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Full Length Research Paper

Genotoxic effects of 5-bromouracil on cytomorphological characters of *Cichorium intybus* L.

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This work was done to study the effect of base analogue 5-bromouracil (5-BU) on the medicinal herb, *Cichorium intybus*. 5-BU induced miss pairing during the DNA replication. The seeds of *C. intybus* were treated with different concentrations of 5-BU. Variations in some parameters such as seed germination, seedling survival, seedling height, pollen fertility, days to flowering, days to maturity, number of leaves per plant, plant height, and chromosome behavior were studied in M1 generation. A positive correlation between increasing concentrations of mutagen and various cytomorphological characters of *C. intybus* was observed.

Key words: Chromosomal aberrations, *Cichorium intybus*, morphological variations, 5-bromouracil.

INTRODUCTION

Mutation breeding makes extensive use of deviations from the norms to improve the characters of important crops. However, an efficient genetic improvement of a cultivar depends on the knowledge of mode of gene action, genetic variability, and the interrelationship among important plant characters. Induced mutagenesis is a significant tool to break through the limitations of variability and to create variability in a short period of time (Kumar and Sing, 2003). The degree of cytological aberrations in either mitosis or meiosis is regarded as one of the dependable criteria for estimating the effect of a mutagen. Mutagen induced chromosomal anomalies are the primary basis of genetic change; therefore, investigations on the mechanism of chromosome breakage, type of aberrations, and their genetic consequences form an integral part of most of the mutation studies (Bhat et al., 2007). Cytogenetic investigation is one of the best documented experimental proofs for the elucidation of the mode of speciation on different groups of plants (Goyal and Khan, 2009). The base analogues which are mutagenic, have structure sufficiently similar to the normal bases so that they are metabolized and incorporated into the DNA during the replication, but sufficiently different such that they increase the frequency of mispairing and thus mutation (Gupta, 1989).

Cichorium intybus L. (2n = 18) is an important medicinal plant belonging to the family Asteraceae, believed to be a native of the temperate part of the Old World. It is grown mainly for medicinal purposes, as food or fodder or, as is more often the case, for the roots, which are commercially valuable. 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 study the effect of response of 5-BU on cytomorphology of *C. intybus.*

MATERIALS AND METHODS

The seeds of *C. intybus* L. (common chicory) were obtained from the store stock of Department of Botany A.M.U. Aligarh. Healthy seed were pre-soaked in distilled water at 25±2°C for 6 h and then treated with different concentrations of 5-BU (0.01, 0.02, 0.03, 0.04 and 0.05%) at 25±2°C for 8 h. During the treatment, the beakers were shaken frequently to provide sufficient aeration to the seeds. Seeds were thoroughly washed in running tape water to remove the 5-BU sticking to the seed coat. One set of seeds soaked in distilled water was kept untreated to act as a 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 to 40 randomly selected M_1 plants of treated as well as

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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.

The observations recorded on days to flowering, days to maturity, number of leaves per plant, and plant height, leaf length etc. in different treatments and control were subjected to statistical analysis with a view to find out the extent of variations induced by the chemical mutagens and their interrelationship. Mean ($\overline{X}$) and standard error (SE) was calculated by using the formula.

Mean: $\overline{\mathrm{X}} = \frac{\sum \mathrm{X}_n}{N}$

Standard error: $\mathrm{S.E.} = \frac{\mathrm{SD}}{\sqrt{\mathrm{N}}}$

Where, X_n = individual readings; N = number of observations, and S.D. = standard deviation.

RESULTS

Cytological effects

Meiosis was normal in the control plants and showed regular formation of nine perfect bivalents (2n = 18) at diakinesis (Figure 1a) and metaphase I (Figure 1b) followed by normal separation at anaphase-I (Figure 1c) and anaphase-II (Figure 1d). However, various chromosomal abnormalities were recorded in the plants raised from 5-BU treatment. Frequency and spectrum of meiotic aberrations induced by 5-BU treatments at different stage of cell division are presented in Table 1. Most frequent chromosomal aberrations were univalents (Figure 1e), multivalent (Figure 1f), stickiness (Figure 1g), stray bivalent (Figure 1h), and precocious separation at metaphase I/II, bridges, laggards (Figure 1i), and none-synchronization (Figure 1j) at anaphase I/II and disturbed polarity (Figure 1k) multinucleate (Figure 1l), and micronuclei at telophase-I/II. A dose dependent increase in meiotic irregularities was observed in 5-BU treatment. Maximum frequencies of meiotic aberrations were recorded at the highest concentration of mutagenetic treatment. The most prominent abnormality induced by 5-BU 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 but their frequency was higher at the highest concentration of 5-BU treatment. Non-synchronization in the divisional stages of PMCs and late separation of bivalents were observed in the 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 5-BU and the percentage of chromosomal anomalies.

