were used. After mutagen treatment, treated seeds (M_1 seeds) were transfer into a small cotton bag and these bags tie on stopcock to remove excess chemical mutagens under running tap water for 2 hrs. Then, twenty-five air dried M_1 and non-treated seeds as control-Wild Type were placed on moistened double whatman filter paper in each Petri plates with three replications in water and 15 % PEG-6000 solution (w/v) for drought tolerance screening (23). 5 mL of 15 % PEG solution was added to each Petri plates under osmotic stress conditions and distilled water was added to each Petri dish under normal conditions every 2 days to compensate for losses through evaporation up to 15 days (24). When seedlings were at stage of first true leaf initiation (after 15 days), successful germination in both water and 15% PEG solution, plants were transferred in the small plastic pots (25). After 20 days, all plants were transferred in the research field (Department of Agricultural Biotechnology, SVPUA&T, Meerut, U.P., India) in a Randomized Block Design (RBD).

Planting methods

All treated (M_1) and control seeds (wild type) were sown in the field in three replications by maintaining row to row and plant to plant distance. The plant-to-plant distances was kept around 15 cm and spacing between adjacent rows was kept 20 cm. The gap between two rows of different genotype (mutant progenies) was kept 50 cm for maintaining proper distance from each genotype to another genotype. After transplanting of all plants, fields were irrigated at regular interval of 20-25 days. The crop was maintained in the field using conventional agronomic practices to keep crop in good condition. All plants (mutant as well as control-wild type) sown in field, were tagged properly with their genotype name along with number and other details. Morphological data was collected for screening M_1 mutant plants and collected M_2 seeds and M_2 seeds further, grow for M_2 generation for desired mutant lines identification.

Data collection and statistical analysis

The impact of Ethyl Methane Sulphonate (EMS) and sodium azide on various morphological traits were analysed in two wheat genotypes during the M_1 generation. Data were recorded from the average of five randomly selected plants from the M_1 population. After seedling stage, drought screening was focused on morphological traits i.e., plant height, total no. of spike bearing tillers per plant, Days to 50 % Heading (DH), day to 50 % Anthesis (DA), days to 50 % maturity (DM), spike length (SL), number of spikelet's per spike and 1000 - Seed weight. The plant height was measured in centimetres from ground level to top of tallest spikes, excluding awn, at the time of 50 % maturity and mean value was calculated. The number of spike-bearing tillers per plant was determined by counting and averaging the tillers from five randomly selected

plants from M_1 population. Reproductive tillers were identified by counting those bearing ear heads. This was recorded as number of days from the appearance of tillers to spike emergence in 50 % of the plants in the plot while 50 % days to anthesis was recorded as number of days from when 50 % of the plants flowered to when spikes emerged in the plot. Moreover, 50 % days to maturity was recorded as number of days from appearance of spikes to day when more than 70 % of the plants reached maturity. Spike length (SL) was measured in centimetres, starting from the neck's base to the topmost spikelet on the largest spike and total number of spikelet's per spike was determined by counting them in each replication. Further, one thousand sun dried matured seeds of M_1 generation were taken manually count as representative sample of each selected plants and used electronic balance to record weight in gram. The collected data were analysed using analysis of variance (ANOVA) with two factors, conducted using OPSTAT software.

Results

In-vitro screening of M_1 populations was carried out by germination of seeds on 15 % PEG-6000 and analysed the morphological mutagenic effects of both EMS and Sodium azide were studied on plant height, total numbers of reproductive tiller per plant, spike length (cm), 50% days of heading, 50 % days of anthesis, 50 % days of maturity, spikelets per spike and 1000-grain weight (g) in M_1 generation of both wheat genotype (Fig. 1).

Effect of chemical mutagens on plant growth characteristics

Plant height, spike length and the number of spikelets per spike are key agronomic traits in wheat, as they are strongly associated with lodging resistance and yield. The plant height of HD-3226 wild type was 69.73 cm in water and 74.33cm in 15% PEG (Fig. 2; Table 1). In EMS treatments, it was varied 66.65cm (EMS 0.25 %) to 75.47cm (EMS 1.0 %) and 72.51cm (EMS 0.75 %) to 78.92cm (EMS 0.5 %) in water and 15 % PEG treatments, respectively. While SA decreased plant height as compared to EMS treatments and ranges from 66.00cm (SA 0.02 %) to 69.50cm (SA 0.08 %) and 63.05cm (SA 0.08 %) to 66.63cm (SA 0.02 %) in water and 15 % PEG treatments, respectively. The average of mean value across all treatments in water was 71.12 cm (EMS) and 68.22 cm (SA) while it was 75.89 cm (EMS) and 64.71cm (SA) in PEG. The plant height of HI -1620 wild type was 71.33 cm in water and 70.53 cm in 15 % PEG treatments. In EMS treatments, it ranges 74.75 cm (EMS 0.25 %) to 76.77 cm (EMS 0.75 %) and 63.39 cm (EMS 0.75 %) to 72.71cm (EMS 1.0 %) in water and 15 % PEG treatments, respectively. SA also decreased plant height as compared to EMS in water treatments and it range from 67.85 cm (SA 0.04 %) to 73.06 cm (SA 0.08 %) and 66.66 cm (SA 0.08 %) to 69.80 cm (SA 0.02 %) 15 % PEG treatments, respectively. Average of mean value across all treatments in water was 75.59 cm (EMS) and 69.77 cm (SA) while it was 65.95 cm (EMS) and 68.50 cm (SA) in 15 % PEG treatments.

