Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN 2320-3862
JMPS 2017; 5(1): 96-100

Received: 15-11-2016
Accepted: 16-12-2016

Saswati Bhattacharya
Department of Botany, Dr A.P.J Abdul Kalam Govt. College, New Town, Rajarhat, Kolkata, West Bengal, India

Aishee De
Division of Plant Biology, Bose Institute, Kolkata, West Bengal, India

Madhubrata Choudhury
Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Correspondence
Madhubrata Choudhury
Department of Life Sciences, Presidency University, Kolkata, West Bengal, India

Cytological Effects of 5- Amino Uracil on *Allium cepa* L. roots

Saswati Bhattacharya, Aishee De and Madhubrata Choudhury

Abstract
5- Amino Uracil is a structural analogue of pyrimidine. In this work the cytological effects of 5-AU has been evaluated in the root tip cells of *Allium cepa* L. following treatment with different concentrations for different durations. Microscopical observation of the cells revealed that 5-AU has considerable clastogenic activities as evidenced by altered MI (Mitotic index) values as well as occurrence of different types of chromosomal abnormalities. Inspite of such abnormalities, the treated cells when allowed to recover for different periods there results a synchronous mitotic wave. Following 32.5 h and 33h of recovery synchronous prophase and metaphase populations were obtained. It has been further concluded that the recovery time required to achieve such synchronization is not independent of the duration of treatment as concluded by some earlier workers.

Keywords: 5-Amino Uracil, *Allium cepa* L. root, clastogenic activity, recovery hours, divisional synchrony

1. Introduction
There are several chemicals which can lead to mitotic cell synchronization in higher plants. One such chemical is 5-Amino Uracil (5-AU) where 5-methyl group is replaced by 5-amino group. This is a structural analogue of the pyrimidine base thymine / uracil. It is found to inhibit DNA synthesis at even low concentration. Initial treatment of roots with such inhibitors followed by withdrawal and subsequent recoveries leads to synchronized cell population (Campo *et al.*, 2005) [1]. Such reports of partial synchronization were obtained from *Vicia faba, Allium cepa*, *Pisum sativum* (Scheuermann and Lobsien, 1973) [2]. However the degree of synchronization is found to vary in different plants i.e. in garlic the rate of synchronization is much higher when compared with onion or barley (Machado et al. 1999) [3]. There are several views regarding the mode of action of 5-AU. There was the initial idea that 5-AU has got identical mode of action with FUDR, another structural analogue that inhibits DNA synthesis by blocking thymidylate synthase (Diez *et al.*, 1976) [4]. But later work (Panda *et al.*, 1995) [5] has shown that the accumulation of cells in S phase and decrease in the number of G_2 phase cells occur in 5-AU treated cells leading to some blocks which occur in some points between S- G_2 transition of the cell cycle. There are three important check-points in cell cycle – in G_1/ S checkpoint the entrance to replication is blocked due to inappropriate cellular growth, in G_2/ M point the cycle is blocked if DNA replication is not complete and finally in M/ G_1 point, which is activated during the metaphase-anaphase transition. Existence of such checkpoints assures that the cell will be able to identify any DNA damage which interferes with DNA replication as well as proper orientation of chromosomes in the metaphase spindle. In the following work, it has been decided to study the effects of 5-AU treatment on *A. cepa* roots. The study includes the effects of 5-AU on the mitotic status of the cell as is reflected from the MI value as well as scanning of different types of chromosomal abnormalities following treatments with different doses for different durations. This may give some idea regarding the clastogenic activities of this chemical which has not been well studied so far. Simultaneously, effort has been made to synchronize the heterogeneous root-tip cell population by 5-AU treatment followed by different sets of recovery.

2. Methods & Materials
5- Amino Uracil (5-AU), 98% was purchased from Sigma- Aldrich, USA (Mol. weight - 127.1). A stock solution of 1000mg/L aqueous solution was prepared which denotes 1000 ppm solution.