Morphological effects

Mutagenic treatment with 5-BU 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 (50%) in 0.05% of 5-BU. The increasing concentrations of 5-BU showed decreasing effect on germination percentage. Plant height was also found to be significantly reduced at higher doses treatment but some of the plants at lower doses showed a slight increase in plant height. Similar trends in other parameters like days to flowering, days to maturity, number of leaves/plant, leaf length and pollen fertility were recorded after 5-BU treatments. It was observed that 0.02% of 5 BU showed better responses in all the morphological parameter.

Thus, this study displayed the minimum percentage of chromosomal anomalies in 0.01% 5-BU concentration while the maximum chromosomal anomalies were found in plants treated with highest concentration of 5-BU , thus treatment of 0.05% concentration showed genotoxic effect on *C. intybus*, however, 0.02% 5-BU displayed better response in all the morphological parameters.

DISCUSSION

Though, the 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 (Sharma, 1965).

Induction of mutation in *C. intybus* has been studied by many researchers (Khan et al., 2009; Haque and Godward, 1985). In this investigation, base analogue was used to understand the mechanism of 5-BU induced damage and biological dosimetry in *C. intybus*.

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 investigation clearly showed that 5-BU 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 multivalent 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

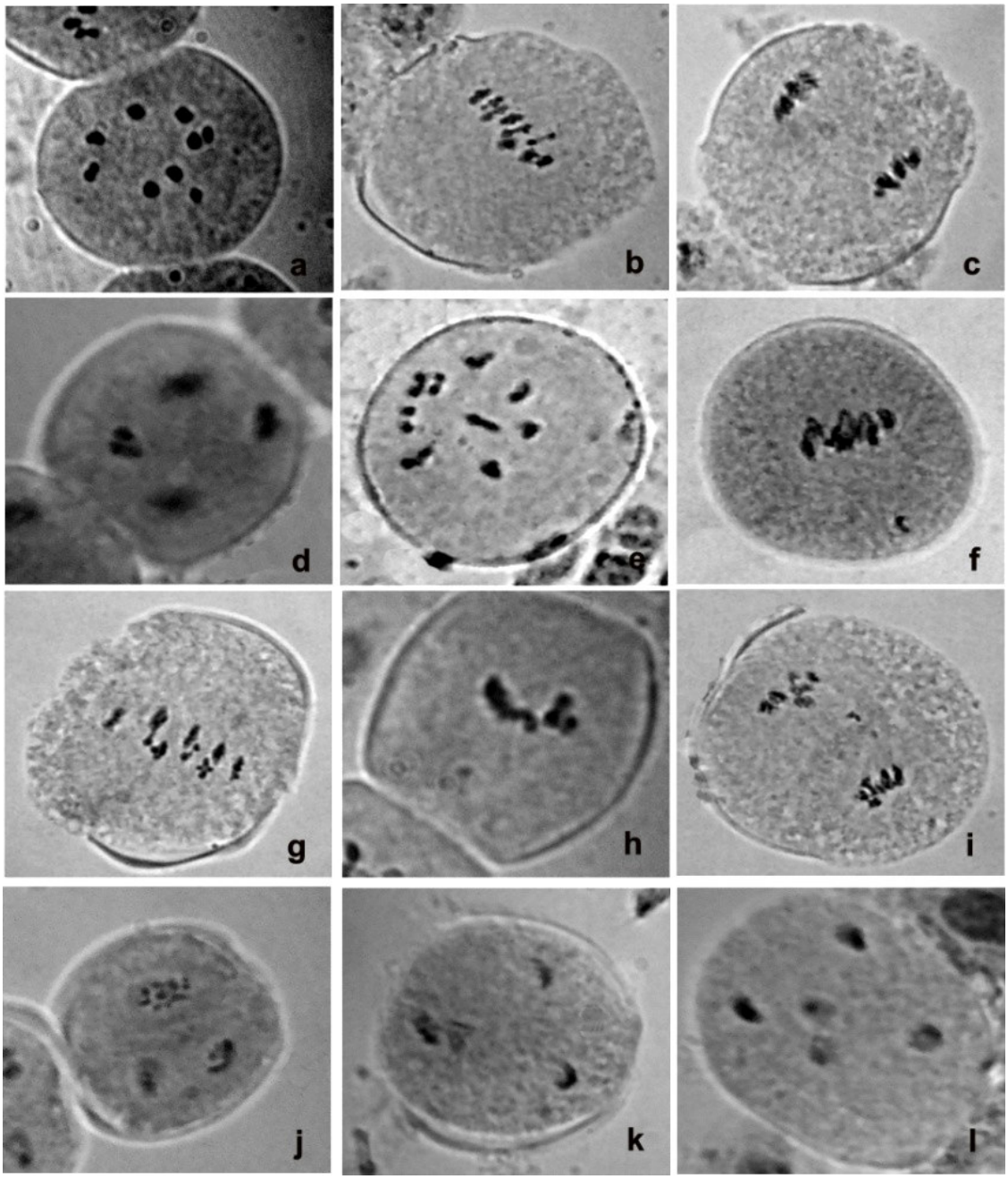

Figure 1. Representative meiotic features observed in the control and mutagen treated plants of C. intybus L. a) Normal diakinesis (n = 9); b) normal metaphase-I (n = 9); c) normal anaphase-I, d) normal telophase-II; e) univalents at early metaphase-I; f) 7^{II} and 1^{IV} at metaphase-I; g) stray bivalent at metaphase-I; h) stickiness at metaphase-I; i) laggard at anaphase-I; j) nonsynchronous division at metaphase-II; k) disturbed polarity at telophase-II; l) multinucleate condition at telophase-II.