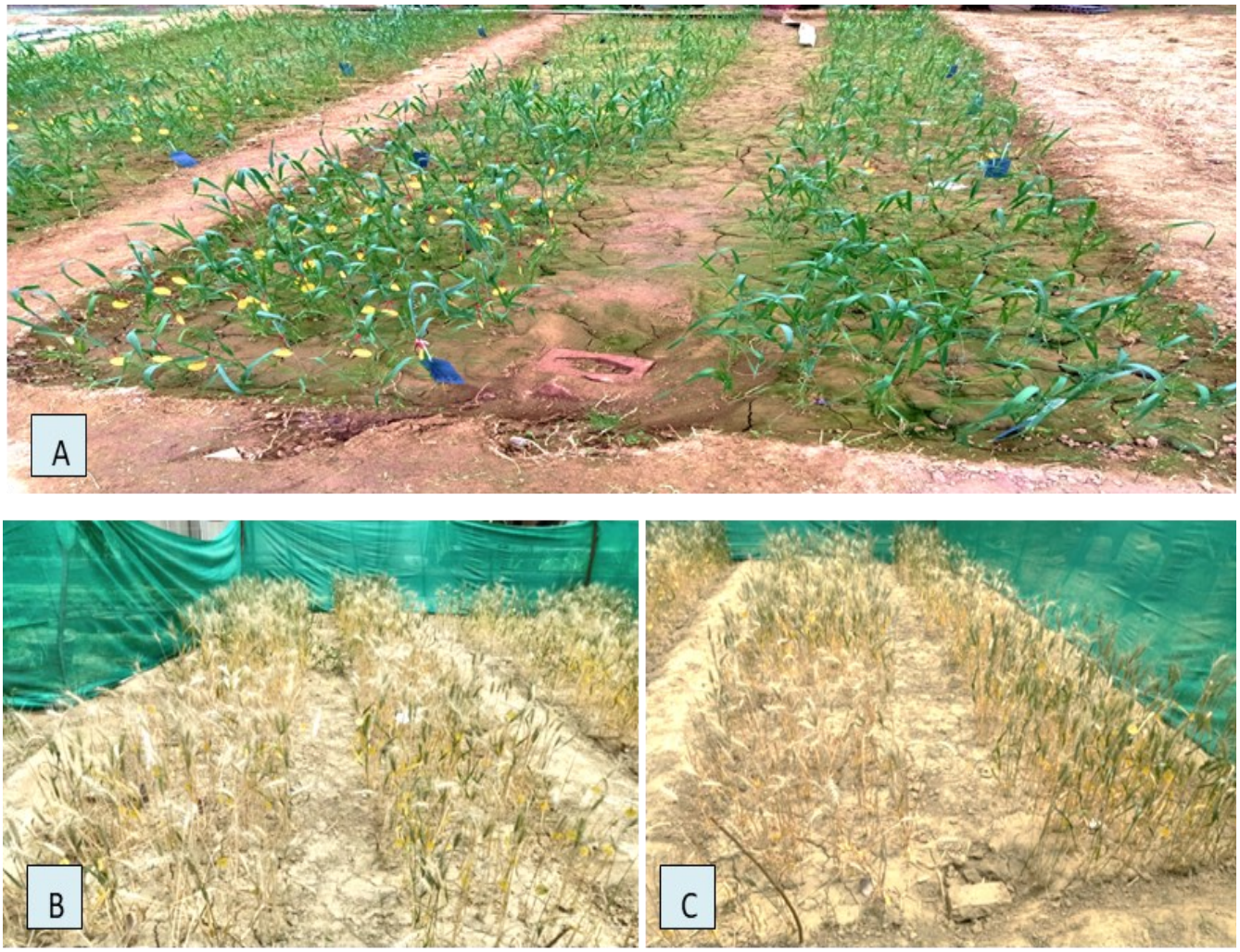

Fig. 1. (A) M_1 generation- plants transfer in the field from pots; (B, C) - M_1 generation during maturity.

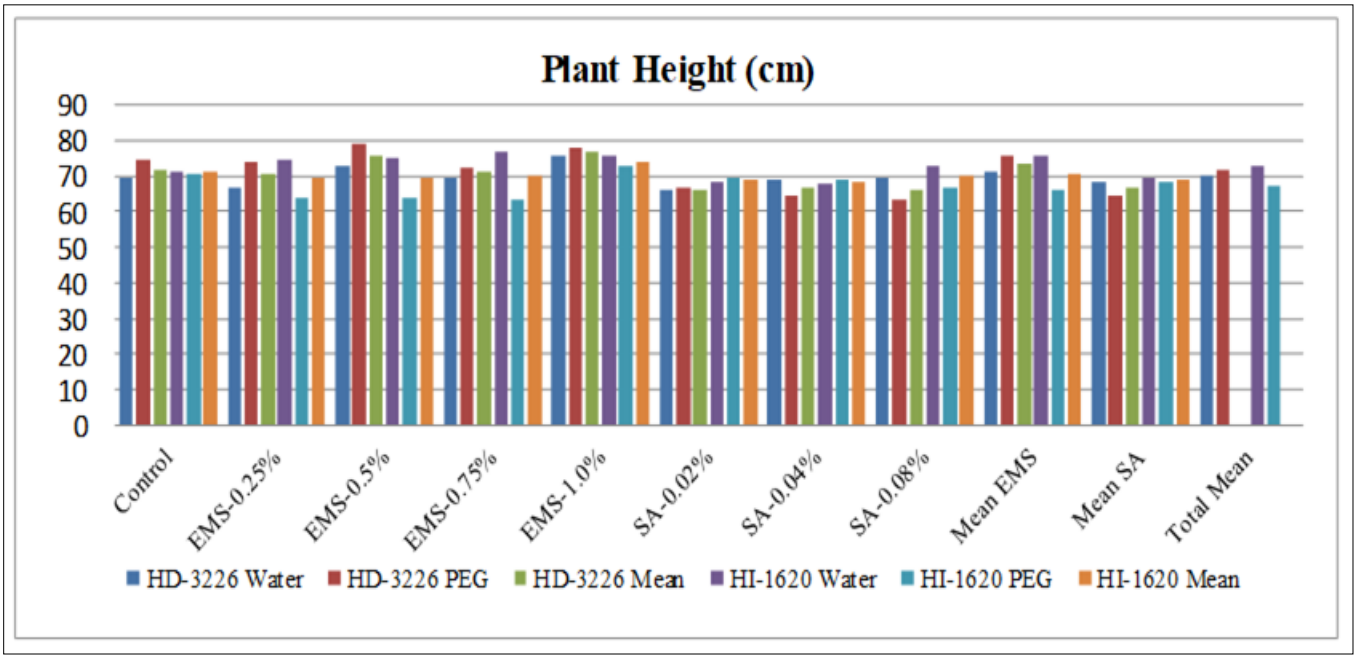

Fig. 2. Mean performance of plant height (cm) of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

Numbers of reproductive tiller per plant in control HD-3226-wild type was 3.93 in water and 4.18 in 15 % PEG. In EMS treatments, it was ranges 2.81 (EMS 1.0 %) to 4.16 (EMS 0.5 %) and 3.2 (EMS 0.25 %) to 3.79 (EMS 1.0 %) in water and 15 % PEG treatments, respectively (Fig. 3; Table 1). While SA increased the total numbers of reproductive tiller per plant as compared to EMS, it ranges 3.50 (SA 0.04 %) to 3.80 (SA 0.02 % and SA 0.08 %) and 4.17 (SA 0.02 % and SA 0.04 %) to 4.67 (SA 0.08 %) in water and 15 % PEG treatments, respectively. Average of mean value across all treatments in water was 3.54 (EMS) and 3.70 (SA) while it was 3.44 (EMS) and 4.34 (SA) in PEG. In HI-1620 genotype, total numbers of reproductive tiller per plant in control-wild type was 3.95 in water and 4.08 in 15 % PEG treatments. In EMS treatments, it ranges 2.10 (EMS 0.25 %) to 3.72 (EMS 0.75 %) and 2.33 (EMS 0.5 %) to 2.95 (EMS 1.0 %) in water and 15 % PEG treatments, respectively. SA increased the total numbers of reproductive tiller per plant as compared to EMS in water treatments and it range from 2.73 (SA 0.04 %) to 3.97 (SA 0.08 %) and it range from 2.83 (SA 0.02 %) to 3.87 (SA 0.08 %) 15 % PEG treatments, respectively. Average of mean value across all treatments in water was 2.63 (EMS) and 3.16 (SA) while it was 2.58 (EMS) and 3.48 (SA) in 15 % PEG treatments.

