Electronic Journal of Plant Breeding, 2(4):578-582 (Dec 2011)
ISSN 0975-928X

Research Note

EMS induced karyomorphological variations in *Cichorium intybus* L.

Iram Fatma Jafri *, Ainul Haq Khan, Mohd Gulfishan And M.Y.K. Ansari
Cytogenetics and Mutation Breeding Laboratory, Department of Botany, A.M.U. Aligarh-202002, India
*E-mail: ifatima.amu@gmail.com

(Received: 25 Jul 2011; Accepted: 20 Nov 2011)

Abstract:
Cytogenetical investigations were carried out in *Cichorium intybus* plants derived from EMS treated seeds at 0.1, 0.2, 0.3, 0.4 and 0.5% concentrations. The plants in the treated populations showed varying degrees of meiotic irregularities almost at all mutagenic concentrations. Variations in some parameters such as seed germination, pollen fertility, days to flowering, days to maturity, number of leaves per plant, leaf length, plant height, and chromosome behavior have been studied in M1 generation. A positive correlation between increasing concentrations of mutagen and chromosomal aberration has been observed.

Key words: *Cichorium intybus*, EMS, chromosomal aberrations and morphological variations.

Cichorium intybus L. (2n=18) is an important medicinal plant belonging to the family Asteraceae, believed to be a native of temperate part of the Old World. It is grown mainly for medicinal purposes, as food or fodder and for commercially valuable roots. Chicory is an excellent mild bitter tonic for the liver and digestive tract; therefore, its extract forms an important constituent in liver medicines like Liv-52 and Geriforte. Leaves and roots are important for curing mouth, breast and face cancer (Hartwell 1967-1971). Dried root powder is used for adulteration in Coffee. In this investigation, an attempt has been made to improve this medicinal plant through mutagenesis. Induced mutagenesis is a significant tool to break through the limitations of variability and to create variability in a short period of time. The degree of cytological aberrations in either mitosis or meiosis is regarded as one of the dependable criteria for estimating the potency of mutagen and to elucidate the response of various genotypes to a particular mutagen (Kumar and Sing 2003). Studies suggested that EMS is an effective mutagen and can be used in inducing genetic variability in a number of crop plants. The present investigation was made to evaluate the effect of EMS on the genetic traits of *C. intybus.*

The seeds of *Cichorium intybus* L. (common chicory) were obtained from the store stock of Department of Botany, Aligarh Muslim University Aligarh. Healthy seeds were pre-soaked in distilled water at 25±2^{0}C for 6h and then treated with different concentrations of EMS (0.01, 0.02, 0.03, 0.04, and 0.05 %) at 25±2-^{O}C for 8 h. During the treatment, the beakers were shaken frequently to provide sufficient aeration for the seeds. Seeds were thoroughly washed in running tap water to remove the EMS sticking to the seed coat. One set of seeds soaked in distilled water was kept as control. Three replicates of 100 seeds were maintained for each dose of treatment and then they were sown under the natural condition. At the time of flowering, the young flower buds of proper size were collected carefully from 30-40 randomly selected M_1 plants of treated as well as control populations and fixed in 1:3 acetic-ethanol with a few crystals of ferric acetate for 24 h. The buds were washed and preserved in 70% ethanol. Anthers were squashed in 1% acetocarmine.

Meiosis was normal in the control plants and showed regular formation of nine perfect bivalents (2n = 18) at diakinesis (fig. 1a) and metaphase I followed by normal separation at anaphase-I and anaphase-II. However, various chromosomal abnormalities were recorded in the plants raised from EMS treatment. Frequency and spectrum of meiotic aberrations induced by EMS treatments at different stage of cell division have been presented in Table-1. Most frequent chromosomal aberrations were univalents (fig.1b, c, & d), multivalents (fig.1e), stray bivalent, precocious separation(fig.1f) and stickiness (fig.1i) at metaphase I/II, bridges(fig.1g), laggards (fig.1i), and non-synchronization (fig.1j) at anaphase I/II and disturbed polarity (fig.1*l*) multinucleate and cytomixis at telophase-I/II. A dose dependent increase in meiotic irregularities was observed in EMS treatment. Maximum frequencies of meiotic aberrations were recorded at highest concentration of mutagenetic treatment. The most prominent abnormality induced by EMS was stickiness of chromosomes at metaphase I and II as well as at anaphase I and II. During stickiness chromosomes

