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COMPARATIVE MUTAGENICITY OF EMS AND GAMMA RADIATION IN WILD CHICKPEA

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Abstract: Mutagenic effectiveness and efficiency of gamma rays, EMS and their combined treatments were studied in the genotype of wild chickpea Cicer reticulatum (Accession Number - ICC 17121). The seeds of wild chickpea were treated with chemical and physical mutagens EMS and gamma radiation independently and in combination. The observation scored in the present study represents EMS and γ radiation induced various types of qualitative and quantitative chromosomal aberration comprising scattered chromosomes, clumping, polyploidy, ring formation, stickiness at metaphase; chromatin bridges, laggard, multipolarity at anaphase. It further revealed that the rate of cell division was affected and measured in term of mitotic index which decreased with the increase in concentration of EMS and increase in gamma rays dose in M_1 and M_2 generation. The combined treatment of chemical and physical mutagens appeared more potent and effective as compared to independent chemical and physical mutagenic treatment. The potency and effectiveness of mutagenic treatment was found to be EMS + γ rays > EMS > γ rays. The wide spectrum of mutation was obtained in higher dose of gamma radiation treatment of M_1 generation and in combined treatment and higher dose of radiation treatment of M_2 generation among all the treatments. The higher concentration of EMS and combined treatment of EMS and gamma radiation treatment revealed deleterious as there was less germination percentage observed in M_1 generation while combination of EMS and gamma radiation treatment revealed comparatively more deleterious effect on the germination percentage in M_2 generation representing the combined treatment is more effective and potent.
Keywords: Wild Chickpea, Mutation, Mitotic Index. EMS and Gamma Radiation.

Introduction

The genus *Cicer* belongs to the family leguminoceae containing 9 annuals and 31 perennial species are distributed worldwide, of which *Cicer arietinum* L. Only one of 9 annuals is under cultivation (Muehlbauer, 1993) and only important annual cultivar species (Van der Maesen, 1987). The diploid chromosomes number has been reported 2n=2x=16 in the cultivar and its wild annual relatives (Ahmad, 2000).

A few incomplete and scattered studies and investigation have been executed to reveal that the morphology and individual chromosomal behavior in cultivated chickpea and its wild species (Ladizinsky and Adler, 1976 a, b, Ahmad and Hymowitz, 1993). The related wild

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species are an important and immense reservoir of useful and beneficial genetic traits (Singh *et al., 1*998). The physical and chemical mutagens are being used in genetic improvement programme of different plants species. Mutagen could be successfully applied to induce the genetic variability in *Len culinaris* (Kumar *et al.,* 2003). Ahmad and Chen (2000) reported the difficulties to recognize most of the chromosome by differences in length due to small size of *Cicer* chromosome. The physical and chemical mutagen induced the chromosomal aberration including breakage and reunion of chromosomes. The studies of chromosomal aberration provide the data for estimation of differential mutagenic sensitivity (Prasad and Das, 1980a,b). The mutation frequency could be increased and mutation spectrum could be altered by the combination treatment of different mutagens (Wani, 2009). The physical and chemical mutagens are known to produce chromosomal aberration (Kumar and Dubey, 1998). Direct effects occur when radiation directly ionizes and damages a macromolecule such as DNA (http://www.Ornl.gov/hgmis). The role of mutation breeding is increasing the genetic variability for qualitative traits in various crop such as *Vigna* (Kozgar *et al.,* 2011).
Cytogenetic studies are important for obtaining information regarding the role and effect of various mutagen and elucidating the response of various genotypes to a particular mutagen. The meiotic consequences of chromosomal aberration induced by individuals and combined application of EMS and gamma rays could be assessed with the cytogenetic study (Sharma *et al.,* 2004). The chromosomes in *Cicer reticulatum* have been reported rather small in size and therefore, they required intense staining of the root tips with stain for cytological study (Ohri and Pal, 1991).