Spike length in control- HD-3226 wild type was 8.40 cm in water and 8.28 cm in 15 % PEG. In EMS treatments, it was ranges 6.85 cm (EMS 0.25 %) to 7.33 cm (EMS 1.0 %) and 7.34 cm (EMS 0.75 %) to 7.80 cm (EMS 0.5 %) in water and 15 % PEG treatments, respectively. While SA slightly decreased spike length as compared to EMS, it ranges from 6.56 cm (SA 0.04 %)

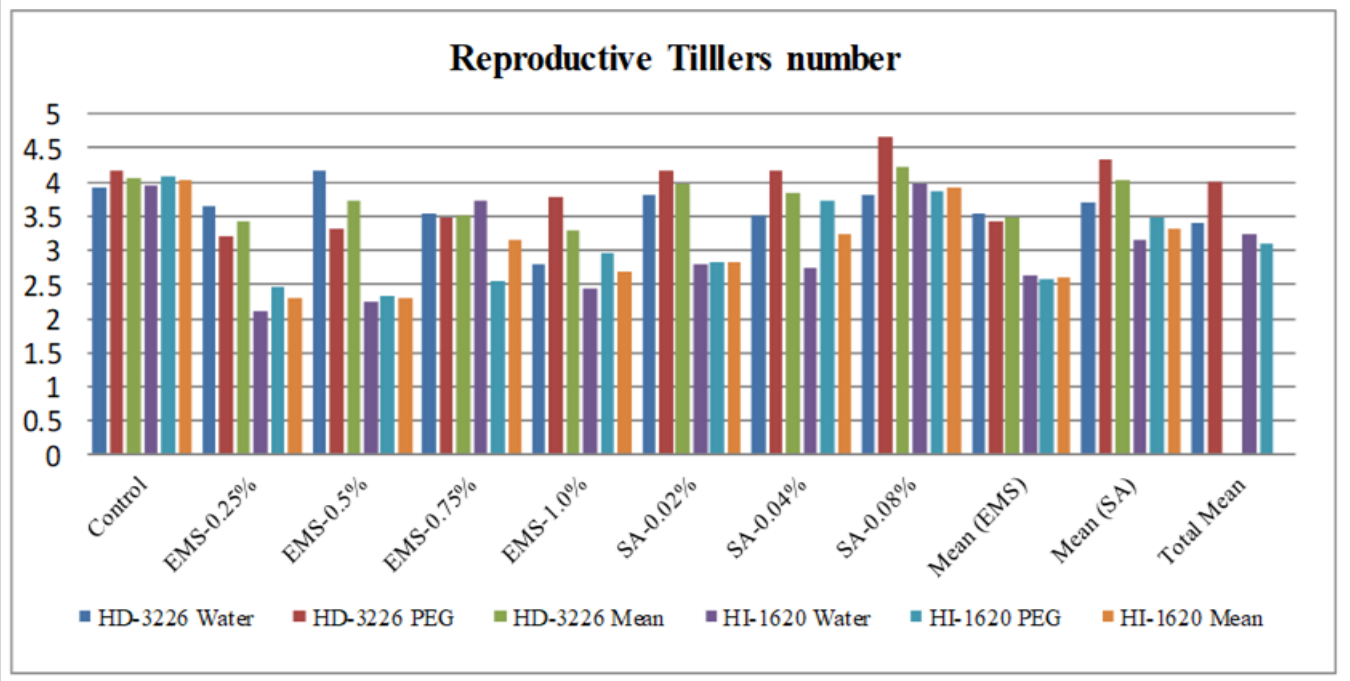

Fig. 3. Mean performance of total numbers of reproductive tiller per plant of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

Table 1. Plant height (cm) and total numbers of reproductive tillers per plant of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation

Treatments	Plant Height (cm)						Reproductive Tillers Number					
	HD-3226			HI-1620			HD-3226			HI-1620		
	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean
Control	69.73	74.33	72.03	71.33	70.53	70.93	3.93	4.18	4.05	3.95	4.08	4.02
EMS-0.25%	66.65	74.14	70.4	74.75	63.73	69.24	3.64	3.2	3.42	2.1	2.48	2.29
EMS-0.5%	72.69	78.92	75.81	75.16	63.98	69.57	4.16	3.32	3.74	2.25	2.33	2.29
EMS-0.75%	69.66	72.51	71.08	76.77	63.39	70.08	3.55	3.47	3.51	3.72	2.56	3.14
EMS-1.0%	75.47	77.92	76.69	75.69	72.71	74.2	2.81	3.79	3.3	2.45	2.95	2.7
SA-0.02%	66	66.63	66.31	68.4	69.8	69.1	3.8	4.17	3.98	2.8	2.83	2.82
SA-0.04%	69.16	64.45	66.81	67.85	69.03	68.44	3.5	4.17	3.83	2.73	3.73	3.23
SA-0.08%	69.5	63.05	66.27	73.06	66.66	69.86	3.8	4.67	4.23	3.97	3.87	3.92
Mean EMS	**71.12**	**75.89**	73.49	**75.59**	**65.95**	70.77	**3.54**	**3.44**	3.49	**2.63**	**2.58**	2.61
Mean SA	**68.22**	**64.71**	66.46	**69.77**	**68.5**	69.13	**3.7**	**4.34**	4.02	**3.16**	**3.48**	3.32
Total Mean	69.86	71.49		72.87	67.48		3.41	4.01		3.24	3.11	
	Factors (A)	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**	**Factor (A)**	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**
CD	2.47	1.23	3.5	2.54	2.11	5.98	0.55	0.27	N/A	0.37	0.24	0.52
SE(d)	1.2	0.6	1.7	2.06	1.03	2.91	0.27	0.13	0.38	0.18	0.09	0.26
SE(m)	0.85	0.42	1.2	2.915	0.72	2.06	0.19	0.09	0.27	0.13	0.06	0.18
Significance at 5%	0	0.01097	0.00001	0.002	0.00001	0.00271	0.00012	0.00009	0.55389	0	0.00475	0.00023

to 7.29 cm (SA 0.02 %) and 6.72 cm (SA 0.08 %) to 7.52 cm (SA 0.04 %) in water and 15 % PEG treatments, respectively (Fig. 4; Table 2). The average of mean values across all treatments in water was 7.11 cm (EMS) and 7.04 cm (SA) while it was 7.55 cm (EMS) and 7.16 cm (SA) in PEG treatments. In HI-1620 genotype, spike length in control-wild type was 8.01 cm in water and 7.79 cm in 15 % PEG treatments. In EMS treatments, it ranges 6.01 cm (EMS 0.5 %) to 7.58 cm (EMS 1.0 %) and 6.19 cm (EMS 0.5 %) to 7.38 cm (EMS 1.0 %) in water and 15 % PEG treatments, respectively. While SA increased spike length as compared to EMS, it ranges from 7.32 cm (SA 0.08 %) to 7.49 cm (SA 0.02 %) and it range from 6.68 cm (SA 0.02 % and SA 0.08 %) to 7.15 cm (SA 0.04 %) in water and 15 % PEG treatments, respectively. Average of mean values across all treatments in water was 6.83 cm (EMS) and 7.38 cm (SA) while it was 6.68 cm (EMS) and 6.83 cm (SA) in 15 % PEG treatments.

Days to 50 % heading in control-HD-3226 wild type were 85.13 in water and 88.43 in 15 % PEG (Table 3). In EMS treatments, it ranges 83.30 (EMS 0.25 %) to 88.30 (EMS 0.5 %) and 86.60 (EMS 0.25 %) to 91.60 (EMS 0.5 %) in water and 15 % PEG treatments, respectively. While SA slightly delay the days to 50 % heading as compared to EMS, it ranges from 90.46 (SA 0.02 %) to 92.80 (SA 0.04 %) and 95.10 (SA 0.04 %) to 96.96 (SA 0.02 %) in water and 15 % PEG treatments, respectively (Fig. 5). Average of mean value across all treatments in water was 86.72 (EMS) and 91.40 (SA) while it was 90.02 (EMS) and 96.10 (SA) in PEG. In HI-1620 genotype, days to 50 % heading in control-wild type was 90.60 in water and 91.62 in 15 % PEG treatments. In EMS treatments, it ranges from 83.37 (EMS 0.75 %) to 87.53 (EMS 0.25 %) and 84.90 (EMS 1.0 %) to 88.06 (EMS 0.25 %) in water and 15 % PEG treatments, respectively. SA also delay the days to 50 % heading as compared to EMS in water treatments and it range from 93.26 (SA 0.04 %) to 100.03 (SA 0.08 %) and

