IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 1 Issue 9, November 2014.
www.ijiset.com
ISSN 2348 – 7968

Comparative Mutagenic Effects of Sodium Azide and X-Ray in the Wild Chickpea *Cicer reticulatum* L.

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Abstract

The comparative mutagenic effect of physical and chemical agents was assessed in the wild chickpea C*icer reticulatum*.The germplasm of *Cicer reticulatum* were subjected for physical and chemical mutagenic treatments separately and in combination. The various qualitative and quantitative chromosomal irregularities were observed such as stickiness, scattered chromosomes, Chromatin Bridge, laggards in the present study. The mitotic index was calculated for each treatment. The combined treatment is more effective than that of independent or separate treatments. The wide spectrum of mutation was observed in higher dose of mutagens independently and in combination.

Key words: - wild chickpea, mitotic index, SA, X-rays radiation, *Cicer reticulatum.*

1.Introduction

The genus *Cicer* belongs to the family Leguminoceae with total 9 annuals and 31 perennials. The only one of nine is under cultivation (Muehlbauer, 1993). The chickpea is one of the important legume crop and ranked third in the world. The diploid chromosome number of wild annual and cultigens has been reported 2n=2x=16 (Ahmad, 2000). The wild species are very important and immense reservoir of the useful genetic traits and variability (Singh *et al.* ,1998). The physical and chemical mutagens are known to generate the chromosomal aberration (Kumar and Dubey, 1998). The role of mutation breeding is increasing to produce the genetic variability in the various plant species (Kozgar *et al.*, 2011).). Mutagenic effectiveness is measured in term of mutation frequency induced by unit dose of mutagen and mutagnic efficiency represents the mutation frequency in relation to biological damage such as injury, sterility, chromosomal aberration etc. caused by mutagenic treatment (Sirsat *et al.*, 2010).The cytogenetic study is significant to derive the valuable information with respect to the role of effectiveness, efficiency and potential of mutagens and assess the response of a genotype to a particular mutagen. The mitotic irregularities could be assessed with the cytogenetic study (Sharma *et al.*, 2004).

2.Material and Method

The germplasm of wild chickpea (*Cicer reticulatum* L.) Accession No. ICC 17164 JM 2106 and ICC 17121 JM 2100 was obtained from ICRISAT, Patancheru (AP) India for the present investigation. The seeds were divided into three sets. The seeds of 1st set treated with three different concentration viz. 0.1%, 0.2%, 0.3%,of Sodium azide (SA) and encoded as T_2, T_3, T_4 respectively. The seeds of 2nd set were treated with combination treatment of SA and X-rays radiation viz. 0.1% SA+5KR, 0.2% SA+10KR, 0.3% and SA +15KR and encoded as T_5, T_6, T_7 respectively. The healthy seeds were first treated with 0.1% to 0.3% SA 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 15 KR X rays. The seeds of 3rd set were treated with different doses 5 KR, 10 KR, 15 KR of X-ray radiation and encoded as T_8, T_9, and T_{10} respectively.While T_1 as the untreated control. The treated seeds were subjected for germination in the petriplates lined with two to three layers of moist filter paper. The 3-4 healthy and actively growing root-tips were excised on reaching the length about 1 to 1.5cm during the time interval of 10.00 am to 11.30 am. The slides were prepared by following standard squash technique (Sharma and Sharma, 1990). The 2% aceto-orcein stain was used in the present

ISSN 2348 – 7968

cytological study. The random 10 counts per slide were scored to cover maximum surface area of the slide for computing the Mitotic Index, standard error for each treatment. Mitotic index was determined for each treatment as Eq. No. (1) (Bhalla et al., 1973).The various mitotic abnormalities were observed in the present study. The data obtained was statistically analyzed as per Eq. No. (2) given by Panse and Sukhatme (1978). The mitotic index is percentage frequency of the dividing cells out of total cells scored.

$$\text{Mitotic index} = \frac{\text{Total No. of dividing cells}}{\text{Total No. of cells scored}} \times 100 \quad (1)$$

$$(\mu) \quad \bar{X} = \frac{\sum \bar{x}_i}{n} \quad (2)$$

Where, $\bar{X}(\mu)$ - mean, $\sum \bar{x}_i$ – Sum of 'i' observation, n- Number of observation.

3.Result and Discussion

The total normal and abnormal cells were scored to calculate the mitotic index for each treatment and presented in the table No. 1. The mitotic index was observed as decreased or depressed as compared to the untreated control treatment. The maximum mitotic index was observed as 7.775 in the untreated control and minimum mitotic index was recorded as 4.28 in the combined treatments of SA and x-ray.