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 investigation was 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 anyone 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

Table 1. Effect of 5-BU on frequency of meiotic aberration in *C. intybus* in M_1 generation.

Treatments (% of 5-BU)	Total number of pollen mother cells scored	Metaphase-I/II (%)				Anaphase-I/II (%)			Telophase-I/II (%)			Total abnormality (%)
		Univalents	Multivalents	Stickiness	Precocious separation	Bridges	Laggards	Disturbed anaphase	Micronuclei	Multinucleate	Disturbed polarity	
Control	215	–	–	–	–	–	–	–	–	–	–	–
0.01	222	0.90	1.80	1.81	1.80	0.45	1.80	2.25	1.35	1.35	1.35	14.86
0.02	226	1.76	1.76	1.76	2.21	o.44	2.21	3.09	1.32	1.32	1.76	17.69
0.03	225	1.77	2.66	2.22	2.22	0.44	2.22	3.10	1.77	2.22	2.66	21.33
0.04	230	2.17	2.60	2.60	2.60	0.43	2.60	2.60	2.17	2.17	2.60	22.60
0.05	232	2.58	3.01	3.01	3.01	0.43	3.01	1.72	2.58	2.58	3.01	25.01

Table 2. Effect of 5-BU on seed germination, days to maturity, days to flowering, plant height, number of leaf per plant and pollen fertility of *C. intybus* in M_1 generation.

Treatment (% of 5-BU)	Germination (%)	Days to flowering ($\overline{X}$ ±S.E.)	Days to maturity ($\overline{X}$ ±S.E.)	Plant height (cm) ($\overline{X}$ ±S.E.)	Number of leaves per plant ($\overline{X}$ ±S.E.)	Leaf length (cm) ($\overline{X}$ ±S.E.)	Pollen fertility (%)
Control	95.00	98.22±0.22	120±23±0.44	170.00±0.22	43.2±3.02	9.02±076	92.33
0.01	82.00	95.19±0.28	115.33±0.22	174.33±0.33	44.8±3.02	9.08±1.04	90.22
0.02	80.00	92.22±0.22	108.44±0.44	178.22±0.44	55.6±4.12	10.0±1.35	84.44
0.03	66.00	90.22±0.36	106.43±0.33	168.23±0.48	47.25±3.35	11.33±0.48	80.33
0.04	58.00	100.33±0.44	126.44±0.22	160.22±0.44	61.16±3.48	14.90±0.84	74.44
0.05	50.00	102.22±0.36	130.22±0.44	162.33±0.36	66.0±4.49	17.0±0.95	70.33

mechanism (Minija et al., 1999). The laggards observed during the 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. Saylor and Smith (1966) suggested that the formation of chromatin bridges might be due to the failure of chiasmata in bivalent to be terminated and chromosome get stretched between the poles. In this study, bridge formation may be attributed to the general stickiness of chromosomes at the metaphase stage or breakage and reunion of chromosomes. Micronuclei might have arisen from the fragments and lagging chromosomes which failed to reach to the poles and get included in the daughter nuclei (Kumar and Dubey, 1998). Disturbed Polarity at anaphase and telophase stages seen in this 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 the primary requirement for seedling growth. Although all doses of mutagen elicited a reducing effect on plant height, however, 0.02% 5-BU displayed an increase in plant height which may be due to the mutation in major or minor genes (Kumar and Rai, 2007). The chromosomal abnormalities will be the effect of the pollen sterility. In this investigation, pollen fertility decreased with the increase in concentrations of the 5-BU. Similar decrease in pollen fertility was reported earlier in *Vicia faba* (Khan et al., 2010), *Capsicum annuum* (Gulfishan et al., 2010) and *C. intybus* (Khan et al., 2009). Reduction in pollen fertility also supports a decrease in seed production

due to the meiotic anomalies.

From the aforesaid result, it becomes clear that 5-BU is genotoxic and able to produce chromosomal aberrations. The relationship between aberration and sterility suggested that induced sterility was mainly the result of chromosomal aberrations. The result shows co-linearity between the concentration of mutagen and percentage of chromosomal anomalies. The result also provides a definite guideline for the improvement of this medicinal herb through the gene manipulations.

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