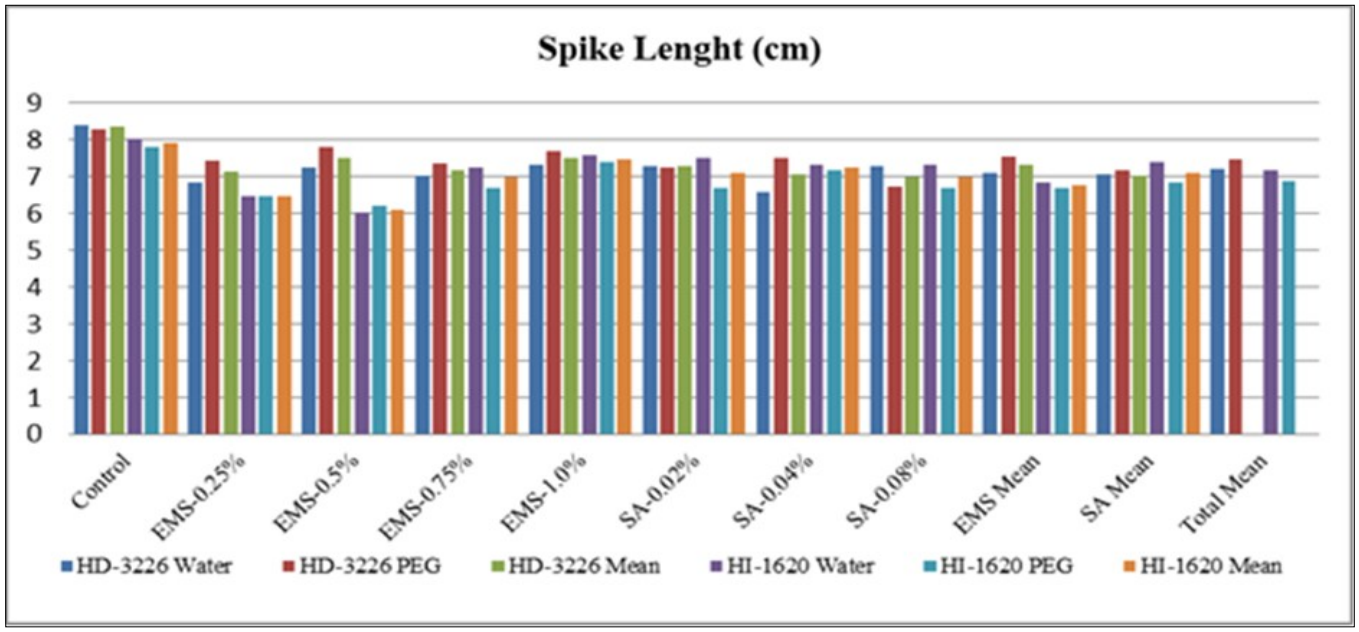

Fig. 4. Mean performance of spike lenght (cm) of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

Table 2. Spike length, Spikelets per spike and 1000 grain weight (g) of HD-3226 and HI-1620 wheat genotype in M_1 generation under moisture stress (mutants) and normal (control-wild type) conditions

Treatments	Spike Length (cm)						Spikelet's per spike						1000 grain weight (g)					
	HD-3226			HI-1620			HD-3226			HI-1620			HD-3226			HI-1620		
	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean
Control	8.4	8.28	8.35	8.01	7.79	7.9	13.2	14	13.6	12.4	11.53	11.96	40.14	36.17	38.15	37.17	37.06	37.11
EMS-0.25%	6.85	7.41	7.13	6.48	6.48	6.48	11.04	11.05	11.04	10.03	10.09	10.06	36.51	36.37	36.44	35.07	35.73	35.4
EMS-0.5%	7.24	7.8	7.52	6.01	6.19	6.1	11.77	12.47	12.12	10.08	11.1	10.59	36.83	35.47	36.15	35.25	35.03	35.14
EMS-0.75%	7.02	7.34	7.18	7.26	6.69	6.98	12.56	11.4	11.98	11.36	10.02	10.69	36.47	35.88	36.17	35.27	35.08	35.17
EMS-1.0%	7.33	7.68	7.51	7.58	7.38	7.48	12.18	12.17	12.17	11.35	11.01	11.18	36.33	35.53	35.93	35.05	34.53	34.79
SA-0.02%	7.29	7.26	7.28	7.49	6.68	7.08	11.17	10.76	10.96	10.23	10.27	10.25	37.13	35.78	36.45	35.07	34.68	34.87
SA-0.04%	6.56	7.52	7.04	7.33	7.15	7.24	11.11	10.85	10.98	9.89	10.5	10.19	36.03	35.63	35.83	34.88	35.03	34.95
SA-0.08%	7.27	6.72	6.99	7.32	6.68	7	11.07	11.27	11.17	10.16	10.46	10.31	35.57	35.47	35.52	34.96	34.08	34.52
EMS Mean	**7.11**	**7.55**	7.33	**6.83**	**6.68**	6.75	**11.88**	**11.77**	11.83	**10.7**	**10.55**	10.63	**36.53**	**35.81**	36.17	**35.16**	**35.09**	35.12
SA Mean	**7.04**	**7.16**	7.01	**7.38**	**6.83**	7.1	**11.11**	**10.96**	11.03	**10.09**	**10.41**	10.25	**36.24**	**35.62**	35.93	**34.97**	**34.59**	34.78
Total Mean	7.21	7.47		7.18	6.88		11.76	11.74		10.68	10.62		36.87	35.78	36.32	35.34	35.15	
	Factors (A)	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**	**Factors (A)**	**Factor (B)**	**Factor (A×B)**
CD	0.37	0.18	0.52	0.36	0.18	0.64	0.55	0.28	0.78	0.55	N/A	0.78	1.45	0.72	2.05	0.68	0.49	0.96
SE(d)	0.18	0.09	0.25	0.17	0.09	0.25	0.27	0.14	0.38	0.27	0.13	0.38	0.71	0.35	1.01	0.33	0.17	0.47
SE(m)	0.13	0.06	0.18	0.12	0.06	0.17	0.19	0.16	0.27	0.19	0.11	0.27	0.5	0.25	0.71	0.23	0.12	0.33
Significance at 5%	0	0.00337	0.01241	0	0.00157	0.0011	0	0	0.02831	0	0.602	0	0.00082	0.02929	0.03907	0	0.00859	0.02848

Table 3. Days to 50 % Heading, Days to 50 % Anthesis and Days to 50 % Maturity of HD-3226 and HI-1620 wheat genotype in M_1 generation under moisture stress (mutants) and normal (control-wild type) conditions