Material and Methods

The seeds of wild *Cicer reticulatum* L. were procured from ICRISAT, Patancheru, (AP), India. The seeds of 1st set treated with four different concentration viz. 0.1%, 0.2%, 0.3%, 0.4% of Ethyl Methane Sulphonate. The seeds of 2nd set were treated with combination treatment of EMS and gamma radiation viz. 0.1% EMS+5KR, 0.2% EMS+10KR, 0.3% EMS +15KR and 0.4% EMS+20KR. The healthy seeds were first treated with 0.1% to 0.4% EMS thereafter washed thoroughly and soaked with blotting paper to remove any residual effect of treating solution then the pre-treated seeds were irradiated with 5KR to 20 KR γ rays. The seeds of 3rd set were treated with different doses 5 KR, 10 KR, 15 KR, 20 KR, 25 KR, 30KR of gamma radiation. The seeds of 1st set were encoded as T_2, T_3, T_4, T_5 according to ascending order. The seeds of 2nd set were encoded as T_6, T_7, T_8, T_9 according to ascending order. The seeds of 3rd set were encoded as T_{10}, T_{11}, T_{12}, T_{13}, T_{14} and T_{15} according to

ascending order of doses whereas the untreated seed formed T_1 treatment. The 2-3 healthy and actively growing root-tip of each mutant types and untreated control of the germinated seeds on reaching the length about 1 to 1.5 cm in the petriplates lined with the 2-3 layer of moist filter paper. The root-tips were excised during the time interval of 10 am to11.30 am.

Squash method - The slides were prepared by following standard squash technique (Sharma and Sharma, 1990). The stain 2% acetoorcein was used in present study. Three slides of each treatment were observed under trinocular microscope (Olympus) and the random 10 counts of each slides were scored to cover maximum surface area of the slide for computing the Mitotic Index. The various abnormalities were observed in metaphase such as sticky chromosomes, scattered chromosomes, change in polarity, disturbed and unoriented chromosomes, polyploidy and ring formation; the abnormalities observed in anaphase as chromatin bridge, disturbed and unoriented chromosomes, multipolarity and change in polarity; while the abnormalities observed in telophase were micronucleus formation, binucleate cell and trinucleate cell in all the treatments of M_1 and M_2 generation except control.

Mitotic Index (MI):-It is computed in term of percentage frequency of dividing cells and mitotic index was calculated by scoring the total dividing cells out of total cells scored.

$$\text{Mitotic Index} = \frac{\text{Total number of dividing cells}}{\text{Total number of cells scored}} \times 100$$

Stastical analysis

Mitotic index was determined for each treatment (Bhalla *et al.,* 1973).

Standard Deviation and Stander Error: The data obtained was statistically analyzed as per procedure given by Panse and Sukhatme (1978) using the formula as undermentioned.

$$(\mu) \quad \overline{X} = \frac{\sum \overline{x_i}}{n}$$

Where, $\overline{X}$ (μ)- mean, $\sum \overline{x_i}$ - Sum of 'i' observation, n- Number of observation.

The standard deviation for mitotic index and standard error of mean were calculated for each treatments of M_1 and M_2 generation.

Results and Discussion

The normal and abnormal dividing cells of prophase, metaphase, anaphase and telophase were counted out of the total cells scored to compute the Mitotic Index and standard deviation for all the treatments and represented in **Table A.** for M_1 generation. The decrease in the mitotic index was observed in all the treatments as compared to the control treatment

T_1. The maximum MI 7.786 was observed in T_1 of M_1 generation while higher MI 6.98 in T_2 was observed and lower MI 4.08 in T_9 among all the treatments of M_1 generation.

The total number of abnormal cells revealed increase along with the increase in the concentration of mutagenic treatment which resulted into the decrease in MI. The maximum number of abnormal cells 29 was found in T_{14} and T_{15} while minimum number of abnormal cells 16 was recorded in T_{10} of M_1 generation.

All the dividing cells including normal and abnormal were counted out of total cells scored for each treatment of M_2 generation and depicted in **Table B**. The mitotic index, total cells and dividing cells at each stage are tabulated in **Table B**. The maximum MI was observed in control T_1 treatment minimum MI 4.72 was observed in T_{15} treatment. The frequency of abnormal cells was observed increased along the increase in the concentration of mutagenic agent. The maximum frequency of aberrant cells was observed in T_9 and T_{15} treatment of M_2 generation. The maximum aberrant cells were observed at metaphase, followed by anaphase and telophase. There was no any specificity of occurrence of specific abnormality in the dividing cells. Similar results like these have been reported by many authors (Ahmad, 1993; Khare, 1994). Also the dose dependent increase and increased along with the increasing concentration of mutagen has been reported in *Cichoriumintybus* L. (Khan *et al.,* 2009).

The maximum number of abnormal cells 20 was observed in T_9 and T_{15} and minimum 10 in T_{10} and T_{11} of M_2 generation. The percent frequency of abnormal cells and mitotic index was calculated for each treatment of M_2 generation. The maximum frequency of abnormal cells 2.02 was observed in T_9 and minimum 0.97 in T_{10} treatment among all the mutants while the parent treatment did not show any abnormalities and abnormal cells.

