

Comparative Analysis on the effect of EMS and MSG on the stomata of *Tinospora cordifolia*(Willd.)

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Abstract: In India, herbal medicines are one of the oldest Medicines. It heals many diseases as mentioned in the Ayurveda and Homeopathy. The plant *Tinospora cordifolia* (Willd) is also called marginal shrub due to its property of curing a lot of diseases. The Plant material of *T. cordifolia* were treated with different concentration of two mutagen i.e EMS and MSG. Stomatal studies were done by usual method of peeling or scratching of epidermis with the help of forceps or sharp razor respectively. Stomatal type were determined based on the classification of stomata on the basis of nature and number of subsidiary cells. The stomata type is anomocytic. Stomatal index, length, breadth and area of stomata of *T. cordifolia* without treatment is highest at the apex (30.85 ± 2.569 , $11.6 \pm 0.382\mu$, $8.2 \pm 0.344\mu$, $150.09\mu\text{m}^2 \pm 9.37$). The stomatal index, Stomatal frequency, length, breadth and area of stomata varied with different regions and concentration of mutagens. It was concluded that effect of mutagens decreases the Stomatal Index, length, width and area of the stomata. Plant treated with MSG performed higher transpiration rate, Stomatal Index, length, width and stomatal area in comparison to EMS treated plant.

Keywords: *Tinospora cordifolia*, Anomocytic, Hypostomatic, Ethyl Methane Sulphonate (EMS), Monosodium Glutamate (MSG), Stomatal Index, Stomatal Area

1. Introduction

The plant *Tinospora cordifolia* (Willd.) Miers ex HK.f. & Th. is a large glabrous woody climber belonging to family Menispermaceae. In Hindi the plant is commonly called Giloe or Amrita which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial being from old age and kept them eternally young. The 40 species of *Tinospora* are distributed in tropical Africa, South East Asia, Indo Malaya Region, China and Australia (Sujit kr. Dalai et al, Dec, 2013). The species is endemic to India and is common throughout tropical and subtropical zones at an altitude of 600m. The plant distributed throughout the tropical region of India upto 1200m above sea level from kurnaon to Assam, in north extending through west Bengal, Bihar, Jharkhand, Deccan, kankan, karnataka and kerala. (Ashish kumar, Jnanasha A.C and Soudamalla, June, 2019).

The stem of *Tinospora cordifolia* (Willd.) is rather succulent with long filiform fleshy aerial roots from the branches. Leaves are membranous and cordate at the base. Leaves have bitter taste and an indistinct odour (Sujit kr. Dalai et al, Dec, 2013). The flower is unisexual in nature. Male flowers are clustered and female flowers are usually solitary (Joshi and Kaur, 2016). The fruits are drupes, ovoid, glossy, succulent, red and pea seed sized.

It is also called as marginal shrub due to its property of curing a lot of diseases in modern system of medicine. It is widely used by tribal's for the treatment of many diseases including gastrointestinal disorder (S.K. Dwivedi and Enepsa, 2016). The leaves are also used as a good fodder for cattle. The plant is considered as one of the most divine herbs in Ayurvedic and folk medicine for its immense medical properties such as antioxidant, antidiabetic, antiperiodic, Antiallergic, anticarcinogenic, antispasmodic,

anti-inflammatory, antiarthritic, antiallergic, antistress, antileproic, antimalarial, hepatoprotective, Immunomodulatory, and antineoplastic activities. It is also used general debility, dyspepsia, fevers and urinary disease. Antiviral properties against Ranikhet disease in poultry have also been ascribed to this climber (Ritu Paliwal, 2016).

The plants mainly contains alkaloids, glycosides, steroids, sesquiterpenoids, aliphatic compounds, essential oils, mixture of fatty acids and polysaccharides. (Garish Joshi and Rajendra kaur, 2016).