Treatments	Days to 50% Heading						Days to 50% Anthesis						Days to 50% Maturity					
	HD-3226			HI-1620			HD-3226			HI-1620			HD-3226			HI-1620		
	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean	Water	PEG	Mean
Control	85.13	88.43	86.78	90.6	91.62	91.11	91.53	97.86	94.7	98	92.33	95.16	124.13	120.93	122.53	118.13	123.06	120.6
EMS-0.25%	83.3	86.6	84.95	87.53	88.06	87.8	93.3	95.33	94.31	93.53	95.26	94.4	114.86	116.33	115.6	114.86	113.63	114.25
EMS-0.5%	88.3	91.6	89.95	83.66	85.3	84.48	92.63	95.7	94.16	93.66	92.96	93.31	114.7	114.7	114.7	114.6	114.63	114.61
EMS-0.75%	87.8	91.1	89.45	83.36	86	84.68	95.13	95.13	95.13	91.36	96	93.68	114.66	116	115.33	114.63	114.96	114.8
EMS-1.0%	87.5	90.8	89.15	83.86	84.9	84.38	94.83	96.23	95.53	93.86	92.23	93.05	114.36	114.96	114.66	114.3	114.4	114.35
SA-0.02%	90.46	96.96	93.71	95.33	96.63	95.98	99	101.7	100.35	98.33	100.96	99.65	119.03	120.6	119.81	118	118.03	118.01
SA-0.04%	92.8	95.1	93.95	93.26	98.73	96	99.46	99.83	99.65	96.93	98.06	97.5	119.43	121.53	120.48	118	119.23	118.61
SA-0.08%	90.93	96.23	93.58	100.03	97.33	98.68	100.23	100.46	100.35	99.03	100.33	99.68	118.96	120.23	119.6	117.86	117.86	117.86
EMS Mean	**86.72**	**90.02**	88.37	**84.6**	**86.06**	85.33	**93.97**	**95.59**	94.78	**93.1**	**94.11**	93.61	**114.6**	**114.4**	114.4	**114.6**	**114.4**	114.5
SA Mean	**91.4**	**96.1**	93.75	**96.21**	**97.56**	96.88	**99.56**	**100.67**	100.11	**98.09**	**99.78**	98.94	**117.86**	**117.86**	117.86	**117.95**	**118.37**	118.16
Total Mean	88.27	92.1		89.7	91.07		95.76	97.78		95.59	96.02		117.52	118.16		116.3	116.97	
	Factor (A)	**Factor (B)**	**Factor (A×B)**	**Factor (A)**	**Factor (B)**	**Factor (A×B)**	**Factor (A)**	**Factor (B)**	**Factor (A×B)**	**Factor (A)**	**Factor (B)**	**Factor (A×B)**	**Factor (A)**	**Factor (B)**	**Factor (A×B)**	**Factor (A)**	**Factor (B)**	**Factor (A×B)**
C.D.	4.335	2.167	4.979	1.838	0.919	2.6	2.1	1.05	N/A	1.07	0.54	1.52	1.59	N/A	2.33	1.76	N/A	2.44
SE(d)	2.112	1.056	2.987	0.896	0.448	1.267	1.02	0.51	1.45	0.52	0.26	0.74	0.79	0.38	1.08	0.84	0.42	1.19
SE(m)	1.494	0.747	2.112	0.633	0.317	0.896	0.72	0.36	1.02	0.37	0.19	0.52	0.54	0.27	0.79	0.59	0.29	0.84
Significance at 5%	0.00072	0.00107	0.0098	0	0.00482	0.01178	0	0.00045	0.07642	0	0.0099	0.07286	0	0.11315	0.04481	0	0.10569	0.04638

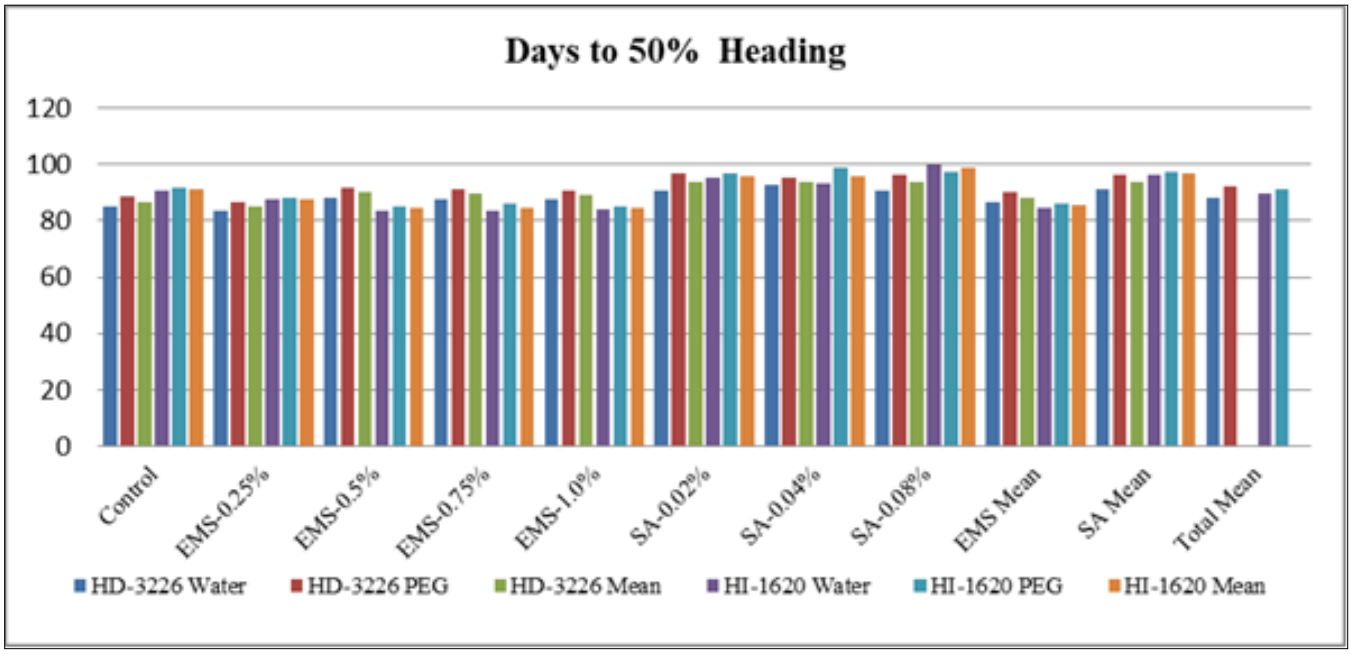

Fig. 5. Mean performance of days to 50 % heading of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

96.63 (SA 0.02 %) to 98.73 (SA 0.04 %) 15 % PEG treatments, respectively. Average of mean value across all treatments in water was 84.60 (EMS) and 96.21 (SA) while it was 86.06 (EMS) and 97.56 (SA) in 15 % PEG treatments.

Days to 50 % anthesis in control HD-3226-wild types were 91.53 in water and 97.86 in 15 % PEG. In EMS treatments, it ranges from 92.63 (EMS 0.5 %) to 95.13 (EMS 0.75 %) and 95.13 (EMS 0.75 %) to 96.23 (EMS 1.0 %) in water and 15 % PEG treatments, respectively. While SA slightly delay the days to 50 % anthesis as compared to EMS, it ranges from 99.00 (SA 0.02 %) to 100.23 (SA 0.08 %) and 99.83 (SA 0.04 %) to 101.70 (SA 0.02 %) in water and 15 % PEG treatments, respectively (Fig. 6; Table 3). Average of mean value across all treatments in water was 93.97 (EMS) and 99.56 (SA) while it was 95.59 (EMS) and 100.67 (SA) in PEG. In HI-1620 genotype, days to 50 % anthesis in control-wild type was 98.00 in water and 92.33 in 15 % PEG treatments. In EMS treatments, it ranges 91.36 (EMS 0.75 %) to 93.86 (EMS 1.0 %) and 92.23 (EMS 1.0 %) to 96.00 (EMS 0.75 %) in water and 15 % PEG treatments, respectively. SA also delay days to 50 % anthesis as compared to EMS in water treatments and it range from 96.93 (SA 0.04 %) to 99.03 (SA 0.08 %) and 98.06 (SA 0.04 %) to 100.96 (SA 0.02 %) 15 % PEG treatments, respectively. Average of mean value across all treatments in water was 93.10 (EMS) and 98.09 (SA) while it was 94.11 (EMS) and 99.78 (SA) in 15 % PEG treatments.