formed a compact mass and the identity of individual chromosomes was lost. Varying numbers of univalents were also observed in higher frequency at the highest concentration of EMS treatment. Non-synchronization in the divisional stages of PMCs and late separation of bivalents were observed in all concentrations. Multivalents at metaphase I were also noted in considerable frequency at the highest concentration (Table-1). The lagging chromosomes and fragments, which usually failed to be included in the daughter nuclei, formed micronuclei. The results showed a co-linearity between the concentration of EMS and the percentage of chromosomal anomalies

Mutagenic treatment with EMS also affected the morphological parameters of the treated sets (Table-2). Germination percentage was found to be maximum (95%) in the control set, while it was minimum (70%) in 0.05% of EMS. The increasing concentrations of EMS showed decreasing effect on germination percentage. Plant height was also found to be significantly reduced at higher concentration of treatment but some of the plants at lower doses showed a slight increase in plant height (0.01 & 0.02%). Similar trends in other parameters like days to flowering, days to maturity, number of leaves per plant, leaf length and pollen fertility were recorded after EMS treatments.

Mutagens have remarkable possibilities of causing variations in various qualitative and quantitative characters of plants by altering the genetic architecture. The chemical mutagens have been reported to be more potent in inducing mutations than the physical ones. Induction of mutation in *Cichorium intybus* have been studied by many researchers (Khan *et al*., 2009; Haque and Godward, 1985)

The chromosomal behavior during meiosis is considered to be one of the most reliable indices for estimating the potency of mutagens and the response of a genotype to mutation. The frequency and spectrum of chromosomal aberrations observed during the present investigation clearly showed that EMS is a very potent mutagen for *C. intybus* Mutagen induced structural changes in chromosomes and gene mutations might be responsible for the failure of pairing among homologous chromosomes and hence the presence of univalents. According to Katiyar (1978) alterations in chromosomal associations, composed of uni-, tri-, tetra-, and multivalents were possibly the outcome of non or irregular pairing of chromosomes due to translocation. Stickiness could be due to depolymerisation of nucleic acid caused by mutagenic treatments or due to partial dissociation of the nucleoproteins and alteration in their pattern of organization (Evans, 1962). Jayabalan and Rao (1987) suggested that stickiness might be due to disturbances in the cytochemically balanced reaction. Precocious movement of chromosome as observed during the present investigation, probably caused by spindle disfunction. According to Sharma *et al*., (2009), precocious chromosome migration to the poles might have resulted from univalent chromosomes at the end of prophase I or precocious chiasma terminalization at diakinesis or metaphase I. Random movement of univalents to any one of the poles leads to the unequal separation of chromosomes (Kumar and Singh, 2003). Non-synchronous movement may be due to severe disturbance in spindle mechanism (Minija *et al*., 1999). The laggards observed during the present study might be due to delayed terminalisation, stickiness of chromosomal ends or because of failure of spindle fiber to bind on kinetochopre (Khan *et al*., 2009). According to Bhattacharjee (1953), acentric fragments or laggards may result in the formation of micronuclei at telophase-II and ultimately variation in number and size of pollen grains resulting from a mother cell. Saylar and Smith (1966) suggested that the formation of chromatin bridges might be due to the failure of chiasmata in bivalent to terminate and chromosome geting stretched between the poles. In the present study, bridge formation may be attributed to the general stickiness of chromosomes at metaphase stage or breakage and reunion of chromosomes**.** Micronuclei might have arisen from the fragments and lagging of chromosomes which failed to reach to the poles and to get included in the daughter nuclei (Kumar and Dubey, 1998). Disturbed Polarity at anaphase and telophase stages seen in the present case may be due to spindle disturbances.

