INTRODUCTION

Wheat (*Triticum aestivum*) is the second-most produced crop on Earth and provides a large fraction of the dietary protein and total food supply. Wheat is one of the major cereals in the world and is one of the main sources of calories and protein. Approximately 85% and 82% of the global population depends on wheat for basic calories and protein, respectively [1]. It is grown all throughout the world in a wide variety of climates. It is a staple food for more than 35% of the world population. Moreover, this cereal is used in the production of a variety of wheat products, such as leavened bread, flat and steamed breads, cakes, pasta, biscuits, noodles, couscous and beer [2]. World trade in wheat is greater than for all other crops combined [3]. Globally, wheat is the leading source of vegetable protein in human food, having higher protein content than either maize (corn) or rice, the other major cereals. Em is a common and effective chemical mutagen, especially recommended to create random mutations in the genetic material by nucleotide substitution, particularly by guanine alkylation. This typically produces only point mutations. Mutation through ems in four varieties of wheat for herbicide resistance and other agronomic traits [5]. Few studies have examined ems differences in different crop species/cultivars. The efficiency of ems not only depends on ems quality but also on genotype [6].

The aim of this study is to determine the sensitivity of wheat cultivars HD 2894 (pusa wheat 109) (timely sown) with different doses of chemical mutagen ethyl-methane sulphonate (ems) by detecting the activity of enzymes and LPO behaviour of mutation in next-generation plants (m1, m2 and m3).

MATERIALS AND METHODS

**Chemicals**

The plant material *Triticum aestivum* (var. HD-2894) was taken from I. A. R. L, New DeBi used in the present study. Em was purchased from Himedia Company. Tris hydrochloride, trichloroacetic acid (TCA) and 2-thiobarbutric acid (TBA) were purchased from SRL (Mumbai, India). All other chemicals used in current investigation were of analytical grade.

**Experimental design**

Presoaked seeds were treated with ems for 6 hr with different doses of mutagen like 0%, 0.1%, 0.2%, 0.3%, 0.4% and 0.5%. The seeds were kept in the mutagenic solution for 6 hr at room temperature with shaking for providing uniform treatment to the dipped seed and equal no. of same genotypes were soaked in distilled water on the other hand, deletion can also occur in lesser extent [4]. Thus, ems is powerful mutagen of changing loci of specific interest without inducing large deletions. Em produces random mutations in the genetic material by nucleotide substitution, particularly by guanine alkylation. This typically produces only point mutations. Mutation through em in four varieties of wheat for herbicide resistance and other agronomic traits [5]. Few studies have examined ems differences in different crop species/cultivars. The efficiency of ems not only depends on ems quality but also on genotype [6].

The aim of this study is to determine the sensitivity of wheat cultivars HD 2894 (pusa wheat 109) (timely sown) with different doses of chemical mutagen ethyl-methane sulphonate (ems) by detecting the activity of enzymes and LPO behaviour of mutation in next-generation plants (m1, m2 and m3).
RESULTS

Ascorbate peroxidase (APX) activity

It was observed that total activity of apx was found to be reduced with increased concentration of mutagen, as compared to control.

M1 generation

After the 6 h time interval, the activity of apx was found to be reduced in variety HD-2894 with increased concentration of ems as compared to a control plant (fig. 1a).

M2 generation

In the m2 generation after given 6 h treatment of variety HD-2894 with different doses of ems, the activity of apx increased till 0.2% (0.1%-15.79 mg/g and 0.2%-15.56 mg/g) compared to control and then decreased with increased concentration of mutagen (fig. 1b).

M3 generation

According to the analysis of 6 h treatment of variety HD-2894 with different doses of ems in an m3 generation, the activity of apx increased till 0.3% [0.1%-14.757 mg/g, 0.2%-14.36 mg/g and 0.3%-14.763 mg/g] and then reduced with increased concentration of mutagen (fig. 1c).

Catalase activity (CAT)

M1 generation

In the first generation observation of variety HD-2894 with 6 h exposure of ems, catalase (CAT) activity was found to be decreased with increased concentration of ems as compared to control (fig. 2a).

M2 generation

According to the second generation observation variety HD-2894 with 6 h exposure of ems mutagen, the further increment was observed till 0.3% (0.1%-0.0095 mg/g, 0.2%-0.0092 mg/g, 0.3%-0.0093 mg/g) as compared to control and then decreased with increased concentration of mutagen (fig. 2b).