Days to 50 % maturity in control- HD-3226 wild type was 124.13 in water and 120.93 in 15 % PEG. In EMS treatments, it ranges 114.36 (EMS 1.0 %) to 114.86 (EMS 0.25 %) and 114.70 (EMS 0.5 %) to 116.33 (EMS 0.25 %) in water and 15 % PEG treatments, respectively (Fig. 7; Table 3). While SA slightly delay the days to 50 % maturity as compared to EMS, it ranges from 118.96 (SA 0.08 %) to 119.43 (SA 0.04 %) and 120.23 (SA 0.08 %) to 121.53 (SA 0.04 %) in water and 15 % PEG treatments, respectively. Average of mean values across all

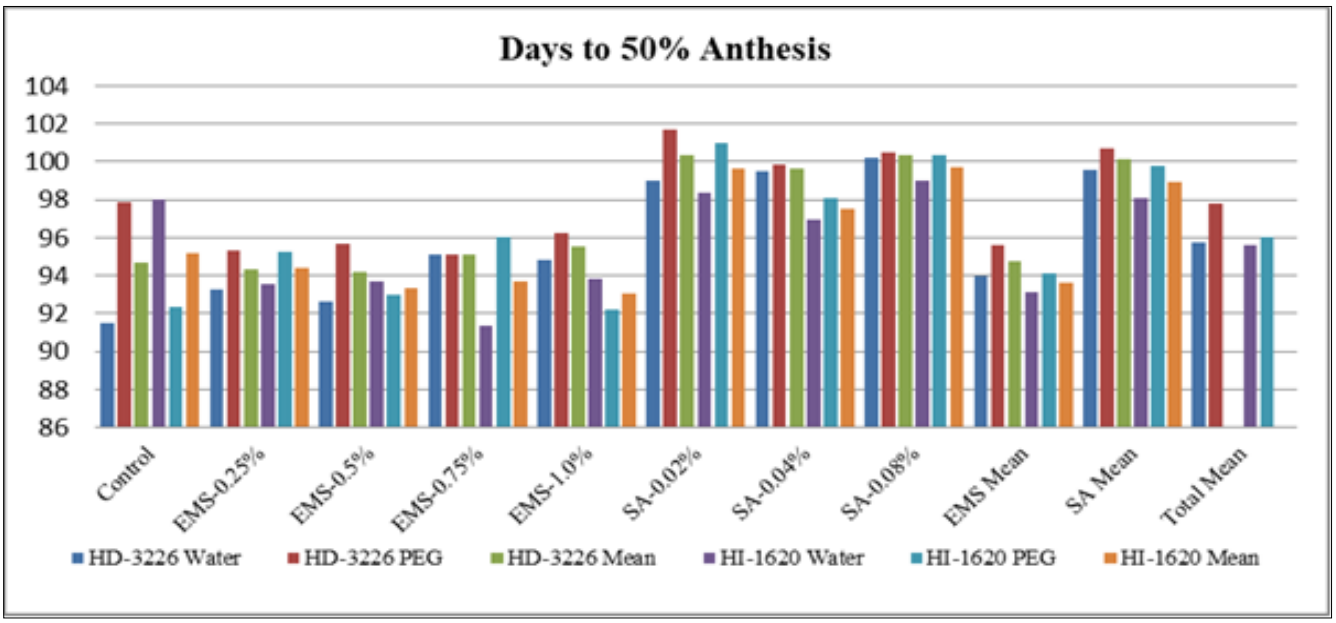

Fig. 6. Mean performance of days to 50 % anthesis of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

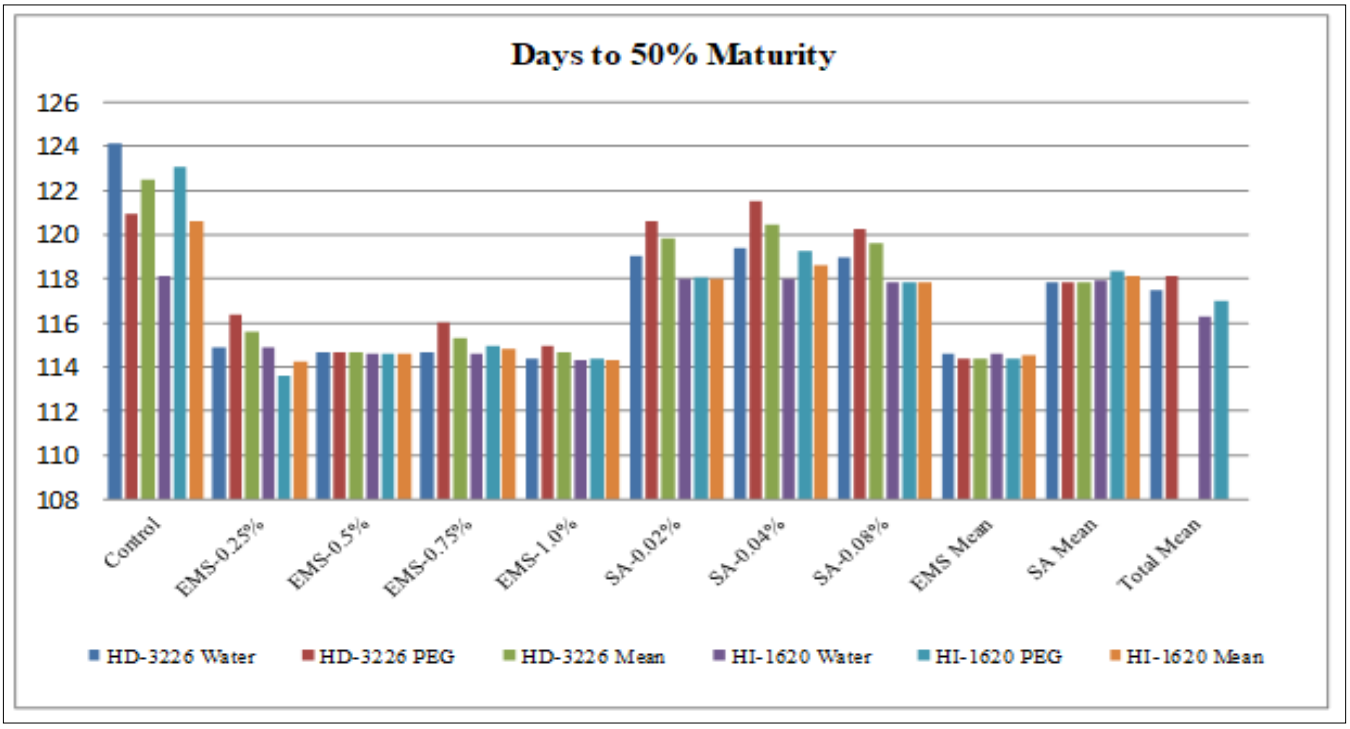

Fig. 7. Mean performance of days to 50 % maturity of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

treatments in water was 114.60 (EMS) and 117.86 (SA) while it was 114.40 (EMS) and 117.86 (SA) in PEG treatments. In HI-1620 genotype, days to 50 % maturity in control-wild type was 118.13 in water and 123.06 in 15 % PEG treatments. In EMS treatments, it ranges from 114.30 (EMS 1.0 %) to 114.86 (EMS 0.25 %) and 113.63 (EMS 0.25 %) to 114.96 (EMS 0.75 %) in water and 15 % PEG treatments, respectively. SA delay days to 50 % maturity as compared to EMS, it ranges from 117.86 (SA 0.08 %) to 118.00 (SA 0.02 % and SA 0.04 %) and it range from 117.86 (SA 0.08 %) to 119.23 (SA 0.04 %) in water and 15 % PEG treatments, respectively. Average of mean values across all treatments in water was 114.60 (EMS) and 117.95 (SA) while it was 114.40 (EMS) and 118.37 (SA) in 15 % PEG treatments.

