Chromosome Botany (2014) 9: 31–34

Comparative investigation of cytological and biochemical analyses among two species of *Cymbopogon* under the influence of ethyl methane sulphonate collected from Ranchi, Jharkhand, India

Suman Kumari and Kamini Kumar

Department of Botany
Ranchi University, Ranchi 834001
Jharkhand, India

Author for correspondence: (kaminikumar@yahoo.co.in)
Received February 8, 2014; accepted March 4. 2014

ABSTRACT. The study provides cytological and biochemical changes (estimation of oil) by inducing chemical mutagen. Various abnormalities were observed after treatment in two species of *Cymbopogon*. *Cymbopogon martinii* var. *Motia* was noted as sensitive and *Cymbopogon flexuosus* as resistant in two generations. Percentage of oil was slightly higher after treatment with EMS in both the species of *Cymbopogon*.

KEYWORDS: *Cymbopogon*, Cytological, EMS

Cymbopogon, a member of the Poaceae with 80 species of grasses is native to warm temperate and tropical regions of the old world oceania. In India *Cymbopogon*, a tall perennial grass is present with 10-12 species. Most of them are aromatic and some yield essential oils of commercial importance (Pullaih 2002). The demand of *Cymbopogon* is for its high citral content. The plant is used in perfumery, soaps and cosmetics, as a mosquito repellent, pharmaceuticals preparations, disinfectants, antibacterial and carminative.

Present paper deals with comparative analysis of cytological and biochemical studies after treatment with ethyl methane sulphonate (EMS) which is being investigated for the first time.

Materials and Methods

The plants of above mentioned species and varieties of *Cymbopogon* were collected from Birsa Agricultural University, Institute of Forest Productivity and local farm of Ranchi district of State of Jharkhand which is situated between latitude 23º45'N longitude 85º30'E of Indian geographical area. EMS was used as chemical mutagen in this study. Similar sized young plants of *Cymbopogon* were treated with five different concentrations of EMS i.e. 0.1%, 0.2%, 0.3%, 0.4% and 0.5% for 6 hours. After treatment, the roots were cut and pretreated with paradichlorobenzene and fixed in 1:3 acetoalcohol for 12 hours and then preserved in 70% alcohol. The treated root apices were stained in 2% acetocarmine and squash preparations were made. The cytological aberrations were studied and recorded.

Biochemical studies were done on above two species of *Cymbopogon* with special reference to estimation of oil. The fresh and dried leaves were cleaned and cut into the small pieces. fifty gm of each of the plant material was taken for the extraction of oil and hydrodistilled in a Clevenger Apparatus at 100ºC for three hours.

Results and Discussion

Mutagenic effects of EMS were recorded in *Cymbopogon flexuosus* and *Cymbopogon martinii* var. *Motia* for two generations. A dose dependent decrease in mitotic index was observed after treatment in both the species of *Cymbopogon*. The marked decrease in the mitotic index and gradual increase in the percentage of chromosomal abnormalities as the concentrations of experimental solution increased which indicated that the drugs inhibited the nucleic acid synthesis. Decline in mitotic index with increasing concentrations of EMS has been reported previously (Kirtane and Dhumal 1999). It was also assumed that the reduction in mitotic index after chemical treatment might be due to prophase poisoning which obstruct the chromosome movement from prophase to metaphase. Such findings were also supported earlier. (Al-Nazzar 1980). While comparing the mitotic index in two species, *C. martinii* var. Motia was found more sensitive and *C. flexuosus* was resistant towards the doses of EMS for both M_1 and M_2 generations.

The mitotic abnormalities after treatment with EMS were recorded at prophase, metaphase, anaphase and telophase stages in two species for two generations. Prophase abnormalities manifested only nuclear vacuolation which might occur due to the disintegration of proteins of nuclear material by the action of EMS.