M3 generation

According to the analysis of 6 h treatment of variety HD-2894 with different doses of ems in an m3 generation, the activity of catalase (CAT) increased till 0.3% (0.1%-0.0095±0.0017, 0.2%-0.0092±0.0001 and 0.3%-0.0093±0.00018) as compared to control (fig. 2c).
M3 generation

In the wheat cultivar of HD-2894 with 6 h exposure of mutagen, catalase (CAT) activity was found to be increased till 0.3% (0.1%-0.0094 mg/g, 0.2%-0.0105 mg/g) compared to control and then found to be decreased with increased concentration of mutagen (fig. 2c).

Glutathione reductase enzyme activity

M1 generation

In the first generation observation of variety HD-2894 with 6 h exposure of ems mutagen, glutathione reductase (GR) activity was found to be reduced with increased concentration of mutagen as compared to control (fig. 3a).

M2 generation

According to the second generation observation of variety HD-2894 with 6 h exposure of ems mutagen, activity of glutathione reductase (GR) was found to be increased till 0.4% (from 0.1%-0.127 mg/g, 0.2%-0.111 mg/g and 0.3%-0.119 mg/g) as compared to control and then decreased with the increased concentration of mutagen (fig. 3b).

M3 generation

In the cultivar of HD-2894 with 6 h exposure of mutagen, glutathione reductase (GR) activity was found to be increased till 0.4% (0.1%-0.209 mg/g, 0.2%-0.212 mg/g, 0.3%-0.219 mg/g and 0.4%-0.118 mg/g) as compared to control and then found to be reduced with the increased concentration of mutagen (fig. 3c).

Lipid peroxidation (LPO) activity

M1 generation

In the first generation observation of variety HD-2894 with 6 h exposure of ems mutagen at 530 and 600 nm, it was observed that the activity was found to be increased in 530 nm till 0.1% (0.1%-0.038 mg/g) as compared to control and then found to be reduced with increased concentration of mutagen (fig. 4a).

M2 generation

According to the second generation observation of variety HD-2894 with 6 h exposure of ems at 530 nm, the increment was observed till 0.3% (from 0.1%-0.045 mg/g, 0.2%-0.053 mg/g, 0.3%-0.056 mg/g) as compared to control and then decreased with the increased concentration of mutagen. According to the observation of variety HD-2894 with 6 h exposure of ems at 600 nm, the increment was observed in 0.2% and 0.3% (from 0.2%-0.052 mg/g, 0.3%-0.057 mg/g) as compared to control and then decreased with the increased concentration of mutagen (fig. 4b).

M3 generation

According to the third generation observation of variety HD-2894 with 6 h exposure of ems at 530 nm, the increment was observed till 0.3% (from 0.1%-0.107 mg/g, 0.2%-0.108 mg/g and 0.3%-0.110 mg/g) as compared to control and then decreased with the increased concentration of mutagen. According to the analysis of HD-2894 with 6 h exposure of ems at 600 nm, the increment was observed till 0.3% (from 0.1%-0.106 mg/g, 0.2%-0.108 mg/g and 0.3%-0.112 mg/g) as compared to control and then reduced with the higher concentration of mutagen (fig. 4c).
DISCUSSION

We observed in the laboratory condition, ems mutagenesis caused significant reduction and stability in the activity of antioxidants and lipid peroxidation in m1, m2 and m3 generation. This was manifested as a significant delay in the activity of antioxidants as the ems concentration was increased. Among the chemical mutagens and alkalinizing agents, ems has especially been demonstrated to be the most potent. Previous studies affirm that while the mutagenesis caused by naked eyes. Therefore, ems should be used to create a mutation in many crops like wheat, which are highly susceptible for harmful pathogens and made them economically inexpensive and beneficial for farmers.

CONCLUSION

The effects of ems appear soon after sowing the seeds and can be observed by naked eyes. Therefore, ems should be used to create a mutation in many crops like wheat, which are highly susceptible for harmful pathogens and made them economically inexpensive and beneficial for farmers.

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AUTHORS CONTRIBUTIONS

Shalija Dubey conducted experiments and prepared manuscript, Dr. Renu Bist helped in the manuscript preparation and Dr. Shrilkeha Misra conceived and oversaw the project, as well as designing of experiments and manuscript preparation.

CONFLICT OF INTERESTS

There is no conflict among the authors.

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