For subsequent experiments different concentrations of solutions ranging from 100ppm to 1000ppm have been applied. Out of these the three significant concentations have been identified which generate variations in the result. These are solutions of 200ppm, 500ppm and 700 ppm concentrations. In this paper only these three concentrations are considered.

For cytological analysis, rooted bulbs of *A. cepa* are kept immersed in the 5- AU solution so that only the roots remain immersed. The treatment is continued as different sets i.e. 200ppm, 500ppm, 700ppm each for 24h, 48h, 72h respectively. Each time after completion of respective treatments, roots are fixed in Carnoy's Fixative (1:3) for overnight, stained by standard Aceto-Orcein schedule and studied for cytological analysis. For cell synchronization, 700ppm of 5-AU is applied as the final dose after several rounds of trial and error with different doses. Rooted bulbs are treated for 24 h in 5-AU followed by different sets of recovery in Knop's solution where the root tips are fixed every 30 mins., the first set being fixed at 30.5h of recovery and the last one after 34h of recovery. Aceto-orcein schedule is followed for cytological studies.

For protein estimations proteins were extracted from the roots of bulbs treated with 700ppm 5-AU for 48 hours and for control set roots of *Allium* that was kept in water for 48 hours were taken. For Extraction, RIPA buffer was used (contains 50mM Tris, 150mM NaCl, 1%non-iodet 40, 0.5% Sodium deoxycholate and 0.1% SLS). Proteins were estimated spectrophotometrically following Lowry's method (Lowry *et al.,* 1951) [6].

3. Results and Discussion

Table 1 shows the comparative account of different divisional stages as well as mitotic frequency obtained by treatment with 200 ppm, 500ppm and 700 ppm of 5-AU for different periods. It is clear that 700 ppm of 5-AU has much pronounced effect on the cell division as compared to 500 ppm. Values of MI decrease sharply with increase in duration of treatment. It is to be noted that the divisional frequency as well as MI becomes very low at 700 ppm for 24h that tells about the extent of clastogenic activity of 5-AU. Similar drop in Mitotic Index has also been reported by Cho *et al.* (2011) [7] on treatment of Wheat roots by increasing concentration of Zebularine, a cytidine analog. There are several reports on the cytological effects of 5-AU (Lydall and Weinart, 1995; Basic-Zaninovic, 1991) [8, 9] manifested as chromosomal abnormalities like fragmentation, micronuclei formation (Fig.1a) as well as anaphase bridge. Chromosome breakage (Fig 1b) is found to be as one of the most notable chromosome abnormality by base analogues. Meador *et al.* (2010) [10] found such breakage in DNA-protein kinase deficient human glioblastoma cells under Zebularine treatment. Cho et al (2011) [7] has found the breakage to involve both euchromatic and heterochromatic region of chromosomes. Another possible mechanism for chromosome breakage as suggested by Tsujimoto (2005) [11] in wheat is by gametocidal genes introduced in the genome as a result of chromosome transfer. No report of such genes is found till date in *Allium*. So the extent of chromosome breakage in the 5- AU treated cells should exclusively be treated as the clastogenic effect of the chemical. Table 2 shows the different types of chromosomal abnormalities found following treatments with 200 ppm, 500 ppm and 700 ppm of 5-AU. Chromosome segregation process is found to be affected by prolonged treatment with 5-AU. This generates laggard, early separations as well as polyploids (Fig. 1c, 1d and 1e) possibly due to some defect in microtubule attachment or due to stickiness of chromatin matter. The latter is confirmed by the occurrence of sticky bridge (Fig. 1f). Such bridge may also result due to chromosome breakage followed by sister chromatid and non-sister chromatid rejoining (EI-Bayoumi *et al.,* 1984) [12]. Lydall and Weinert (1995) [8], Heddle and Carravo (1977) [13] have observed aneuploid nuclei and other chromosomal aberrations which they attributed to the chemical effect of 5-AU on spindle microtubules as observed in yeast. It is clear from the table that 5-AU mainly induces chromosome breaks which is manifested as fragmented chromosomes. It is known that these fragments during cell division, are either lost or appear as dot-like extranuclear entities designated as micronuclei. Micronuclei are the result of acentric chromosome fragments as well as of changed spindle function (Hogstedt and Karlson, 1985) [14]. It is already established that *Allium* micronucleus assay is an authentic parameter for genotoxicity assay (Ondrej, 1979) [15].The occurrence of micronuclei further strengthens the clastogenic activity of this chemical (Handschumacher and Welch, 1970) [16]. Such chromosome breaks which leads to Micronucleus formation could be interpreted at molecular level, due to absence of some chromosomal proteins or alteration of DNA replication process (Socher and Davidson, 1970) [17]. Presence of laggards indicates that 5-AU interferes with the tubulin proteins of the spindle. Thus the interference in protein function may be one of the principle reasons for its clastogenic action and this possibly takes place in the form of inhibition of synthesis of spindle proteins as well as chromosomal proteins. The total protein content of treated and control set when compared, it was found that the value is 422µg/g of tissue in untreated set and 364µg/gm of tissue in treated set. This reduction in protein content may confirm the inhibition of spindle and chromosomal proteins as well on 5-AU treatment.