Metaphase abnormalities were observed very frequently in both generations in two species. Stickiness in chromosome might occur due to disturbances in the nucleic acid metabolism of the cell (Darlington 1942). The occurrence of fragments at metaphase might be attributed to the failure of broken chromosomes to recombine due to the effect of chemical mutagen. Tropokinesis and diagonal arrangements observed might be due to spindle abnormality. The

Table 1. Types and frequency of chromosomal abnormalities during different phasef mitosis at varying concentration of ethyl methane sulphonate in *Cymbopogon flexuosus* in M_1 Generation

Concentration	Total No. of cells observed	Resting cells	Total No. of dividing cells	Mitotic Index	Physiological Aberrations								Clastogenic Aberrations							No. of abnormal cells
					Prophase Abnormality	Percentage Metaphase Abnormality							Percentage Anaphase Abnormalities				Percentage Telophase Abnormalities			
					Nuclear vacuolation	Fragment	Stickiness with fragment	Stickiness	Elongated cell	Tropokinesis	Diagonal arrangement	Triploid cell	Laggard	Unequal separation	Single Bridge	Double Bridge	Binucleate	Trinucleate	Micronuclei	
Control	916	315	601	65.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.10%	907	411	448	49.39	2.67 ± 0.28	0.44 ± 0	0.89 ± 0.33	1.33 ± 0.57	0.66 ± 0	0.22 ± 0	0.22 ± 0	0.44 ± 0	0.44 ± 0	0.44 ± 0	0.22 ± 0	-	1.56 ± 0.33	0.66 ± 0.50	0.44 ± 0	48
0.20%	926	466	428	46.22	3.27 ± 0.53	0.23 ± 0	0.46 ± 0	0.70 ± 0.50	0.46 ± 0	0.23 ± 0	0.23 ± 0	0.23 ± 0	0.23 ± 0	0.70 ± 0.50	-	0.23 ± 0	1.16 ± 1.50	1.40 ± 0.57	0.70 ± 0.50	44
0.30%	923	400	467	50.59	2.56 ± 0.81	0.21 ± 0	0.42 ± 0	1.71 ± 0.40	2.35 ± 0.37	0.21 ± 0	0.21 ± 0	0.21 ± 0	0.42 ± 0	0.85 ± 0	0.21 ± 0	0.64 ± 0.50	1.49 ± 0.47	0.21 ± 0	0.21 ± 0	56
0.40%	912	396	481	52.74	1.45 ± 0.24	0.20 ± 0	0.41 ± 0	1.03 ± 0.50	0.20 ± 0	0.62 ± 0.50	0.20 ± 0	0.41 ± 0	-	0.20 ± 0	-	-	1.03 ± 0.33	0.62 ± 0.50	0.83 ± 0.33	35
0.50%	921	436	456	49.51	2.19 ± 0.33	0.21 ± 0	-	1.31 ± 0.57	0.21 ± 0	-	0.65 ± 0.50	0.43 ± 0	0.21 ± 0	-	0.21 ± 0	-	0.43 ± 0	0.21 ± 0	0.21 ± 0	29

Table 2. Types and frequency of chromosomal abnormalities during different phases of mitosis at varying concentration of ethyl methane sulphonate in *Cymbopogon flexuosus* in M_2 generations