In present study, the germplasm revealed the varied and difference response of mutagenic treatment. The time required for the germination ranged between 3 to 11 days for all the treatments including the control treatment. The varied degree of effectiveness and efficiency varied between different mutagens and also between varieties has been reported in the chickpea (Wani, 2009). The similar differences in mutagenic response have also been reported by many workers (Kharkwal, 1998; Bhat *et al.,* 2007; Dhanvel *et al.,* 2008). Combination treatment of different mutagens increase the mutation frequency and alter the mutation spectrum (Wani, 2009), maximum high frequency of abnormal dividing cells followed by the combination treatment with EMS and gamma rays has been reported in chickpea (Wani and Anis, 2008). The observation scored in the study represents EMS and γ radiation induced various types of qualitative and quantitative chromosomal aberration

comprising scattered chromosomes, clumping, polyploidy, ring formation, stickiness at metaphase. Chromatin bridges, laggard, multipolarity at anaphase. It is further revealed that the rate of cell division was affected which in turn the mitotic index decreased with the increase in concentration of EMS and doses of γ rays. The number of cells with various anomalies has been scored at different stages of mitosis (Kumar and Dubey, 1997). Mitotic cell division, in present study was normal in control seeds, which showed regular 16 chromosomes at metaphase and their normal separation at anaphase in the *Cicer reticulatum* L. Major abnormalities displayed an increasing trends alongwith the increasing doses of gamma rays and EMS treatments has been reported alongwith the frequency of stickiness was higher at 20KR dose, bridges and chromosomal association in greater frequency at highest doses (Kumar *et al.,* 2003).

Dose dependant decrease in MI and increase in frequency of various abnormalities in all the treatment was observed in both the generation $M_1$and M_2 represented in **Table A**, The mitoclastic abnormalities like chromosome bridge at anaphase, diagonal orientation of chromosomes, misorientation of chromosomes, laggard, disturbed anaphase, fragmentation of chromosomes, early movement of chromosomes, clumping of chromosomes, nonsynchronous movement of chromosomes at anaphase have been reported in onion root meristem (Sreeranjini and Thoppil, 2001).

In present study, all the treatment applied reduced mitotic index over than that in control. The dividing cell revealed anomalies at different stage of mitosis. Similar observation with dose dependant increase in mitotic aberration following chemical and physical mutagenic treatment has been reported by different workers (Vandana and Dubey, 1992a, b; Kumar and Dubey, 1997). The percentages % of abnormalities as an index of effectiveness of mutagen and the combined treatment has been reported to be the most effective (Kumar *et al.,* 2003).

Stickiness

The stickiness was more common abnormality found in almost all the treatments of M_1 and M_2 generation and presented in the **Table C** and **Table D**. The frequency was more in M_1 generation as compared to M_2 generation and stickiness was observed more frequent at metaphase in both the generation. Stickiness of chromosomes could be the result of disturbances in cytochemically balanced reaction by the secondary effects of radiation (Jayabalan and Rao, 1987) while stickiness due to depolymerization of nucleic acid caused by mutagenic treatment (Tarar and Dnyansagar, 1980a, b). The abnormalities such as laggard, multipolarity, bridges, nondisjunction of chromosomes etc. at anaphase also increased with

increasing treatments of EMS (Sharma and Kumar, 2004).This stickiness of chromosomes was resulted due to depolymerization of DNA (Darlington, 1942) partial dissolution of nucleoprotein (Kaufmann, 1956).

Polyploidy

The polyploid cells were observed at metaphase. The frequency of polyploidy cells was observed higher in all the treatments of the M_1 generation than M_2 generation. The formation of polyploids cell might be attributed to inactivation of spindle apparatus and failure to separation of chromatids, several chemicals having cytotoxic effects on spindle apparatus in different plants have been reported by many workers (Sharma and Sharma, 1990; Sudhakar *et al.,* 2001).

Ring Formation

The ring formation was observed in all the treatments that is T_3 to T_{15} of M_1 generation while T_5, T_7, T_8, T_9, T_{12}, T_{13}, T_{14} and T_{15} treatments of M_2 generation. Clastogenic chromosomal aberration like fragment, ring chromosome; fragmentation attributed to chromosomal breakage due to effect of HgCl2; the formation of ring chromosome might be result of broken chromosome end (Kumar and Tripathi, 2003).