It was reported earlier by several workers that when plant root meristems are incubated in 5-AU for some hours and then transferred to normal culture media, a mitotic wave is observed characterized by maximum mitotic index several times higher than in normal untreated meristems. When *A. cepa* roots are subjected to 5-AU treatment followed by recovery for different periods in Knop's solution, significant increase in mitotic frequency was observed, the value being gradually increased from 30.5 h to 32 h of recovery. After that, there seems to be a sudden increase in MI at 32.5 h of recovery where the value reaches to as high as 88.68 (Table 3). It is to be noted that 5-AU treatment was performed for 24 h which almost covers two cell cycles in *A. cepa*, each having the duration of 13.5 h comprising of 12h interphase followed by 1.5 h to complete mitosis. Similarly while using a lower concentration of 5-AU, there is also a gradual increase and decrease of mitotic index. Due to 5-AU treatment, progressions of most of the cells are blocked in S-G_2 transition probably due to the consequences of preferential inhibition of replicating DNA (Campo *et al.,* 2005). As soon as the cells are allowed to recover, there results a mitotic wave which is responsible for the initial increase in MI. Fig. 2a shows a population of cells from such preparation where most of the cells belong to prophase. This may represent a stage of cell cycle comprising of synchronous population of prophase cells. At the 33.0 h recovery most of the cells are found to achieve metaphase synchrony (Fig. 2b). A graphical representation of cell synchronization following 5-AU treatment is shown in Fig. 3. It has been found that lower

concentration of 5-AU than usually employed for cell synchronization induces chromosomal abnormalities and formation of micronucleus but does not fully block the cell flow in mitosis (Machado *et al.,* 1999).

Synchrony in *A. cepa* roots was also achieved by earlier workers (Campo *et al.,* 2005) [1]. But in that particular study they have used much lower concentration of 5-AU i.e. 0.5mM and have achieved maximum synchrony during recovery period of 18h. They have observed that the value of maximum MI is directly proportional to the duration of treatment up to 18 h, after which the toxicity level disturbs the cell undergoing prolonged treatment. As a consequence the value of the maximum MI decreases with longer treatment. Moreover they have found the recovery time required to achieve maximum MI is roughly constant and is independent of the duration of treatment. However when the 5-AU treatment is applied in much higher concentration as is given in this study, the cell has to spend much more time in recovery to counteract the action of this chemical. As a consequence, prolonged recovery period is needed to initiate the divisional phase so that maximum MI is achieved. This delay in the progression of arrested cells may be attributed to the inhibition of DNA repair routes as suggested by some earlier work (Zaninovic, 1991) [9]. In the present study 32.5 h recovery is required to achieve synchronized prophase population and 33 h to attain synchronized metaphase population. It has already been confirmed by cytological analysis of 700ppm of 5- AU treated cells that the different abnormalities signify its clastogenic action. Therefore it seems that such treated cells remain arrested at the S-G_2 transition and needs much more time in recovery before it attains divisional synchrony.