The reduction in percentage of germination might be due to the effect of mutagen on meristematic tissues of the seed. The mutagenic treatments might have also delayed the germination process. Kleinhofs *et. al.*, (1978) reported a delay in the initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth, ATP and DNA synthesis. Reduction in seed germination in 5-BU treated populations has been explained due to delay or inhibition in physiological and biological processes necessary for seed germination which include hormonal imbalance and inhibition of meiotic process (Ananthaswamy *et. al*., 1971). Reduction in plant height may be due to inhibition of energy supply caused by mutagen and also a result of inhibition of mitosis which is primary requirement for seedling growth. Although all doses

of mutagen elicited a reducing effect on plant height, 0.02% EMS displayed an increase in plant height which may be due to the mutation in major or minor genes (Kumar and Rai 2007). In the present investigation, pollen fertility decreased with the increase in concentrations of the EMS. Similar decrease in pollen fertility was reported earlier in *Vicia faba* (Khan *et al*., 2010), *Capsicum annuum* (Gulfishan *et al* ,2010) and *Cichorium intybus* (Khan *et al*.,2009) Reduction in pollen fertility also supports a decrease in seed production due to the meiotic anomalies.

Thus, the result of the present investigation clearly reveal that the EMS has a vary significant mutagenic potential in *C. intybus* despite its narrow genetic base. The significant cytological and morphological abnormalities induced through the mutagen (EMS), provide enough scope for further improvement in this crop through mutagenesis. The results also provide a definite guideline for improvement of this medicinal herb through the gene manipulations.

References:

Ananthaswamy, H.M., Vakil, V. and Srinivasan, A. 1971. Biochemical and Physiological changes in gamma irradiated wheat during germination. *Rad. Bot.,* **11**: 1-2.

Bhattacharjee, S.K. 1953. Cytogenetics of *Lens esculenta* Monesh. *Caryologia,* **5**: 159-166.

Evans, H.J. 1962. Chromosome aberrations induced by ionizing radiations. *Int. Rev. Cytol.,* **13**: 221-321.

Gupta, P.K. 1989. *Cytology, Genetics and Evolution*. 5th Ed., Rastogi Publications, Meerut.

Haque, M.Z. and Godward, M.B.E. 1985. Effect on seed irradiation on M_1 achenes of *lactuca* and *cichorium*. *Envir. Exp. Bot.,* **22**: 359.

Hartwell, J.L. (1967-1971). Plants used against cancer. A survey. *Lloydia,* 30-34

Jayabalan, N. and Rao, G.R. 1987. Gamma radiation induced cytological abnormalities in *Lycopersicon esculentum* Mill. Var. Pusa ruby, *Cytologia,* **52**: 1-4.

Katiyar, R.B. 1978. Radiocytogenetical studies on *Capsicum* 1 Meiotic anomalies. *Cytologia,* **43:** 415-421

Khan, A.H., Sharma, M. and Jafri, I.F. 2010. Clastogenci Effects of single and combined treatments of MMS and gamma rays in *Vicia faba* L. *Nucleus,* **52**:145-152

Khan, Z., Ansari, M.Y.K., Gupta, H. and Chaudhary S (2009). Dynamics of 2,4-D in generation of cytomorphological variants in an important anticancerous and antihepatotoxic herb – *Cichorium intybus* L. *Turkish J. Bot.,* **33**:383-387

Khan, Z., Ansari, M.Y.K., Gupta, H., and Chaudhary, S. 2009. Methyl methane sulphonate induced chromosomal variations in a medicinal plant *Cichorium intybus* L. during microsporogenesis. *Biology and Medicine*, **1**: 66-69.

Kleinhofs, A., Owais, W.M. and Nilan, R.A. 1978. *Azide Mut. Res.,* **55**: 165-195.