Yield related attributes of wheat

Gaining insight into the genetic basis of these traits is essential for improving plant structure and boosting yield potential in wheat breeding efforts. Spikelets per spike and 1000-grain weight is very important morphological character decided to the magnitude of plants and follow to produce overall yield and it varies one variety to another. Spikelets per spike in control-HD-3226 wild type were 13.20 in water and 14.00 in 15 % PEG. In EMS treatments, it ranges 11.04 (EMS 0.25 %) to 12.56 (EMS 0.75 %) and 11.05 (EMS 0.25 %) to 12.47 (EMS 0.5 %) in water and 15 % PEG treatments, respectively. While SA decreased spikelets per spike as compared to EMS, it ranges 11.07 (SA 0.08 %) to 11.17 (SA 0.02 %) and 10.76 (SA 0.02 %) to 11.27 (SA 0.08 %) in water and 15 % PEG treatments, respectively (Fig. 8; Table 2). Average of mean values across all treatments in water was 11.88 (EMS) and 11.11 (SA) while it was 11.17 (EMS) and 10.96 (SA) in PEG treatments. In HI-1620 genotype, spikelets per spike in control-wild type were 12.40 in water and 11.53 in 15 % PEG treatments. In EMS treatments, it ranges 10.03 (EMS 0.25 %) to 11.36 (EMS 0.75 %) and 10.02 (EMS 0.75 %) to 11.10 (EMS 0.5 %) in water and 15 % PEG treatments, respectively. SA also decreased spikelets per spike as compared to EMS, it ranges 9.89 (SA 0.04 %) to 10.23 (SA 0.02 %) and 10.27 (SA 0.02 %) to 10.50 (SA 0.04 %) in water and 15 % PEG treatments, respectively. Average of mean values across all treatments in water was 10.70 (EMS) and 10.09 (SA) while it was 10.55 (EMS) and 10.41 (SA) in 15 % PEG treatments.

1000 grain weight (g) of two genotypes with their treatments were recorded after maturity and results presented in Table 2. 1000-grain weight in control-HD-3226 wild type was 40.14 g in water and 36.17 g in 15 % PEG. In EMS treatments, it ranges 36.33 g (EMS 1.0 %) to 36.83 g (EMS 0.5 %) and 35.47 g (EMS 0.5 %) to 36.37 g (EMS 0.25 %) in water and 15 % PEG treatments, respectively (Fig. 9; Table 2). SA slightly decreased 1000 grain weight as compared to EMS and ranges 35.57 g (SA 0.08 %) to 37.13 g (SA 0.02 %) and 35.47 g (SA 0.08 %) to 35.78 g (SA 0.02 %) in water and 15 % PEG treatments. Average of mean values across all treatments in water was 36.53 (EMS) and 36.24g (SA) while it was 35.81 g (EMS) and 35.62 g (SA) in PEG treatments. In HI-1620 genotype, 1000-grain weight in control-wild type was 37.17 g in water and 37.09 g in 15 % PEG treatments. In EMS treatments, it ranges 35.05g (EMS 1.0 %) to 35.27 g (EMS 0.75 %) and 34.53 g (EMS 1.0 %) to 35.73 g (EMS 0.25 %) in water and 15 % PEG treatments, respectively. SA also decreased 1000-grain weight as compared to EMS, it ranges from 34.88 g (SA 0.04 %) to 35.07 g (SA 0.02 %) and 34.08 g (SA 0.08 %) to 35.03 g (SA 0.04 %) in water and 15 % PEG treatments, respectively. Average of mean values across all treatments in water was 35.16 g (EMS) and 34.97 g (SA) while it was 35.09 g (EMS) and 34.59 g (SA) in 15 % PEG treatments.

Discussion

Wheat provides over 20 % of the total calories and protein in the human diet. It is rich in protein and dietary fiber, with grain containing 8-15 % protein and flour containing 8-13 %, along with 60-80 % starch. Beyond its nutritional role, wheat is vital in baking, as its gluten proteins give dough its unique stickiness and bread-making characteristics (26). India is the second largest

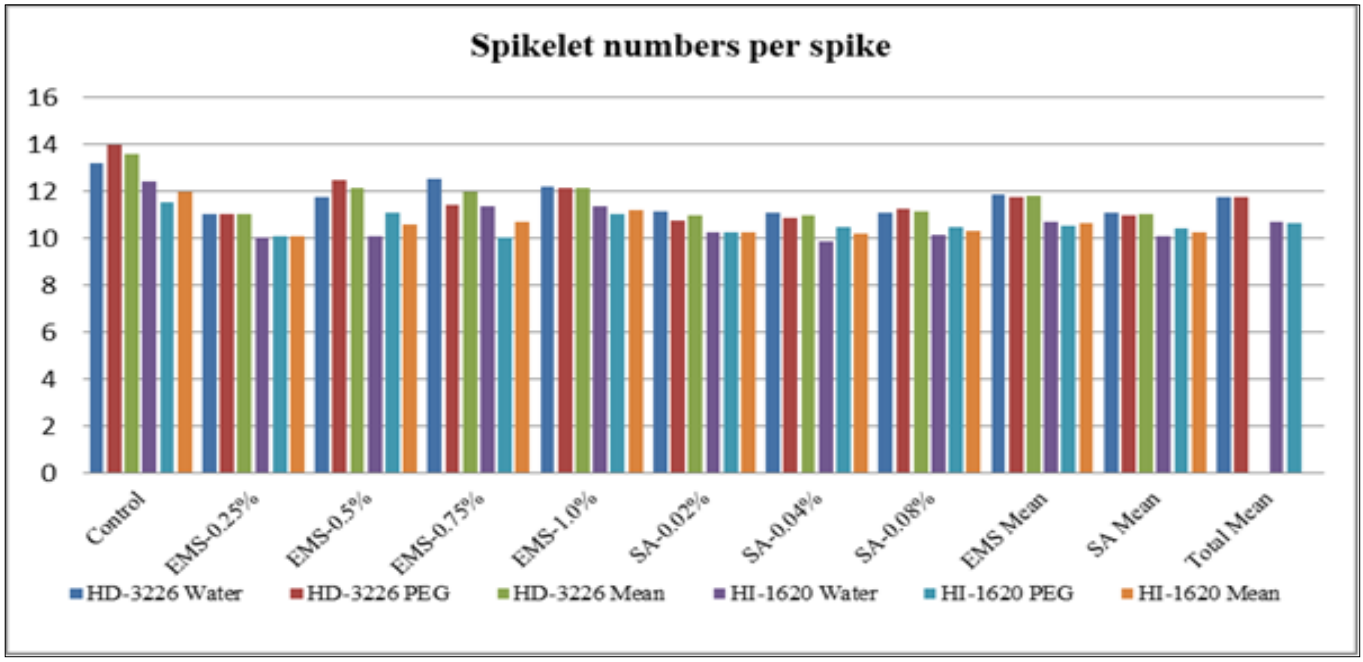

Fig. 8. Mean performance of spikelet numbers per spike of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

