



Mutagenic effects of Ethyl Methane Sulphonate on the pollen grains of *Urginea indica* Kunth cytotype I

Richa Sinha

AMITY University Jharkhand
Ranchi, India

ABSTRACT

Urginea indica Kunth commonly called as Jangli Pyaz or jangli dungli is a medicinal plant having very high therapeutic value. Due to over exploitation, it has become vulnerable in some places. So, development of genetically advanced varieties of this plant is needed so that we can avail the benefits of this plant for a longer period of time, with great ease to mitigate several human ailments. To develop such variations in the plants through mutation breeding, screening of the plant is the most important aspect. Therefore, in this research sensitivity of *Urginea indica* Kunth to Ethyl Methane Sulphonate was assessed through the measurement of the mutagenic effects on pollen grains. Dose dependent decrease in the pollen fertility was observed. In addition, variation in the shape and size of the pollen grains were also observed after treatment with different concentrations. Thus, *Urginea indica* Kunth was found to be sensitive to Ethyl Methane Sulphonate.

Keywords: *Urginea indica* Kunth, pollen fertility, Ethyl Methane Sulphonate, mutation breeding

I. INTRODUCTION

Urginea indica Kunth is a treasured wild herb which has long been used to mitigate human sufferings. It is used extensively by locals as well as pharmaceutical companies to cure numerous human ailments. This wild poisonous herb belongs to the Liliaceae family having a very high therapeutic value. Therefore, to develop genetic variability in such an important medicinal plant, mutation breeding is needed to be applied. Induction of mutation can improve and increase the yield of this medicinal plant.

The induction of mutation requires screening of the mutagens and assessment of sensitivity of the plant to that particular mutagen. Plants play an important role in various aspects of mutagenesis research. Moreover, plant system is reported to display numerous genetic and chromosome changes to determine the effects of mutagens. It is used extensively for mutagen screening (detection and verification of mutagenic activity), mutagenic monitoring and determining mutagen effects and mechanisms of mutagen action of certain mutagen^[1].

Mutation breeding is reported to be an established method for inducing genetic materials for several desired characters. The main advantage of this method is the possibility of improving one or few characters without changing the rest of the cytotypes^[2]. Additionally, it can increase yield as well as improve several other qualitative traits in the investigated plant species. Both physical and chemical mutagens are reported to be applied for this purpose; however, the chemical mutagens are used extensively because of its greater potency over physical mutagens and ease with which it can be used^[3].

Therefore, in this research attempt has been made to find out the sensitivity of *Urginea indica* Kunth to Ethyl Methane Sulphonate. For this, the effects of Ethyl Methane Sulphonate on some of the pollen characters including percentage pollen fertility, pollen shape and size of *Urginea indica* Kunth were assessed after treatment with different concentrations. Pollen fertility is considered as one of the important, stable genetically controlled characteristic of the plant which can be used to assess the effectiveness and efficiency of mutagens for

hybridization and for the study of cytological effects [4].

II. MATERIAL AND METHODS:

The bulbs of *Urginea indica* Kunth were collected from Birsa Agriculture University, Ranchi, Jharkhand. The bulbs were of larger size and white in color bearing pinkish orange flower.

A chemical mutagen, Ethyl Methane Sulphonate was used to find out its mutagenic effects on the pollens of *Urginea indica* Kunth. Five different concentrations of Ethyl Methane Sulphonate (0.1%, 0.2%, 0.3%, 0.4% and 0.5%) were prepared under aseptic conditions. Fresh and healthy *Urginea indica* Kunth bulbs of uniform size were treated with these five concentrations for six hours. Then the treated bulbs were thoroughly washed in running tap water to remove the residual effect of the chemicals used. The control and the treated bulbs were grown in the experimental plots in the randomized block design to raise M₁ generation.

Pollen studies were performed from flowers of *Urginea indica* Kunth raised from the bulbs treated with Ethyl Methane Sulphonate. Pollens were dusted on the glass slides for acetocarmine staining. Stained pollen grains were considered to be fertile and unstained one as sterile. The percentage pollen fertility and sterility was determined by the formulae:

$$\begin{aligned} \text{Percentage Pollen Fertility} \\ &= \frac{\text{(Number of Fertile Pollens)}}{\text{(Total Number of Pollens studied)}} \\ &\times 100 \end{aligned}$$

Percentage Pollen Sterility

$$\begin{aligned} &= \frac{\text{Number of Sterile Pollens}}{\text{Total Number of Pollens studied}} \\ &\times 100 \end{aligned}$$

Shape of the pollen grains of treated flowers were determined by using Erdtman (1952) [5] table based on the relation between polar axis and equatorial axis (P/E) of the pollen grains of *Urginea indica* Kunth.