In the present study all the treatment including independent and combined treatment has revealed the depressed mitotic index as compared to the control. The dose dependent increase mitotic aberration following chemical and physical mutagenic treatment has been reported in a fababean (Vandana and Dubey,1992a,b). The values of mitotic aberration in SA treatment has been reported as increasing in horsegram (Sirsat *et al.*,2010).The percent frequency of abnormal or aberrant cells has been reported as index of efficiency and effectiveness of mutagen (Kumar *et al.*, 2003). The chemical mutagen sodium azide has been reported as more effective (Sirsat *et al.*,2010). The various mitotic abnormalities or irregularities observed in the each treatments is represented in the table No. 2

Stickiness- it is most common abnormality observed in all the treatment and depicted in table No. 2. The frequency of this abnormality was more at metaphase. The abnormality might be attributed to the result due to cytochemically balanced reactions disturbances by the radiation effect (Jayabalan and Rao, 1987) while Tarar and Dnyansagar (1980a) reported it was due to depolymerization of nucleic acid caused by mutagenic treatment.

Polyploidy - It was observed more in all the treatment. The abnormality might be due to inactivness of spindle apparatus and failure of chromatid separation. The numbers of chemical have been reported as cytotoxic effect on spindle apparatus (Sharma and Sharma, 1990; Sudhakar *et al.*, 2001).

Ring formation – The ring formation was recorded in all the treatment. The clastogenic chromosomal aberration such as fragment, ring chromosome, which might be attributed to breakage and ring formation, might be due to the broken chromosome end (Kumar and Tripathi, 2003).

Chromatin bridge - The chromatin bridge was observed in all the treatment and the frequency is more in higher concentration of SA and x-ray. It might be attributed to the failure of chiasmata terminalization and chromosome would get stretched between poles (Saylor and Smith, 1966).

Laggard - The frequency of laggard was more in some of the independent mutagenic treatment and combined treatment. The occurrence of lagging chromosome might be attributed to the failure to carry the chromosome to respective pole (Tarar and Dnyansagar, 1980b). The fragment might be due to broken chromosome fragment fail to reunite with the chromosome (Kaur and Grover, 1985).

Micronucleus formation – The micronucleus was observed in the various treatments and mentioned in the table No.2. The micronucleus formation may be attributed to the failure of movement of lagging chromosome (Kumar and Dubey, 1998) fragment which fail to move toward the respective pole as well as the irregular distribution of acentric fragment (Bhattacharya, 1983).

Mutagenic potentiality of combined treatment of SA and gamma rays has been reported as more efficient than their individual ones in cowpea (Kumar and Verma, 2011).Similar observation have been reported in wild chickpea (Kamble and Patil, 2014).

4.Conclusion

SA and X-ray reveal the mitotoxic and clastogenic effect in the present study. Both mutagenic agents showed the mitodepressive activity independently and in combination treatment. The potential and effectiveness of mutagenic treatment was found to be X-ray >S A> SA + X- ray in present study.

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Ackowledgement

The authors is very much grateful to the UGC, New Delhi for providing financial assistance for the present study and due sincere thank to ICRISAT, Patancheru (AP)India for supplying the wild germplasm to the present study.

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Table No. 1: Mitotic Index and Standard Deviation of the dividing cells in Mitotic cell division in wild *Cicer reticulatum* L.

Sr. No.	Treatment	Total Number of Dividing cells Mean (Normal + Abnormal)	Total cells scored Mean	Mitotic Index Mean ± Standard Deviation (δ)
1	T_1	90	1158	7.78(±0.1)
2	T_2	71	1232	5.76(±0.02)
3	T_3	72	1276	5.64(±0.02)
4	T_4	62	1128	5.49(±0.02)
5	T_5	64	1345	4.75(±0.06)
6	T_6	64	1422	4.50(±0.04)
7	T_7	61	1423	4.28(±0.05)
8	T_8	69	1015	6.79(±0.02)
9	T_9	67	1027	6.52(±0.01)
10	T_{10}	68	1068	6.36(±0.02)

Table No.2 Various mitotic abnormalities and irregularities in wild *Cicer reticulatum* L.

Sr. No.	Treatment	Stickiness	Polyploidy	Ring formation	Chromatin bridge	Laggard	Micronucleus formation	Total
1	T_1	---	--	--	--	--	--	--
2	T_2	2	1	1	1	2	2	9
3	T_3	2	2	1	1	3	2	11
4	T_4	3	2	2	2	3	1	13
5	T_5	3	3	2	2	2	1	13
6	T_6	4	3	2	3	3	3	18
7	T_7	4	3	3	3	3	3	19
8	T_8	1	1	1	1	1	2	7
9	T_9	1	1	1	1	1	2	7
10	T_{10}	2	1	2	1	1	2	9

Mitotic Chromosomal Abnormalities in wild chickpea treated with SA and X-rays.