Table 1: Comparison of mitotic frequency of different divisional stages in *A. cepa* roots obtained after treatment with 5-AU

Mitotic frequency of Control Set		Concentration of 5- AU used (ppm)	Duration of 5-AU treatment (hrs)	Mitotic Frequency of 5-AU treated cells				Mitotic Frequency
				Percentage of different divisional phase				
Duration (hrs.)	Mitotic Frequency			Prophase	Metaphase	Anaphase	Telophase	
3	11.50±2.12	200	3	11.47±3.22	1.57±0.38	0.91±0.21	0.95±0.03	14.90±1.12
8	9.25±1.45		8	6.87±0.98	2.48±0.45	2.16±0.22	0.60±0.23	12.11±2.11
24	14.05±0.98		24	0.88±1.45	0.71±0.12	0.62±0.15	0.09±0.12	2.30±0.21
3	12.00±2.67	500	3	8.92±2.56	1.49±0.15	0.83±0.23	0.94±0.07	12.18±1.29
8	8.76±3.88		8	5.73±1.02	2.13±0.54	1.86±0.19	0.74±0.06	10.46±0.26
24	16.01±1.76		24	1.37±0.34	0.79±0.12	0.48±0.34	0.41±0.03	3.05±0.19
3	11.85±1.20	700	3	6.98±0.76	1.27±0.09	0.70±0.17	0.39±0.01	9.35±1.27
8	9.20±2.06		8	2.05±0.56	0.49±0.10	0.17±0.10	0.32±0.01	3.03±0.94
24	14.24±2.11		24	0.00±0.00	0.00±0.00	0.00±0.00	0.000±0.00	0.00±0.00

*Data presented as mean ± Standard deviation; n=20 replicates

Table 2: Chromosomal abnormalities obtained in *Allium cepa* roots following treatment with different concentrations of 5-AU

Concentration of 5-AU (ppm)	Duration of treatment (hrs)	Total no. of cells scored	Total no. of abnormal cells	% of abnormality	Remarks
200	24	1025	0	0	-
	48	848	21	2.40	Laggard chromosomes, early separations
	72	1102	41	3.74	Micronuclei, polyploidy
500	24	983	44	4.46	Fragmentation, micronuclei, early separations
	48	1012	59	5.72	Anaphase bridges, laggard chromosomes,
	72	1205	92	7.60	Anaphase bridges, multipolarity
700	24	893	58	6.46	Micronuclei, anaphase bridges, clumping of chromosomes
	48	797	86	8.82	Sticky bridge and fragmentation of chromosomes
	72	1182	124	10.49	Fragmentation, micronuclei

n=20 replicates.

Table 3: Summarized result of cell synchronization of the cells of *Allium cepa* followed by 700ppm dose of 5-AU treatment for 24 hrs

Sl. No.	Duration of Recovery (in hours)	Prophase Frequency among all the cells scored	Metaphase Frequency among all the cells scored
1	30.50	11.02±1.21	0.674 ±0.02
2	31.00	12.11±1.02	1.22±1.1
3	31.50	12.89±2.34	3.18±0.81
4	32.00	13.98±1.32	4.37±0.96
5	32.50	82.02±3.54	4.60±0.22
6	33.00	58.03±2.59	15.30±1.69
7	33.50	9.22±1.67	4.50±0.96
8	34.00	7.34±1.12	1.86±0.13
9	CONTROL	8.24±0.17	3.34±0.09