III. RESULTS:

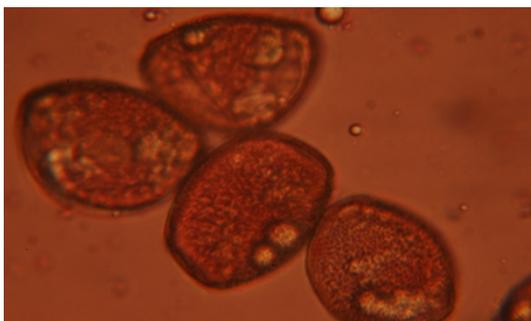
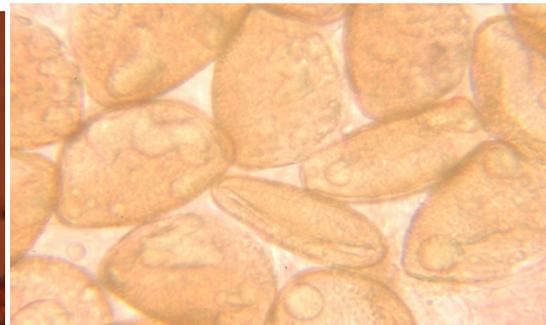
The results are depicted in table 1-2 and fig.1-7. The shape of pollen grains in *Urginea indica* Kunth was reported to show minor variations after treatment with different concentrations of Ethyl Methane Sulphonate (table 1). The percentage pollen fertility was found to decrease significantly with the increased concentration of Ethyl Methane Sulphonate. The percentage pollen fertility was 85.025% in control, which showed linear decrease with increased concentrations of Ethyl Methane Sulphonate (table 2). Similarly, percentage pollen sterility was reported 14.975 in control, which showed considerable increase with increased concentrations and ranged from 20.941% at lowest concentration to 41.026% at highest concentration (table 2; fig. 3-4 and 7).

Table-29: Pollen morphology in *Urginea indica* Kunth Cytotype I after treatment with different concentrations of Ethyl Methane Sulphonate (EMS)

Concentration	Pollen Grains			
	Polar Diameter (P) μ	Equatorial Diameter (E) μ	P/E	Shape
Control	33.5 ± 0.830	26.3 ± 1.219	1.3 ± 0.583	Sub prolate
0.1%	32.4 ± 0.829	25.0 ± 0.850	1.3 ± 0.111	Sub prolate
0.2%	33.2 ± 0.667	22.5 ± 0.563	1.5 ± 0.119	Prolate
0.3%	34.3 ± 1.074	23.0 ± 0.726	1.5 ± 0.150	Prolate
0.4%	35.7 ± 0.788	24.3 ± 0.397	1.5 ± 0.078	Prolate
0.5%	36.2 ± 0.590	35.1 ± 0.771	1.4 ± 0.143	Prolate

Table-31: Percentage Pollen Fertility and Percentage Pollen Sterility in *Urginea indica* Kunth after treatment with different concentrations of Ethyl Methane Sulphonate (EMS)

Concentration	Total No. of Pollen grains studied	No. of fertile pollen grains	Percentage Fertility (%)	No. of sterile pollen grains	Percentage Sterility (%)
Control	808	687	85.025	121	14.975
0.1%	850	672	79.059	178	20.941
0.2%	890	605	67.978	285	32.022
0.3%	816	530	64.951	286	35.049
0.4%	885	539	60.904	346	39.096
0.5%	819	483	58.974	336	41.026

**Fig.1: Bulb of *Urginea indica* Kunth****Fig.2: Flower of *Urginea indica* Kunth****Fig.3: Fertile pollens (control)****Fig.4: Sterile Pollens (after EMS Treatment)**

