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EFFECT OF Co^{60} GAMMA RAYS ON PEARL MILLET

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Radiations are known to induce phenotypic, genotypic and cytological changes in different organisms. Among the induced cytological aberrations, chromosomal interchanges are the most common (Burnham 1956). Translocations have been widely used by various workers as gene markers, for determining the inheritance of economic characters and for crop improvement through various cytogenetical techniques. They have also been used in producing large chromosome rings and for creating new genetic recombinations.

Radiation induced translocations involving four or six chromosomes have been reported in pearl millet by Krishnaswamy and Ayyangar (1941), Burton and Powell (1966) and Pantulu (1958, 1967). The present study was carried out to assess the response of an inbred line of pearl millet to different doses of gamma rays in producing various chromosomal and other changes which can be further used in breeding programmes.

Material and Methods

Dry dormant seeds of BIL 4, an inbred line of pearl millet variety T. 55, were exposed to 15, 25, 35 and 45 Kr of Co^{60} gamma rays. The treated and untreated (control) seeds were sown in March 1968 in nursery in small rows and transplanted in the field after 30 days.

One hundred seeds in each treatment were grown in petri-dishes under laboratory conditions for germination and root tip studies. The observations on germination were taken after 8 days, the seedling height after 7 and 30 days and the survival in M_1 generation at maturity. The root tips from 3 day old seedlings were fixed in Carnoy's fluid and stained in basic fuchsin (feulgen) after hydrolysis in 1N HC1 at 60°C for 10 minutes and acetocarmine squashes made for roitotic studies.

Spikes of suitable stages were fixed in acetic-alcohol (1 : 3) and smeared in 1 per cent acetocarmine for meiotic studies. Chromosomal abnormalities were scored at metaphase and anaphase stages of mitosis and meiosis in the various treatments. Pollen stainability in different treatments was recorded by the acetocarmine glycerine method.

Results

Table I shows the percentage germination, seedling height and survival in different treatments of gamma rays and control.

Table 1

Germination, seedling height and survival in Coco treated pearl millet

Dose (Kr)	Germination Per cent	Seedling height 7 days Mean (cm)	30 days Mean (cm)	Survival Per cent
0	88.0	7.1	19.1	85.2
5	81.0	8.3	31.5	80.2
15	83.0	5.3	22.7	72.3
25	87.0	6.1	19.9	59.8
35	73.0	1.2	11.0	37.0
45	81.0	1.9	9.4	48.1

Germination was slightly reduced in the irradiated seeds, the percentage reduction in germination over the control being 1.1 (25 Kr) to 17.0 (35 Kr). Height of 7 day old seedlings was reduced in all treatments except in the lower dose (5 Kr) where an increase of 16.9 per cent over the control was observed. However, after 30 days the height of the seedlings increased over the control in case of 5, 15 and 25 Kr treatments. The higher doses of 35 and 45 Kr showed a reduction in height of 42.4 and 50.8 per cent respectively over the control. Survival at maturity was markedly low in irradiated populations as compared to control, the maximum reduction being 56.6 per cent in 35 Kr.

Mitosis: Root tip studies revealed various types of chromosomal abnormalities in different treatments (Table 2).

Table 2

Mitotic abnormalities in Coco treated pearl millet.

Type of abnormality	Frequency of cells showing abnormality in 0 Kr	5 Kr	15 Kr	25 Kr	35 Kr	45 Kr
Ring chromosomes	0	0	0	0	0	-
Fragment	0	2	0	[illegible]	4	8
Bridge	0	0	1	3	7	6
Fragment + Bridge	0	0	0	0	0	1
Laggards	0	0	0	0	1	2
Unequal distribution	0	0	1	0	0	1
Aneuploid	0	0	2	2	0	1
Number of aberrant cells	0	1	4	[illegible]	12	22
Number of normal cells	291	280	402	180	194	339
Total number of cells	291	282	406	191	206	361
Percentage of aberrant cells	...	0.71	0.98	5.76	5.82	6.09

Fragments and/or bridges were of common occurrence (Figs. 1 and 2). Mostly, the aberrant cells showed a single bridge; rarely interlocked and criss-cross bridges were seen in 35 Kr and 45 Kr. Ring chromosomes resembling the meiotic metapbase chromosomes (bivalents) were observed in 45 Kr (Fig. 3). Aneuploid and lagging chromosomes were also seen in a few cells. The chromosome abnormalities taken together exhibited a linear relationship with the dose. highest frequency of aberrant cells was in 45 Kr being 6,09 per cent. Lower doses (5 Kr and 1 Kr) of gamma rays caused even less than 1 per cent of the cells to be aberrant.