Concentration	Total No. of cells observed	Resting cells	Total No. of dividing cells	Mitotic Index	Physiological Aberrations								Clastogenic Aberrations							No. of abnormal cells
					Prophase Abnormality	Percentage Metaphase Abnormality							Percentage Anaphase Abnormalities				Percentage Telophase Abnormalities			
					Nuclear vacuolation	Fragment	Stickiness with fragment	Stickiness	Elongated cell	Tropokinesis	Diagonal arrangement	Triploid cell	Laggard	Unequal separation	Single Bridge	Double Bridge	Binucleate	Trinucleate	Micronuclei	
Control	916	315	601	65.61	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.10%	923	471	408	44.20	1.96 ± 0.40	0.73 ± 0.50	0.98 ± 0	0.24 ± 0	0.49 ± 0	0.24 ± 0	0.24 ± 0	-	0.49 ± 0	0.49 ± 0	0.73 ± 0.50	0.49 ± 0	1.71 ± 0.33	0.98 ± 0.33	0.98 ± 0	44
0.20%	919	444	427	46.46	2.10 ± 0.25	0.23 ± 0	0.46 ± 0	0.70 ± 0.50	0.70 ± 2.00	0.23 ± 0	0.23 ± 0	0.70 ± 0.50	0.46 ± 0	0.93 ± 0	0.70 ± 0.50	-	1.63 ± 0.33	1.40 ± 0.28	0.70 ± 0.50	48
0.30%	914	296	556	60.83	3.59 ± 0.32	0.71 ± 0.33	0.53 ± 0.50	1.97 ± 0.37	0.89 ± 0.50	0.53 ± 0.50	0.35 ± 0	0.17 ± 0	0.17 ± 0	-	0.17 ± 0	-	0.89 ± 0.50	0.35 ± 0	0.71 ± 1.00	62
0.40%	927	389	488	52.64	1.024 ± 0.33	0.40 ± 0	0.20 ± 0	1.02 ± 0.33	1.63 ± 0.40	0.81 ± 0.33	0.81 ± 1.00	0.40 ± 0	0.20 ± 0	0.40 ± 0	-	0.20 ± 0	1.63 ± 0.40	0.61 ± 0.50	0.81 ± 0	50
0.50%	940	430	476	50.63	1.05 ± 0.25	-	-	1.47 ± 0.47	1.05 ± 0.33	0.42 ± 0	-	-	-	0.21 ± 0	0.21 ± 0	-	-	1.47 ± 0.24	1.26 ± 0.57	34

Table 3. Types and frequency of chromosomal abnormalities during different phases of mitosis at varying concentration of ethyl methane sulphonate in *Cymbopogon martinii* var. *Motia* in M_1 generation.

Concentration	Total No. of cells observed	Resting cells	Total No. of dividing cells	Mitotic Index	Physiological Aberrations								Clastogenic Aberrations							No. of abnormal cells
					Prophase Abnormality	Percentage Metaphase Abnormality							Percentage Anaphase Abnormalities				Percentage Telophase Abnormalities			
					Nuclear vacuolation	Fragment	Stickiness with fragment	Stickiness	Elongated cell	Tropokinesis	Diagonal arrangement	Triploid cell	Laggard	Unequal separation	Single Bridge	Double Bridge	Binucleate	Trinucleate	Micronuclei	
Control	930	274	656	70.53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.10%	927	504	374	40.34	2.40 ± 0.22	0.53 ± 0	0.26 ± 0	1.06 ± 0.33	1.33 ± 0.25	0.53 ± 0	0.26 ± 0	0.26 ± 0	0.26 ± 0	0.53 ± 0	1.06 ± 0.33	0.80 ± 0.50	2.13 ± 0.40	1.33 ± 0.25	0.26 ± 0	49
0.20%	906	483	379	41.83	1.58 ± 0.28	0.52 ± 0	0.52 ± 0	1.31 ± 0.25	1.58 ± 0.28	0.52 ± 0	0.26 ± 0	0.26 ± 0	0.26 ± 0	0.52 ± 0	0.26 ± 0	-	1.58 ± 0.28	1.31 ± 0.33	1.05 ± 0.33	44
0.30%	905	425	428	47.29	1.63 ± 0.25	-	-	1.40 ± 0.28	1.86 ± 0.40	0.93 ± 0.33	0.46 ± 0	0.93 ± 0.33	-	0.23 ± 0	-	-	2.10 ± 0.37	0.70 ± 0.50	1.86 ± 0.40	52
0.40%	918	479	392	42.70	1.53 ± 0	0.25 ± 0	0.25 ± 0	1.02 ± 0.33	1.53 ± 0.28	0.51 ± 0	1.02 ± 0.33	0.76 ± 0.50	0.51 ± 0	0.51 ± 0	0.51 ± 0	0.25 ± 0	1.78 ± 0.47	1.53 ± 0.28	0.25 ± 0	47
0.50%	910	484	384	42.19	1.30 ± 0.25	1.04 ± 0.33	0.52 ± 0	1.04 ± 0.33	1.04 ± 0.33	0.78 ± 0.50	0.78 ± 0.50	0.52 ± 0	0.26 ± 0	0.26 ± 0	-	-	1.30 ± 0.33	1.04 ± 0.33	1.04 ± 0	42

Table 4. Types and frequency of chromosomal abnormalities during different phases of mitosis at varying concentration of ethyl methane sulphonate in *Cymbopogon martinii* var. *Motia* in M_2 generation.