It was to investigate and determine the shape, size and frequency, of stomata and its variation in leaf epidermis caused by the application of two mutagen EMS (Ethyl methane sulphonate) and MSG (Monosodium Glutamate) on *Tinospora cordifolia* (Willd.). As a basis for casual analysis of data of Stomatal index, length, width and area, studies on functional leaf anatomy are becoming more important for evaluating biosystematics. (Vijay Paul, Laxmi Sharma, Rakesh Pandey and R.C Meena, 2019)

2. Materials and Methods

The plants were collected from the house of Dr. Kamini kumar, University professor, Pro V.C (on lien), University Department of Botany Ranchi, Jharkhand and Birsa Agriculture Kanke, Ranchi, Jharkhand.

The Plant material of *T. cordifolia* were treated with different concentration of two mutagen i.e EMS and MSG. The treated plant material were then washed under tap water and planted in the soil. They were also planted with the help of Rootex power, by applying Rootex power at the base of stem and kept them into water inside a glass or any container for early growth and development of root and leaves. Fresh leaves were then taken for stomatal studies. Both adaxial and

abaxial surface of leaves are divided into three regions i.e apex, middle and base. Stomatal studies were done by usual method of peeling or scratching of epidermis with the help of forceps or sharp razor respectively. The scratched or peeled off epidermis was then stained by safranin and mounted by glycerin. They were observed under microscope and necessary measurement was done with the help of stage micrometer and ocular micrometer.

Stomatal Type:- Stomatal type were determined based on the classification of stomatal on the grounds of nature and number of subsidiary cells. (Metcalf CR, Chalk L., 1950).

Stomatal index :- The stomatal index was calculated by using the following formula

$$SI = \frac{\text{no of stomata per unit area}}{\text{no of stomata + no of epidermal cell}}$$

Area of Stomatal Aperture:- The length and breadth of Stomatal aperture was calculated by ocular micrometer and the area of stomatal aperture was calculated by the formula (Metcalf CR, Chalk L., 1950),

$$A = \frac{\pi}{2} \times l \times b \mu m^2 \text{ (Since it is a semicircle)}$$

Where, A = Area, l = length, b = breadth, $\frac{\pi}{2}$ = constant

3. Result

The stomata type was anomocytic (Sachet Hedge, M.Jayaraj, A.V.Bhandarkar, 2015) (Fig 3). The stomatal index, stomatal frequency, length, breadth and area of stomata varied with different regions and concentration of mutagen. Stomatal index (30.85 ± 2.569), length ($11.6 \pm 0.382 \mu$), breadth ($8.2 \pm 0.344 \mu$) and Area ($150.09 \pm 9.37 \mu m^2$) of *T.cordifolia* without treatment was highest at the apex. It was lowest at the base (SI = 24.153 ± 0.791), (L = $10.6 \pm 0.401 \mu$), (B = $7.5 \pm 0.382 \mu$), (A = $125.31 \pm 8.36 \mu m^2$), [Table 1]. (where SI = Stomatal Index, L = length of stomata, B = breadth of stomata, A = Area of stomata). The epidermal cells are wavy and irregular in Shape [Fig 3]. On the Adaxial surface of leaf stomata was

absent and shape of epidermal cell was pentagonal to hexagonal [Fig 4].

After comparison of both mutagen treated plant, it was observed that Stomatal Index ($a = 28.4 \pm 0.601, m = 28.7 \pm 0.792, b = 27.48 \pm 0.75$), length ($a = 9.06 \pm 0.277 \mu, m = 8.98 \pm 0.344 \mu, b = 9.28 \pm 0.171 \mu$), Width ($a = 6.4 \pm 0.277 \mu, m = 6.5 \pm 0.333 \mu, b = 6.14 \pm 0.315 \mu$) and area ($a = 94.03 \pm 6.581 \mu m^2, m = 91.61 \pm 7.762 \mu m^2, b = 90.2 \pm 5.907 \mu m^2$) was maximum at all regions of leaf in MSG treated plant. It was minimum at all regions of plant treated with EMS (S.I at $a = 27.3 \pm 0.731, m = 28.4 \pm 0.735, b = 26.91 \pm 1.054$), (L at $a = 8.66 \pm 0.166 \mu, m = 8.56 \pm 0.180 \mu, b = 8.9 \pm 0.172 \mu$), (B at $a = 6.2 \pm 0.126 \mu, m = 6.44 \pm 0.251 \mu, b = 6.1 \pm 0.283 \mu$), (A at $a = 86.04 \pm 2.666 \mu m^2, m = 87.8 \pm 4.199 \mu m^2, b = 85.56 \pm 2.639 \mu m^2$), [Table 1] [Fig 1, Fig 2]