Meiosis : The pollen mother cel (PMC) studies were made from randomly seleeted M_1 plants. Meiosis in BIL 4, was normal showing seven bivaleats (Fig. 4) at diakinesis. Univalents, bivalents, trivalents, quadriva'.ents and hexavalents occurred with a varied frequency in different treatments of radiation. Table 3 shows the percentage of cells showing various abnormalities at metaphase. The highest association recorded was a hexavalent (Fig. *5) in* one plant grown from seeds exposed to 25 Kr. Both ring and chain type hexavaients were seen. Among the observed chromosome associations, quadrivalents were of most frequent occurrence (Fig. 6). There was no cell showing any interchange in 5 Kr (Table 3). In 15 Kr and 25 Kr treatments, cells with two separate rings of four chromosomes were observed (Fig.7). Trivalents along with quadrivalents were also recorded in a few cells (Fig. 8). The percentage of cells showing interchanges was maximum in 15 Kr (64.9) followed by 45 and 25 Kr radiations, where the observed values were 52.9 and 52.1 respectively. Maximum abnormalities were recorded in the highest dose (45 Kr).

Table 3

Meiotic abnormalities at metaphase in Co^{60} treated pearl millet

Dose (Kr)	Number of cells studied	Percentage of cells			Total aberrant cells (%)
		7 II	Inter-* changes	Others**	
0	294	97.4	0	2.6	2.6
5	281	96.1	0	3.9	3.9
15	388	26.6	64.9	8.3	73.2
25	409	42.8	52.1	5.1	57.2
35	294	48.6	34.1	17.3	51.4
45	289	24.6	52.9	22.5	75.4

* include configurations like trivalents, quadrivalents and hexavaients

** include univalents, fragments, micronuclei etc.

Table 4 shows the percentage of plants showing interchanges and other abnormalities in different treatments of radiation. Percentage of plants showing interchanges was maximum at a dose of 25 Kr (21%). No interchange was recorded in 5 Kr, but frequency of plants showing other abnormalities was maximum, being 15.6 per cent.

Table 4

Meiosis as affected by gamma rays in Co^{60} treated pearl millet

Dose Kr	Percentage of plants showing			
	7II	IV	VI	Other abnormalities
0	97.8	0	0	2.2
5	84.4	0	0	15.6
15	85.7	8.6	0	5.7
25	72.0	17.6	3.4	6.9
35	81.8	10.1	0	9.1
45	86.4	9.1	0	4.5

Anaphase studies revealed abnormalities such as laggards, bridges, fragments, late separation, micronuclei and unequal distribution of chromosomes in the various treatments (Table 5). Control cells showed normal anaphases. Among the aberrant cells, laggards followed by unequal distribution of chromosomes occurred with the highest frequency. Figures 9 and 10 show cells with bridge and unequal distribution chromosomes on either side of the poles at anaphase I. Fragments occurred only in higher doses of gamma radiation. Micronuciei were seen in a few cells. The percentage of aberrant cells varied from 4.88 (5 Kr) to 16.30 (45 Kr). Table 5 gives the pollen fertility as determined by the aceto-carmine stainability method. It was low in all treatments as compared to control, the lowest being 35.85 per cent at 45 Kr as compared to 85.11 per cent in control.