Concentration	Total No. of cells observed	Resting cells	Total No. of dividing cells	Mitotic Index	Physiological Aberrations								Clastogenic Aberrations							No. of abnormal cells
					Prophase Abnormality	Percentage Metaphase Abnormality							Percentage Anaphase Abnormalities				Percentage Telophase Abnormalities			
					Nuclear vacuolation	Fragment	Stickiness with fragment	Stickiness	Elongated cell	Tropokinesis	Diagonal arrangement	Triploid cell	Laggard	Unequal separation	Single Bridge	Double Bridge	Binucleate	Trinucleate	Micronuclei	
Control	930	274	656	70.53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.10%	923	430	461	49.94	1.08 ± 0.50	0.86 ± 0	-	0.43 ± 0	-	0.86 ± 1.0	1.08 ± 0.25	0.65 ± 0.50	0.43 ± 0	-	0.43 ± 0	-	0.86 ± 0.33	0.21 ± 0	-	32
0.20%	905	385	482	53.25	1.45 ± 0.33	1.45 ± 0.47	-	1.03 ± 0.33	-	0.41 ± 0	1.03 ± 0.33	-	-	-	-	-	1.24 ± 0.57	0.82 ± 1.0	0.41 ± 0	38
0.30%	927	321	560	60.40	1.60 ± 0.37	0.71 ± 0.33	-	1.78 ± 0.31	0.71 ± 0.33	0.17 ± 0	0.71 ± 0.33	0.17 ± 0	0.17 ± 0	-	-	-	1.60 ± 0.37	-	0.53 ± 0.50	46
0.40%	920	459	432	46.95	1.15 ± 0.50	0.23 ± 0	0.92 ± 1.0	1.62 ± 0.16	0.04 ± 0	0.69 ± 0.50	0.92 ± 0.33	-	-	-	-	-	0.46 ± 0	0.23 ± 0	-	29
0.50%	902	464	406	45.01	1.97 ± 0.40	0.98 ± 0.33	-	1.72 ± 0.47	-	1.23 ± 0.50	1.23 ± 0.25	-	-	0.24 ± 0	0.24 ± 0	-	0.49 ± 0	-	-	33

Table 5. Estimation of oil in *Cymbopogon flexuosus* and *C. martinii* var. *Motia* after treatment with ethyl methane sulphonate in M_1 and M_2 generations.

Species	Concentration	M_1 Generation		M_2 Generation	
		Fresh sample	Dried sample	Fresh sample	Dried sample
Cymbopogon flexuosus	Control	29.73 ± 0.17	0.46 ± 0.06	29.73 ± 0.17	0.46 ± 0.17
	0.10%	32.93 ± 0.69	0.60 ± 0.11	32.73 ± 0.58	0.46 ± 0.06
	0.20%	33.93 ± 0.17	0.66 ± 0.06	32.40 ± 0.30	0.53 ± 0.06
	0.30%	33.40 ± 0.30	0.73 ± 0.06	32.33 ± 0.26	0.73 ± 0.11
	0.40%	33.90 ± 0.15	0.86 ± 0.06	32.60 ± 0.17	0.80 ± 0.11
	0.50%	34.00 ± 0.11	1.06 ± 0.06	32.86 ± 0.70	0.93 ± 0.06
Cymbopogon martinii var. Motia	Control	29.33 ± 0.17	0.33 ± 0.06	29.33 ± 0.17	0.33 ± 0.06
	0.10%	33.46 ± 0.60	0.36 ± 0.03	31.13 ± 0.17	0.40 ± 0.11
	0.20%	33.26 ± 0.46	0.46 ± 1.13	31.53 ± 0.35	0.46 ± 0.06
	0.30%	32.60 ± 0.11	0.60 ± 0.11	31.33 ± 0.29	0.53 ± 0.06
	0.40%	33.66 ± 0.17	0.66 ± 0.06	31.73 ± 0.66	0.66 ± 0.06
	0.50%	33.33 ± 0.37	0.86 ± 0.24	32.80 ± 0.72	0.73 ± 0.06

presence of triploid cells indicated that EMS is a potential spindle poison. Similar results have been recorded in *Vicia faba* (Chandra *et al* 2002).