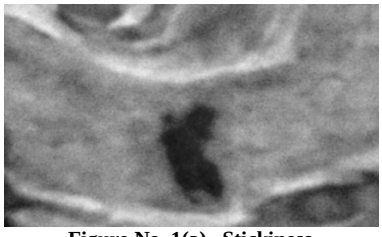

Figure No. 1(a). Stickiness

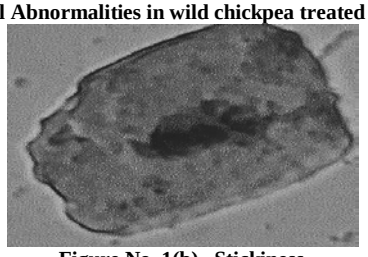

Figure No. 1(b). Stickiness

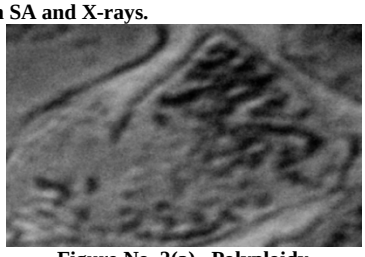

Figure No. 2(a). Polyploidy

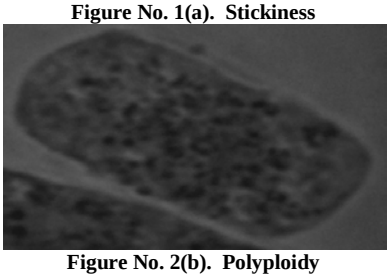

Figure No. 2(b). Polyploidy

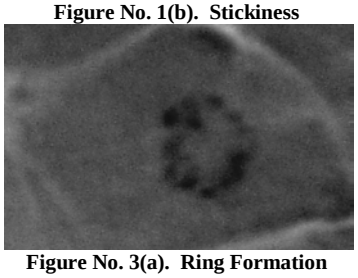

Figure No. 3(a). Ring Formation

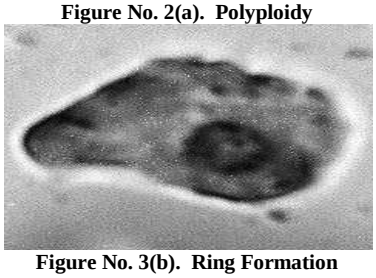

Figure No. 3(b). Ring Formation

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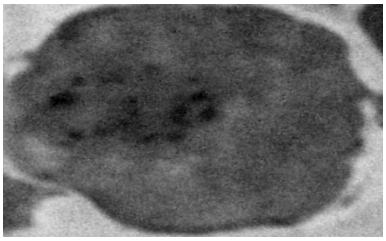


Figure No. 4(a). Chromatin Bridge

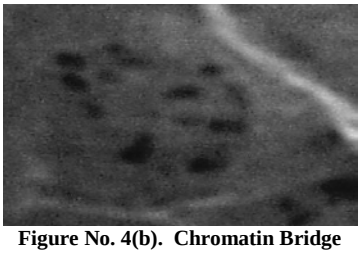


Figure No. 4(b). Chromatin Bridge

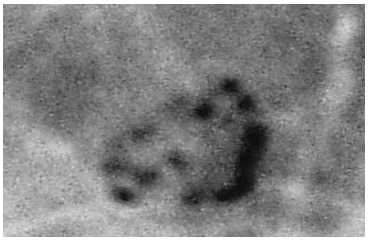


Figure No. 5(a). Laggards

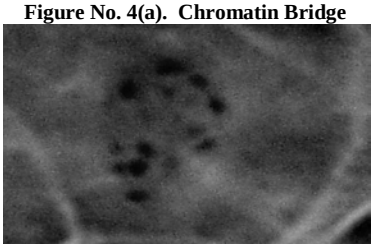
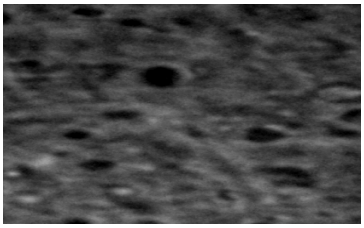


Figure No. 5(b). Laggards



FigureNo. 6(a). Micronucleus Formation

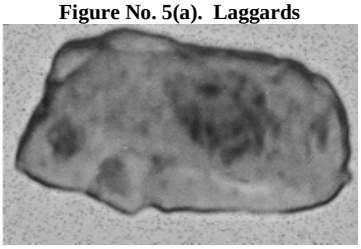


Figure No. 6(b). Micronucleus Formation