Kumar, G. and Singh, V. 2003. Comparative analysis of meiotic abnormalities induced by gamma rays and EMS in barley. *J. Indian Bot. Soc.,* **82:** 19-22

Kumar, G. and Rai, P. 2007. EMS Induced Karyomorphological Variations in Maize *(Zea mays L)* Inbreds. *Turkish J. Biol.,* **31**: 187-195.

Kumar, S. and Dubey, D. K. 1998. Effect of separate and simultaneous application of gamma rays and EMS on germination, growth, fertility and yield in cultivars Nirmal and LSD-3 of khesari (*Lathyrus sativus* L.). *J. Phytol. Res.,* **11**: 165-17

Minija, J., Tazo, A., and Thoppil, J.E. 1999. Mitoclastic properties of *Menthe rotudifolia* L. *J. Cytol. Genet.,* **34:**169-171.

Saylor, L.G. and Smith, B.N. 1966. Meiotic irregularities in species of inter-specific hybrids in Pisum. *American J. Bot.,* **53**: 453-468

Sharma, M., Khan, A.H. and Bhat, T.A. 2009. Assessment of mutagenicity of individual and combination treatments of gamma rays and MMS in broad bean (*Vicia faba* L.) *Cytologia,* **74**(2): 235-241.

Table 1. Effect of EMS on frequency of meiotic aberration in *C. intybus* in M_1 generation.

Treatments (% of EMS)	Total no. of PMCs scored	Metaphase-I/II (%)				Anaphase-I/II (%)			Telophase-I/II (%)			Total number of abnormal PMCs (%)
		Univalents	Multivalents	Stickiness	Precocious separation	Bridges	Laggards	Disturbed anaphase	Micronuclei	Multinucleate	Disturbed polarity	
Control	215	–	–	–	–	–	–	–	–	–	–	–
0.01%	217	0.92	1.38	1.84	1.84	1.38	1.38	1.84	0.92	0.92	0.92	13.36
0.02%	216	1.38	1.38	1.85	2.31	1.85	2.31	2.70	1.38	1.38	1.38	18.05
0.03%	224	1.78	2.23	2.23	2.23	2.23	2.23	2.67	1.78	1.78	1.78	20.98
0.04%	228	1.75	1.75	2.63	2.63	2.63	2.63	3.07	1.75	1.75	2.19	22.8
0.05%	230	2.17	2.17	2.60	3.04	2.60	3.04	3.47	1.73	0.17	2.60	25.62

Table 2. Effect of EMS on seed germination, days to maturity, days to flowering, plant height, no. of leaf per plant and pollen fertility of *C. intybus* in M_1 generation

Treatments (% of EMS)	Seed germination (%)	Days to flowering $\overline{X}$ ±S.E.	Days to maturities $\overline{X}$ ±S.E.	No. of leaves/plant $\overline{X}$ ±S.E.	Leaf length (cm) $\overline{X}$ ±S.E.	Plant height (cm) $\overline{X}$ ±S.E.	Pollen fertility (%)
Control	95.00	98.22±0.22	120.23±0.44	43.2±3.02	9.02±0.76	170.00±0.22	92.33
0.01%	84.00	96.22±0.33	116.22±0.33	44.4±3.55	9.35±0.95	172.22±0.44	88.44
0.02%	82.00	92.24±0.44	110.22±0.44	57.6±4.4	9.43±0.49	174.33±0.36	84.22
0.03%	78.00	90.28±0.22	108.23±0.36	60.2±5.777	13.82±2.41	168.22±0.24	80.44
0.04%	74.00	100.44±0.36	124.22±0.36	77.4±2.85	15.50±0.60	167.23±0.28	78.22
0.05%	70.00	104.21±0.44	128.33±0.56	82.0±2.74	19.53±0.46	135.33±0.44	74.44
Pooled mean	77.60	96.68±0.35	11740±0.41	64.32±3.86	13.52±0.98	163.44±0.35	81.15