Chromatin Bridge Formation

The chromatin bridge was observed in all the treatments while the frequency of abnormality was more in higher concentration and doses of EMS and γ rays in M_1 generation. According to Saylor and Smith (1966), the bridge formation could be due to the failure of chiasmata in a bivalent to terminalize and the chromosomes get stretched between the poles.

Laggard

The occurrence of laggard chromosome was more frequents in T_2, T_3, T_9, T_{12} and T_{13} treatments in M_1 generation at lower concentration of EMS, higher concentration and doses of EMS and γ radiation in combination and higher doses of γ rays. The occurrence of lagging chromosomes may be due to abnormal spindle formation and as a result spindle fibres failed to carry the respective chromosomes to the polar region and resultantly lagging chromosome appeared (Tarar and Dnyansagar, 1980a, b). The occurrence of fragments at mitosis may be attributed to the failure of broken chromosome to recombine it (Kaur and Grover, 1985).

Multipolarity

The multipolarity was found to be more frequent in both the generation while it was greater in M_1 generation than M_2 generation. The frequency of multipolarity was observed more in higher concentration of EMS and higher doses of γ radiation and higher doses of combined

treatment of EMS and γ radiation. The precocious movement of chromosomes might have been caused by the early terminalization, stickiness, or because of movement of the chromosome ahead of the rest during anaphase (Permjit and Grover, 1985).

Micronucleus Formation

The micronucleus was more frequency observed in T_2, T_3, T_6, T_{13} and T_{14} of M_1 generation at higher concentration and doses of the treatments whereas the frequency was lower in M_2 generation as compared to M_1 generation and represented in **Table C** and **Table D.** Micronuclei generally arise from fragments and lagging chromosomes, fail to reach the pole and get included into the nuclei (Kumar and Dubey, 1998). The lagging chromosomes and fragment failed to get included in the daughter nuclei formed micronuclei (Sharma *et al.,* 2004). Bhattacharya (1953) suggested irregular distribution of acentric fragments or laggard may also result in the formation of micronuclei.

Conclusion

Both the mutagen represents mitodepressive property independently and in combination increasing the frequency of abnormal dividing cells. However, the frequency of the particular abnormality did not show dose dependant relatedness. The combined treatment of chemical and physical mutagens appeared more potent and effective as compared to independent chemical and physical mutagenic treatment. The potency and effectiveness of mutagenic treatment was found to be EMS + γ rays > EMS > γ rays.

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http://www.Ornl.gov/hgmis

Table A. Mitotic Index and Standard Deviation of the dividing cells in Mitotic cell division of M_1 seeds in wild *Cicer reticulatum* L.

Sr. No	Treatment	Mitotic Index Mean ± Standard Deviation (δ)	Total Cells scored Mean	Dividing cells Mean (Normal+ Aberrant)	Prophase Mean (Normal+ Aberrant)	Metaphase Mean (Normal+ Aberrant)	Anaphase Mean (Normal+ Aberrant)	Telophase Mean (Normal+ Aberrant)
1	T_1	7.786(±0.1)	1288	101	27	30	26	18
2	T_2	6.94(±0.02)	1242	87	28	25	20	14
3	T_3	6.74(±0.02)	1018	69	25	19	15	10
4	T_4	6.53(±0.02)	1025	67	24	20	14	9
5	T_5	6.18(±0.05)	1034	65	25	20	11	8
6	T_6	5.08(±0.07)	1125	57	22	14	11	10
7	T_7	4.75(±0.05)	1022	48	16	13	11	8
8	T_8	4.38(±0.05)	1169	52	16	16	13	8
9	T_9	4.08(±0.03)	1001	41	10	14	11	6
10	T_{10}	6.14(±0.02)	1387	85	27	23	19	16
11	T_{11}	5.71(±0.01)	1429	82	26	22	18	16
12	T_{12}	5.31(±0.02)	1528	82	28	21	17	16
13	T_{13}	5.17(±0.02)	1416	74	27	20	14	13
14	T_{14}	4.89(±0.07)	1386	68	21	20	17	10
15	T_{15}	4.21(±0.07)	1366	58	19	18	12	9

Table B. Mitotic Index and Standard Deviation of the dividing cells in Mitotic cell division of M_2 seeds in wild *Cicer reticulatum* L.