*Data presented as mean ± Standard deviation; n=20 replicates

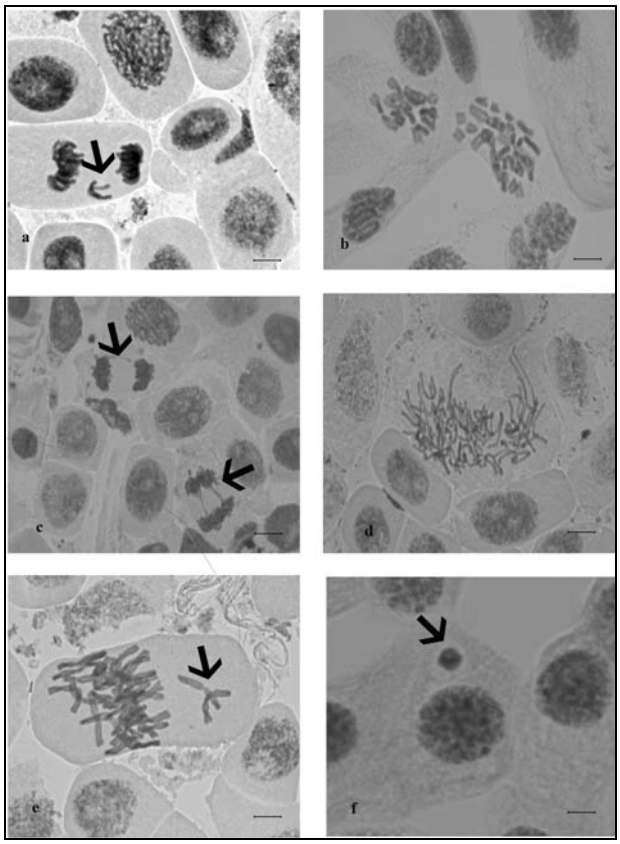

Fig 1: Cytological abnormalities due to 5-AU treatments in *A. cepa* roots: a: Laggard; b: Fragmentation of chromosomes; c: Sticky Bridge; d: Polyploidy; e: Early separation; f: Micronuclei (Bar=5µm)

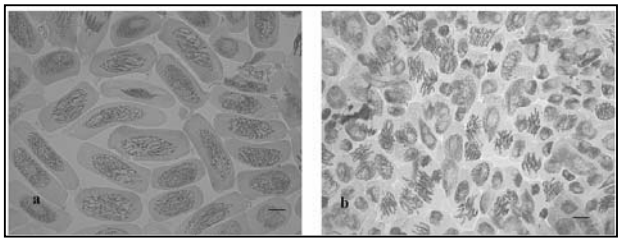

Fig. 2: Synchronization of mitotic division by 5-AU treatment (700ppm for 24 hrs.) in *A. cepa* roots followed by recoveries as mentioned below: a: Synchronized prophase (32.5h); b: Synchronized metaphase (33h) (Bar=10µm)

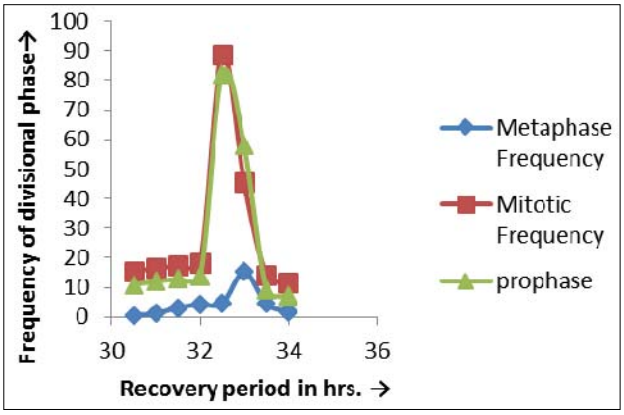

Fig 3: Synchronization of root tip cells of *A. cepa* following 5- AU treatments

4. Conclusion

This work explores the clastogenic activities of 5- aminouracil on onion root cells. Among the different concentrations of 5-AU applied, 700 ppm shows maximum decrease in mitotic index values. The chemical is also found to induce different types of chromosomal abnormalities like fragmentation, micronuclei formation, missegregation of chromosomes, occurrence of polyploids, stickiness of chromatin, laggards etc. This points out the extent of clastogenic activities of this chemical as a result of interference in protein function. Reduction in the total protein content in treated cells confirms the assumption. The root cells when treated for 24 h in 5-AUfollowed by prolonged recovery in Knop's solution results in synchronization of divisional phases. Prophase synchrony occurs in 32.5 h and Metaphase synchrony occurs in 33h of recovery. It has been concluded that the pronounced clastogenicity of 5-AU blocks the treated cells at S-G2 transition of cell cycle which can be recovered by subjecting the cells to long treatment of recovery. During this period due to removal of the block, a mitotic wave is generated leading to cell synchronization at prophase and metaphase stages.