4. Discussion

The leaf had anomocytic or Ranunculaceous type of stomata. In anomocytic type, the stomata are only present on abaxial surface, adaxial surface is devoid of stomata. The leaves of plant were hypostomatic with different stomatal frequency, stomatal length, stomatal width and stomatal area.

The investigation showed that the mutagen EMS and MSG affected the epidermal cell shape, converted them from more wavy to less wavy, in which MSG affected most [Fig 5, Fig 6]. The leaves without treatment had higher stomatal index, length, width and area. So It may be concluded that effect of mutagens decreases the stomatal index, length, width and area of the stomata. While comparing effect of two mutagen EMS and MSG on plant, Plant treated with MSG perform higher transpiration rate, stomatal index, length, width and Stomatal area.

5. Acknowledgements

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Table 1: Stomatal index data and data related to length, width and area of stomata (in μ) of abaxial and adaxial surface of *Tinospora cordifolia*.

surface	Plant treatment	Apex				Middle				Base			
		S.I	Length (μ)	Width (μ)	Area (μm^2)	S.I	Length (μ)	Width (μ)	Area (μm^2)	S.I	Length (μ)	Width (μ)	Area (μm^2)
Abaxial surface	Normal	30.85 ± 2.569	11.6 ± 0.382	8.2 ± 0.344	150.09 ± 9.37	25.63 ± 1.375	11.3 ± 0.321	8.1 ± 0.223	144.45 ± 7.67	24.15 ± 0.791	10.6 ± 0.401	7.5 ± 0.382	125.31 ± 8.36
	EMS	27.3 ± 0.731	8.66 ± 0.166	6.2 ± 0.126	86.04 ± 2.666	28.48 ± 0.735	8.56 ± 0.180	6.44 ± 0.251	87.8 ± 4.199	26.91 ± 1.054	8.9 ± 0.172	6.1 ± 0.283	85.56 ± 2.639
	MSG	28.4 ± 0.601	9.06 ± 0.277	6.5 ± 0.279	94.03 ± 6.581	28.7 ± 0.792	8.98 ± 0.344	6.5 ± 0.333	91.61 ± 7.762	27.48 ± 0.750	9.28 ± 0.171	6.14 ± 0.315	90.2 ± 5.907
Adaxial surface		0	0	0	0	0	0	0	0	0	0	0	0