Sr. No	Treatment	Mitotic Index Mean (±SE)	Total Cells scored Mean	Dividing cells Mean (Normal+ Aberrant)	Prophase Mean (Normal+ Aberrant)	Metaphase Mean (Normal+ Aberrant)	Anaphase Mean (Normal+ Aberrant)	Telophase Mean (Normal+ Aberrant)
1	T_1	7.806 (±0.08)	994	78	21	23	20	14
2	T_2	7.10 (±0.04)	975	70	21	20	17	12
3	T_3	6.81 (±0.03)	978	67	21	19	16	10
4	T_4	6.65 (±0.03)	991	66	23	19	13	11
5	T_5	6.40 (±0.06)	998	64	22	19	12	10
6	T_6	5.43 (±0.06)	1006	55	21	13	11	10
7	T_7	5.20 (±0.01)	1012	53	20	13	11	9
8	T_8	4.96 (±0.04)	1005	50	19	13	11	7
9	T_9	4.55 (±0.03)	979	45	16	11	10	8
10	T_{10}	6.21 (±0.04)	1007	63	21	16	14	12
11	T_{11}	5.95 (±0.04)	974	58	21	14	12	11
12	T_{12}	5.75 (±0.03)	983	57	20	14	12	11
13	T_{13}	5.43 (±0.06)	1006	54	20	14	11	9
14	T_{14}	5.14 (±0.03)	1010	52	19	13	11	9
15	T_{15}	4.72 (±0.06)	1030	49	16	14	11	9

Table C. Various mitotic abnormalities and irregularities in M_1 generation of wild *Cicer reticulatum* L.

Sr. No.	Mitotic Irregularities	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
1	SM	--	3	3	3	2	2	3	3	4	2	2	2	3	4	4
2	SCM	--	2	2	2	3	1	1	2	1	1	2	2	1	2	2
3	CPM	--	1	--	1	2	2	1	1	2	1	1	1	2	1	2
4	DUM	--	2	2	1	1	2	1	1	--	2	1	1	1	2	2
5	PC	--	1	1	2	2	1	1	3	2	1	2	3	2	2	3
6	RF	--	--	1	2	2	3	3	3	3	1	3	3	3	3	3
7	CB	--	2	2	2	1	2	1	3	3	1	2	2	2	3	2
8	DUA	--	1	2	1	2	1	2	2	1	2	1	2	1	2	1
9	MA	--	2	1	1	2	2	2	3	2	1	1	3	2	2	3
10	CPA	--	2	2	3	3	2	1	--	1	1	2	1	1	1	2
11	LA	--	3	3	2	2	1	2	2	3	1	2	3	4	3	1
12	MF	--	2	2	1	1	2	1	1	1	1	1	1	2	2	1
13	BC	--	1	--	1	1	1	1	2	2	1	1	1	1	1	1
14	TC	--	--	--	1	1	--	1	1	--	--	--	1	--	1	2
15	**Total**	**--**	**21**	**21**	**23**	**25**	**22**	**21**	**27**	**25**	**16**	**21**	**26**	**25**	**29**	**29**

Table D. Various mitotic abnormalities and irregularities in M_2 generation of wild *Cicer reticulatum* L.

Sr. No.	Mitotic Irregularities	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}	T_{13}	T_{14}	T_{15}
1	SM	--	2	1	2	1	2	2	1	1	1	1	1	1	2	1
2	SCM	--	1	2	1	2	1	2	2	1	2	1	1	1	1	2
3	CPM	--		1	1	2	2	1	1	1	1		1	2	1	1
4	DUM	--	1	1	2	1	1	1	2	2	1	2	1	1	2	1
5	PC	--	--	--	1	1	1	1	2	2	--	1	1	1	1	2
6	RF	--	--	--	--	1	--	1	1	2	--	--	1	1	2	2
7	CB	--	1	1	1	1	1	1	--	1	1	1	1	1	1	1
8	DUA	--	1	1	1	1	1	--	1	1	--	--	--	--	1	1
9	MA	--	1	2	1	2	1	1	2	2	1	1	1	1	1	2
10	CPA	--	1	1	1	--	--	2	2	2	--	--	1	1	1	1
11	LA	--	1	1	2	2	1	2	2	1	1	1	1	2	1	2
12	MF	--	1	1	2	1	1	1	1	1	1	1	1	1	1	2
13	BC	--	2	2	1	2	1	1	1	2	1	1	1	1	1	1
14	TC	--	--	--	--	1	--	--	1	1	--	--	--	1	1	1
15	**Total**	**--**	**12**	**15**	**17**	**19**	**13**	**16**	**19**	**20**	**10**	**10**	**12**	**15**	**18**	**20**

SM-Sticky metaphase, SCM-Scattered metaphase, CPM- Change in polarity, DUM-Disturbed and unoriented metaphase, PC-polyploid cell, RF- Ring formation, CB-Chromatin bridge, DUA-Disturbed and unoriented anaphase, MA- Multipolarity, CPA-Change in polarity, LA-Laggard MF- Micronucleus formation, BC-Binucleate cell, TC-trinucleate cell.