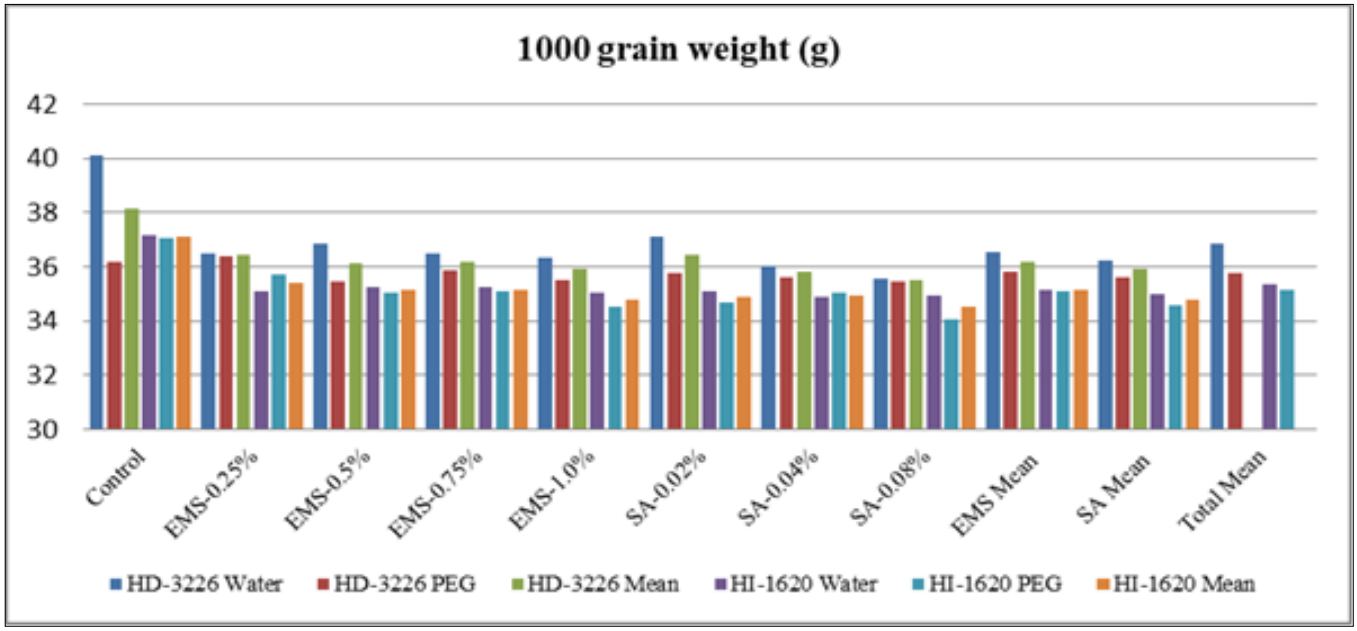

Fig. 9. Mean performance of 1000 grain weight (g) of HD-3226 and HI-1620 wheat genotype after Ethyl Methane Sulphonate and Sodium azide (SA) treatments in water and 15 % PEG under moisture stress (mutants) and normal (control-wild type) conditions in M_1 generation.

producer of wheat in the world after China with about 12 % share in total world wheat production (27). Water is essential for seed germination, seedling growth, vegetative period of crop, flowering at translocation of minerals and nutrition incorporate throughout the plants, from root to leaf and vice versa in the plants (28). Water stress is a major limiting factor for crop production and estimated the 50 % of the global wheat production is affected by water deficit conditions (1). *In vitro* screening method using PEG has been proved to be very effective method for studying the effect of water stress on seed germination and seedling growth characters (29, 19, 20) and simple cost-effective method to screen large set of germplasm within very less time and accurately (21). However, artificial induction of drought using PEG is dependent on concentration and varies with crop and genotype. PEG-induced water stress adversely affected germination, shoot-root length and key physiological traits in wheat (30). Previous research has investigated the effects of varying PEG concentrations on drought tolerance (31). Earlier study has identified 11 drought-tolerant wheat mutant lines following treatment with 15 % PEG and gamma radiation (32). It was reported notable morphological alterations in wheat subjected to EMS under different treatment conditions (33). EMS treatment developed a drought-resistant wheat mutant characterized by broader leaves and a denser fibrous root system (34). Sodium azide treatment significantly influenced agronomic traits such as plant architectures, spikelet count, yield and protein content (35). In present research, plant height and spike length showed an increase under PEG treatment compared to water in EMS-treated plants, whereas SA treatments led to a reduction in both wheat genotypes relative to the control-wild types. Variations in plant height i.e., dwarf and giant plants were produced through sodium azide and ethyl methane sulphonate treatments (7, 36, 37). Early flowering and

maturation times are vital for enhancing wheat production in water-stressed environments. The number of productive tillers in wheat is an essential agronomic characteristic that impacts biomass production and grain yield potential (38). In the current study, total number of reproductive tillers per plant decreased under PEG in EMS treatments but increased in SA treatments compared to the control-wild types. Tillers also support development of spikes which directly influence the number of kernels harvested per plant and thus grain yield (39, 40). Several studies have alluded that wheat genotypes were more drought tolerant due to their ability to maintain a high number of productive tillers under drought stress (37, 41). The time required for 50 % heading, anthesis and maturity was delayed under PEG treatment compared to water in both EMS and SA treated wheat genotypes, as well as in the control-wild types. Similarly, early and late flowering and maturity was reported in mutant plants which developed for salt tolerance in wheat through 1 % EMS treatments (7). Sodium azide also accelerates maturity while reducing tiller count, spikelets and spike length in wheat (42). Water stress accelerates the early booting, heading and maturity in wheat genotypes (43). Spikelets per spike and 1000-grain significantly affected the yield and these were decreased under PEG treatment in both EMS and SA treated wheat genotypes and, in the control, wild types and several studies support these findings. It was reported that 1000 grain weight decline due to moisture stress at milking stage (44). Similarly, desirable mutants of wheat genotype which produced more seed and high-test weight have been found (36, 45, 38). Moreover, loss in biological yield at the booting stage stress were also reported (46, 47).

Conclusion

Drought is the leading factor limiting agricultural growth and yield, as water shortages affect plants in both cultivated fields and natural ecosystems. Water stress causes toxicity, reduces photosynthesis, leads to leaf bleaching, curling, wilting and can ultimately result in plant death. Wheat crops are highly susceptible to water scarcity, leading to reduced yield and overall production. The present study was focused to develop the mutant population with enhanced agronomic traits towards water stress through chemical mutagens and observed the effects of mutagens on morphological traits under osmotic stress conditions in both wheat genotypes. In this investigation, we found that SA reduced plant height, delayed days to 50 % heading, 50 % anthesis and 50 % maturity under PEG conditions compared to water, in both EMS and SA treatments. However, the total number of reproductive tillers per plant increased with SA treatment. Spike length increased under PEG conditions in HD-3226 wheat genotype as compared to water, while it decreased in HI-1620 wheat genotype compared to the control (wild type). Additionally, the number of spikelets per spike and 1000 grain weight decreased under PEG conditions in both EMS and SA treatments. Mutagenic treatment combined with osmotic stress had a significant effect on the morphological characteristics of both wheat genotypes.

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Authors' contributions

RR and MKY drafted the manuscript. AT, LKG, V & RSS participated in design of the methodology and PK performed the statistical analysis. AKS and S assisted in compiling the data. All authors reviewed and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors have stated that they have no competing interests.

Ethical issues: None

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