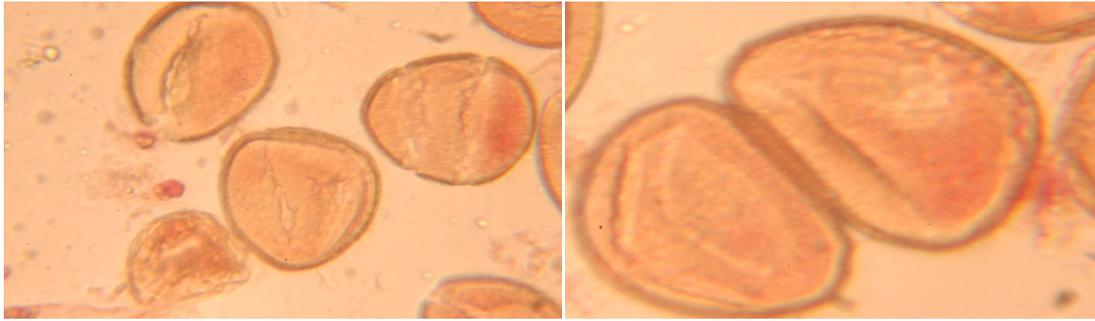
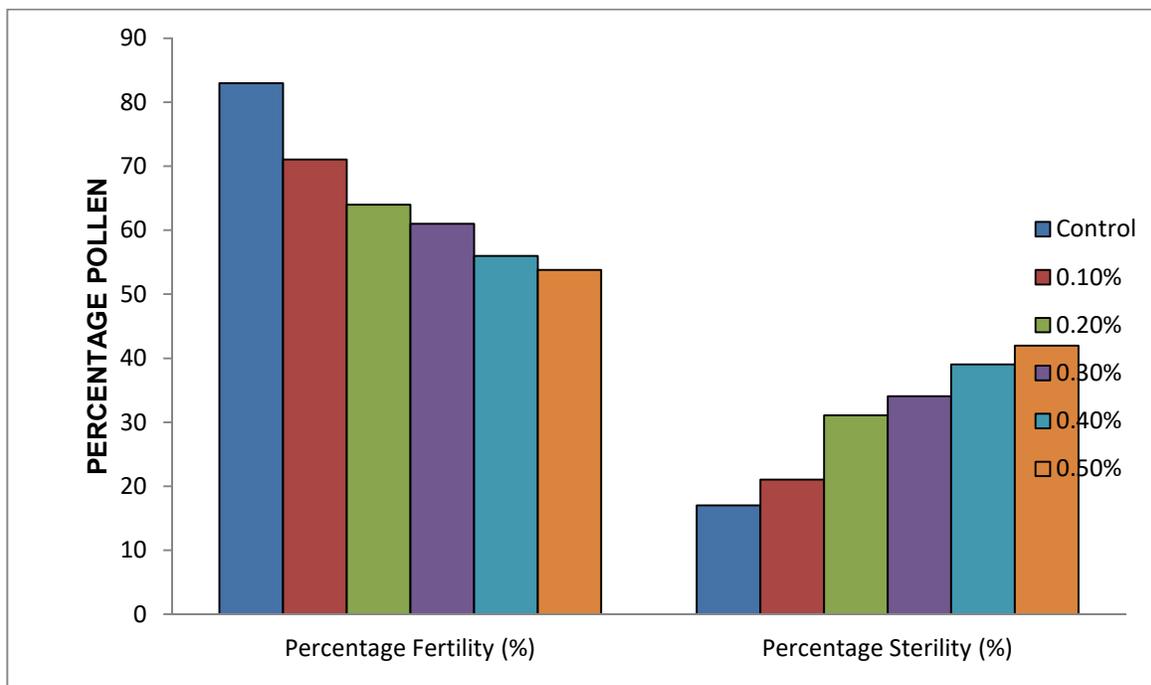


Fig.5: Distorted Pollens (after EMS Treatment) Fig.6: Distorted Pollens (after EMS Treatment)

Fig. 1-6: Photographs and Photomicrographs of *Urginea indica* Kunth Cytotype I
*(EMS: Ethyl Methane Sulphonate)

Fig.-7: Column graph showing Percentage Pollen Fertility and Percentage Pollen Sterility in *Urginea indica* Kunth after treatment with different concentrations of Ethyl Methane Sulphonate



IV. DISCUSSION:

Different species differs with respect to their sensitivity to different mutagens and the assessment of mutagenic sensitivity is an important feature of mutation breeding. Similarly, in the present study, the sensitivity of *Urginea indica* Kunth was assessed on the basis of measurement of the effects of Ethyl Methane Sulphonate on different pollen characters, especially pollen fertility rate in M₁ Generation.

The changes observed in the percentage pollen fertility and pollen shape and size of *Urginea indica* Kunth after treatment with different concentrations of Ethyl Methane

Sulphonate was significant in the present investigation. Increased percentage of pollen sterility of *Urginea indica* Kunth, in a dose dependent manner was observed in different concentration of the mutagens. It has been reported that the changes induced in the shape, size and other properties of pollen grains is caused by the irregular or abnormal meiosis. This happens due to the fact that the structure and physiology of pollen grains is under genetic control [6, 7].

Pollens were reported to show significant decrease in percentage pollen fertility, while increase in percentage

pollen sterility. It is considered that sterility observed after mutagenic treatment is due to detectable chromosomal aberrations and cryptic deficiencies^[8, 9]. The increase in the percentage sterility of pollen grains after treatment with Ethyl Methane Sulphonate might be the result of gene mutation or invisible deficiencies^[10]. It has also been reported that pollen fertility is directly correlated with meiotic anomalies. Fertility depends on the efficiency of the meiotic process^[11, 12].

Aberrations induced by mutagens in the pollen mother cells could be detected in the microspores in the form of pollen sterility. The pollen sterility count is a better way and a dependable parameter to find out the mutagenic effects^[4]. Therefore, the relationship between aberrations and sterility suggests that mutagen induced sterility is mainly the result of chromosomal aberrations which might have passed from somatic to germ cells.

Thus, *U. indica* Kunth of this variety was found sensitive to Ethyl Methane Sulphonate.

CONCLUSION:

The shape of pollen grains of *Urginea indica* Kunth Cytotype I was noticed to show variations after treatment. The increase or decrease in the polar and equatorial diameter of pollen grains was not in linear fashion. The percentage pollen fertility decreased dose dependently after treatment with Ethyl Methane Sulphonate.

Thus, variations in pollen size and shape; and decline in percentage pollen fertility showed the sensitivity of *Urginea indica* Kunth Cytotype I to Ethyl Methane Sulphonate.

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