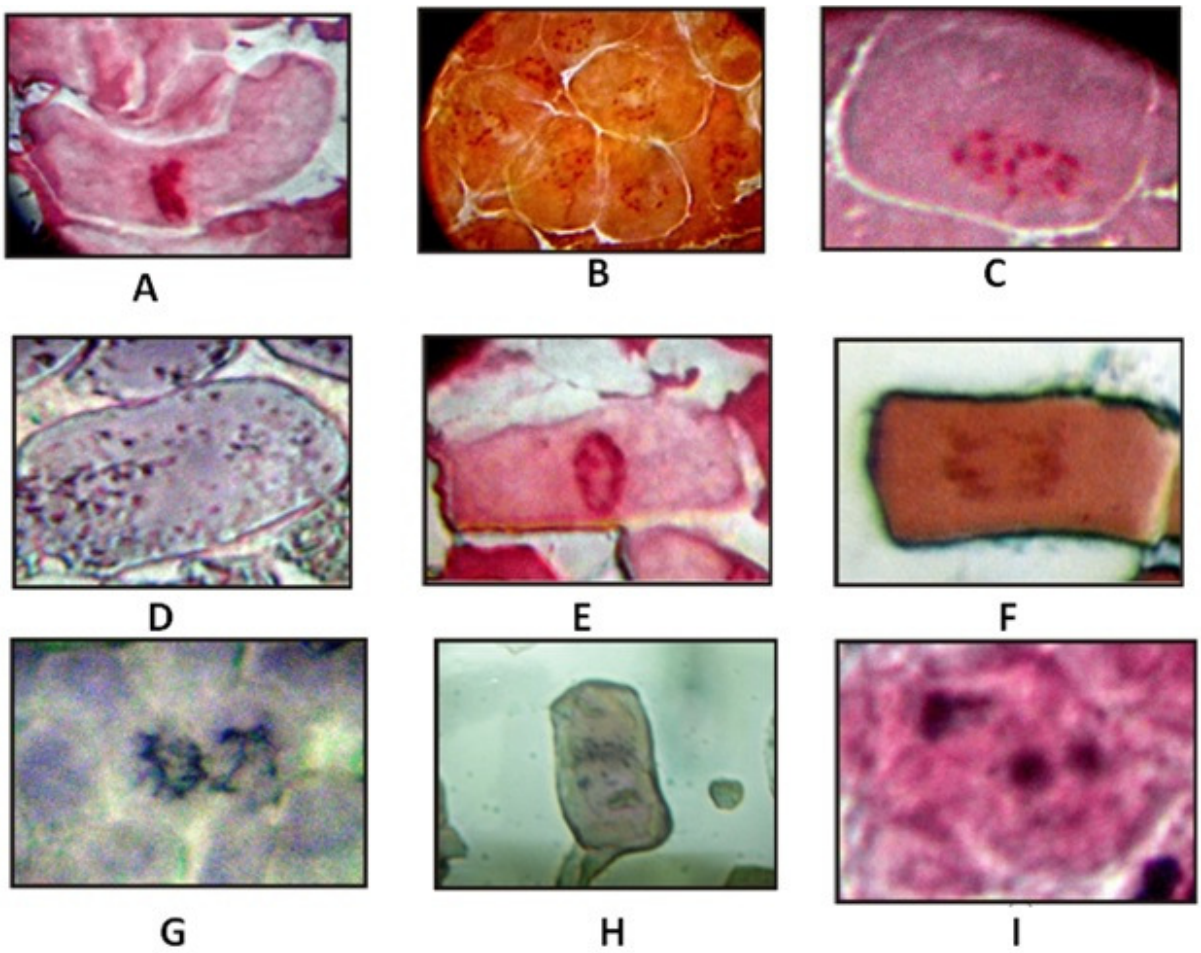

Figure 1: A-Sticky metaphase, B- Scattered metaphase, C- Disturbed metaphase, D-Polyploid, E-Ring formation, F- Anaphasic chromatin bridge, G- Multipolarity, H-Laggard chromosome, I- Trinucleate cell.