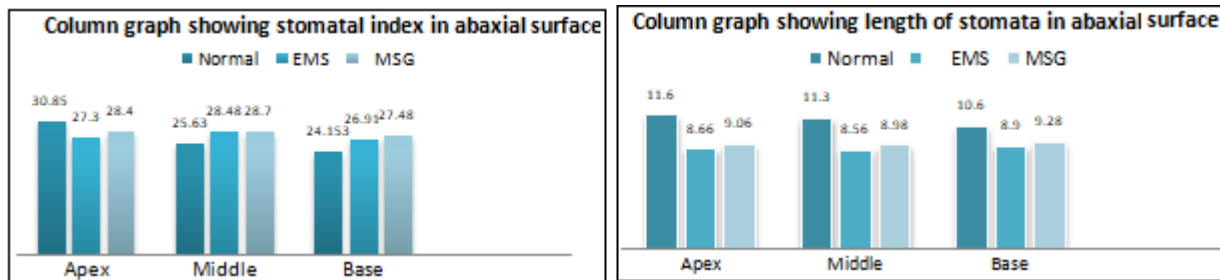


Figure 1: Column graph showing stomatal index and length affected by two mutagen

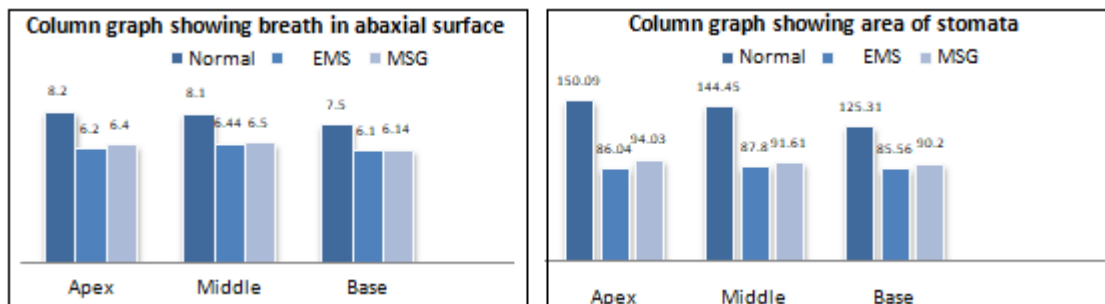


Figure 2: Column graph showing breath and area affected by two mutagen

Photomicrographs of stomata without treatment of mutagen on abaxial (Fig -3) and adaxial surface(Fig- 4).



Figure 3

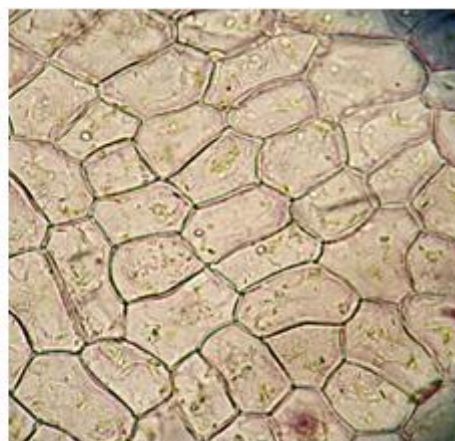


Figure 4

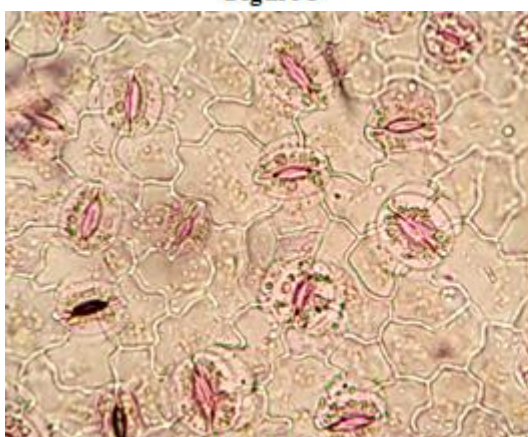


Figure 5

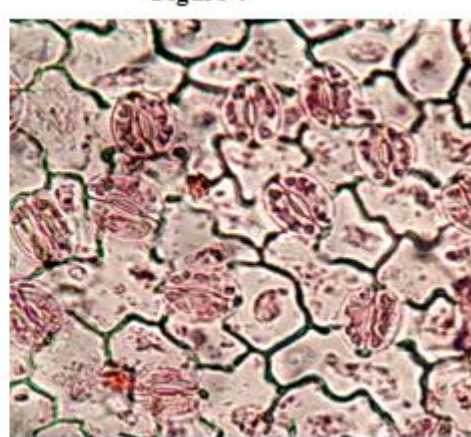


Figure 6

Photomicrographs of stomata after treatment by two mutagen EMS (Fig -5)and MSG (Fig -6)

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