The occurrence of lagging chromosome at anaphase might be explained on the basis of abnormal spindle formation and failure of chromosomal breakage by binding DNA regions (Lawly and Brookers 1963). Anaphasic bridges might be formed as a result of stickiness of chromosomes at anaphase.

Telophase abnormalities include binucleate, trinucleate and micronuclei. Arrest of cytokinesis leading to the formation of binucleate and trinucleate cells were induced by some clastogenic chemicals supports the present finding. Binucleate and trinucleate cells were also reported in cancer cells (Graham 1963). Micronuclei arise from lagging chromosomes and fragments. Micronuclei are the true mutagenic effects and they may lead to the loss of genetic material (Auerbach 1962).

While comparing the two species of *Cymbopogon* after treatment EMS, it was observed that the total number of aberrant cells were slightly lower in *Cymbopogon flexuosus* in comparison to *Cymbopogon martinii* var. *Motia*.

The data for estimation of oil are depicted in table 5. In *C. flexuosus*, it was noticed that there was tremendous increase in percentage of oil as compared to control in both fresh and dry samples. While comparing the two species of *Cymbopogon* after treatment with EMS, it was noticed that the percentage of oil was slightly higher in *Cymbopogon flexuosus* in comparison to *Cymbopogon martinii* var. Motia.The oil percentage was higher in M_1 generation.

Essential oil and citral content were influenced by factors such as temperature, light intensity, soil moisture,

fertilizer and maturity stage (Miyazaki 1965). Other relevant factors that effect production of essential oil are leaf position and different plant parts of lemongrass (Singh *et al* 1989). *Cymbopogon martinii* when exposed to physical mutagen (Gamma radiation at 15Kr) and two chemical mutagens (EMS - 0.4% and EI - 0.04%) together with their relative effectiveness for enhanced biomass growth and oil yield in M_1 generation were reported earlier in 2011 (Srivastava 2011). These findings are in confirmity of this research work. Therefore, it may be concluded that Ethyl Methane Sulphonate definitely enhanced the citral content.

Acknowledgements. The authors are grateful to the Head, University Department of Botany, Ranchi University, Ranchi, Jharkhand, India for providing all the necessary laboratory facilities.

Literature Cited

AL-Najjar, Nahla, R., Soliman and Alef, S. 1980. Cytological effects of fungicides I Mitotic effect of Vitavase-200 and Dithane S-60 on wheat and two related species. Cytologia. 45: 163-168.

Auerbach, C. 1962. Mutation, An introduction to research on mutagenesis. Part 1 Methods Oliver and Boyd Edinburgh, B. 62: 284-291.

Chandra Ravi, B., Sharan, R. P. and Sareen, P. K. 2002. Clastogenic effects of trifluralin in *Vicia faba*. J. Cytol. Genet. 3 (NS): 201-203.

Darlington, C. D. 1942. Chromosome Chemistry and gene action. Nature 149: 66-69.

Graham, M. R. 1963. The cytological diagnosis of cancer WB Saunders Co., Philadelphia, London.

Kirtane, S., Laware, S. L. and Dhumal, K. N. 1999. Effects of ultragin and tribhuvankirti on root tip cells of *Allium cepa* L. J. Cytol. Genet. 34 (2): 147-151.

Lawely, P. D. and Brookers, P. 1963. Further studies on alkylation of nucleic acid and their constituent nucleotides. Biochem. J. 89: 137-138.

Miyazaki, Y. 1965. Grass and oil yields from lemongrass and the quality of oil. Japanese J. Trop. Agric. pp. 37-41.

Pullaiah, T. 2002. Medicinal Plants in India. Vol.-I.

Singh, N. Luthra, R. and sangwan, R. 1989. Effect of leaf position and age on the essential oil quantity and quality in Lemongrass (*Cymbopogon flexuosus*)[1]. Planta Medica. 55 (3): 254-256.

Srivastva, H. K. and Satpude, G. K. 2011. Induction of mutations for enhanced essential oil in palmarosa (*C. martinii*). Journal of Essential oil Research. 10 (3).