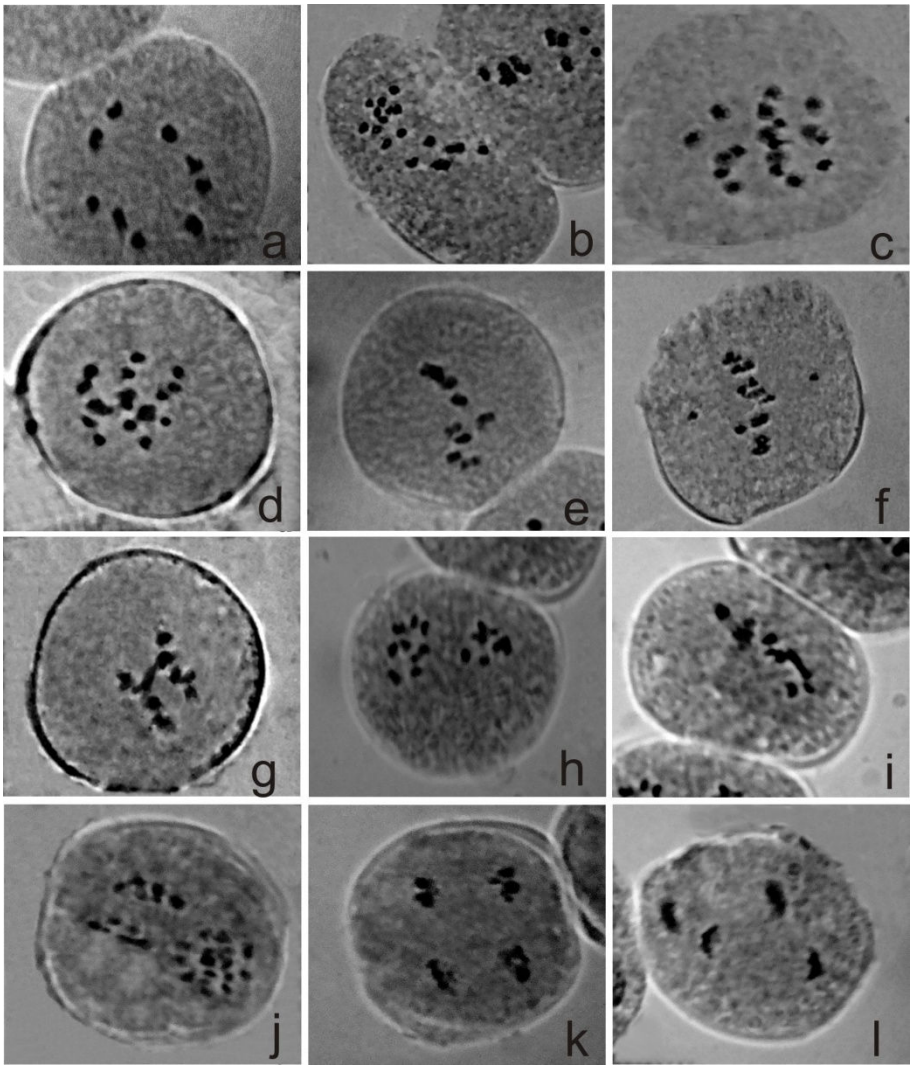

Figure 1: Representative meiotic features observed in control and mutagen treated plants of *C.* intybus L. a) Normal diakinesis (2n=18), b) 10^{I} and 4^{II} at diakinesis, c) 18^{I} at diakinesis d) 10^{I} and 4^{II} at diakinesis e) 6^{II} and 1^{VI} at metaphase-I, f) Precocious seperation at metaphase-I, g) Bridge formation at anaphase-I, h) Normal Metaphase-II, i) Stickiness at metaphase-II j) Nonsynchronous division at metaphase-II, k) Normal anaphase-II) l) Disturbed polarity at telophase-II,