5. Acknowledgement

Authors are grateful to the Department of Biological Sciences, Presidency University for providing all the facilities for the experiments. Special thanks to Sri Sukhendu Maity and Smt. Debarati Pramanick for their tireless assistance and support.

6. Funding

This work was financially supported by the Faculty Research Program and Development Fund, Presidency University, Kolkata (FRPDF/DBS/2015-16).

7. References

1. Del Campo A, Bracho M, Marcano L, Guinez J, De la Torre. DNA injury induced by 5- Aminouracil and Caffeine in G_2 checkpoints path of higher plant cells. Biocell (Mendoza). 2005; 29(2):1-10.
2. Scheuermann W, Klaffke-Lobsien G. On the influence of 5- Aminouracil on the cell cycle of root tip meristems Exp Cell Res. 1973; 76:428-436.
3. Machado MFPS, Silva MF, Vicentini VEP. Effect of 5-Aminouracil on mitotic cell division in *Allium cepa.* Acta Scientiarum. 1999; 21(2):225-228.
4. Diez JL, Gonzalez-Fernandez A, Lopez-Saez JF. Mechanism of mitotic synchronization induced by 5-Aminouracil. Exp Cell Res. 1976; 98:79-89.
5. Panda BB, Subhadra AV, Panda KK. Prophylaxis of antioxidants against the genotoxicity of methyl mercuric chloride and maleic hydrazide in *Allium* micronucleus assay Mutat Res. 1995; 343:75-84.
6. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951; 193(1):265-75.
7. Cho SW, Ishii T, Matsumoto N, Tanaka H, Eltayeb AE, Tsujimoto H. Effects of the cytidine analogue zebularine on wheat mitotic chromosomes. Chromosome Science. 2011; 14(1-2):23-8.
8. Lydall D, Weinart TA. Yeast checkpoint genes in DNA damage processing implications for repair and arrest Science. 1995; 270:1488-1491.
9. Basic-Zaninovic T, Papes D, Franekic J. Cycloheximidegenotoxicity in *in vitro* and *in vivo* test systems Mutat Res. 1991; 263:203-210.

10. Meador JA, Su Y, Ravanat JL, Balajee AS. DNA-dependent protein kinase (DNA-PK)-deficient human glioblastoma cells are preferentially sensitized by Zebularine. Carcinogenesis. 2010; 31(2):184-91.
11. Tsujimoto H. Gametocidal genes in wheat as the inducer of chromosome breakage. Wheat Inform Serv. 2005; 100:33-48.
12. El-Bayoumi AS, Habib AA, Halieem AS. Mitotic disturbances induced by drugs in *Allium cepa* root tips Qatar. Univ. Sci. Bull. 1984; 4:95-115
13. Heddle JA, Carravo AV. The DNA content of micronuclei induced in mouse bone marrow by γ-irradiation; evidence that micronuclei arise from acentric chromosomal fragments. Mutat Res. 1977; 44:63-69.
14. Högstedt B, Karlsson A. The size of micronuclei in human lymphocytes varies according to inducing agent used. Mutation Research/Genetic Toxicology. 1985; 156(3):229-32. doi.: 10.1016/0165-1218(85)90067-9
15. Ondrej M. The influence of 5- Aminouracil on the mitotic index of barley root meristems. Biol Plantarum. 1979; 21(6):440-445.
16. Handschumacher RE, Welch AD. The Nucleic Acids 3rd ed. E Chargaff & J N Davidson Acacdemic Press. New York. 1970.
17. Socher SH, Davidson D. 5- Aminouracil treatment: A method for estimating G_2. J Cell Biol. 1971; 48:248-252