Table 5

Frequency of anaphase abnormalities (meiotic) and pollen stainability in Co^{60} treated pearl millet

Type of abnormality	Frequency of cells in					
	0 Kr	5 Kr	15 Kr	25 Kr	35 Kr	45 Kr
Laggard	0	11	20	55	39	52
Unequal distribution	0	5	13	15	20	41
Bridge	0	2.	7	14	18	8
Fragment	0	0	0	2	4	1
Bridge and Fragment	0	1	0	0	2	0
Laggard and Bridge	0	0	0	2	1	5
Micronuciei	0	2	0	0	0	5
Number of aberrant cells	0	21	40	88	84	112
Number of normal cells	406	409	371	527	652	575
Total number of cells	406	430	411	615	736	687
Percentage of aberrant cells	0	4.88	9.73	14.31	11.41	16.30
Percentage pollen stainability	85.11	78.02	70.13	66.91	43,14	35.85

Discussion

The effects of a mutagen are usually measured by certain parameters such as percentage germination, seedling height survival at maturity, chromosomal aberrations, pollen and seed fertility, morphological changes in M_1 and the mutation frequency in M_2. During the present study, the maximum reduction in germination over the control was only 17.0 per cent in 35 Kr, which was rather low. Similar results were reported by Kaukis and Webster (1956) and Wu and Pi (1968) in sorghum. The growth of the seedlings was found to be stimulated at lower doses of the radiation and suppressed at higher doses. Similar responses were reported earlier by Kaukis and Webster (1956) and Wu and Pi (1968) in sorghum and by Jagathesan and Sastry (1963) in cotton. Sharma and Boyes (1962) reported stimulation of seedling growth in buck wheat. Johnson (1936) reported growth acceleration in X-ray treatments in sub-lethal doses while suppression of the vegetative activity was recorded by Gunckel and Sparrow (1953).

The reduction in the survival at maturity of the plants due to irradiation treatments observed in the present studies have parallels in earlier observations of workers in this field. For example, Wu and Pi (1968) observed such reduction in sorghum after X-ray treatment.

The chromosomal fragments, bridges and laggards were the most common abnormalities recorded at various stages of mitosis in the present studies. Such changes were reported earlier by Gelin (1941) in barley and Kaukis and Webster (1956) in sorghum. Occurrence of ring chromosomes observed at the highest dose has a parallel in rice as observed by Korah (1958) after X-ray treatment.

At metaphase 1 of meiosis, translocations involving 4 to 6 chromosomes were common in different treatments of gamma rays.

Caldecott and Smith (1952) in barley observed rings of four chromosomes in 371 spikes out of a total of 3509 spikes examined. Patil and Bora (1961) also observed large number of reciprocal translocations after X-irradiation leading to the formation of chains and ring quadrivalents in *Arachis hypogaea*. They further observed bridges at anaphase I and anaphase II. The highest association in the present study was a ring chain of six chromosomes in one plant (25 Kr). Krishnaswamy and Ayyangar (1941) reported chromosomal aberrations in 3 plants of pearl millet in a population raised from X-rayed seeds. They reported ring and chain of four chromosomes and in one plant a ring of six chromosomes. In the ring forming cells unequal distribution of chromosomes was observed. Pantulu (1958, 1967) also recorded chromosomal interchanges in pearl millet after irradiation.

In the present study anaphase abnormalities like unequal distribution of chromosomes on either side of the poles, laggards, bridges were common. Such abnormalities have also been reported by Wu and Pi (1968) in sorghum after X-irradiation. In pearl millet also, Krishnaswamy and Rangaswamy (1941) reported such abnormalities in X-ray treated population.

Pollen sterility was found to be higher in different irradiated treatments as compared to that of control. These findings are in agreement with Wohrmann (1955) who reported positive relationships between doses and increased pollen sterility in *Alopecurus pratensis.*

The irradiation of pearl millet with Co^{60} gamma rays evidently reduced survival and pollen fertility. The effect of irradiation on germination was not very large. The higher doses of gamma rays induced interchanges in the chromosomes and also other mitotic and meiotic abnormalities.

Summary

The irradiation of seeds of [illegible] 4, an inbred line of pearl millet, with various [illegible] f Co^{60} gamma rays reduced survival of the plants and their pollen fertility. There was a stimulating effect on plant height recorded after 7 days and 30 days of germination with lower doses whereas the effect was inhibitory in case of higher doses. A number of chromosome abnormalities were induced in mitosis as well as in meiosis after irradiation. The higher doses of gamma rays induced interchanges in chromosomes which have basic applied implications in crop improvement.

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Fig. 7. Meiotic cell showing
3II + 2IV

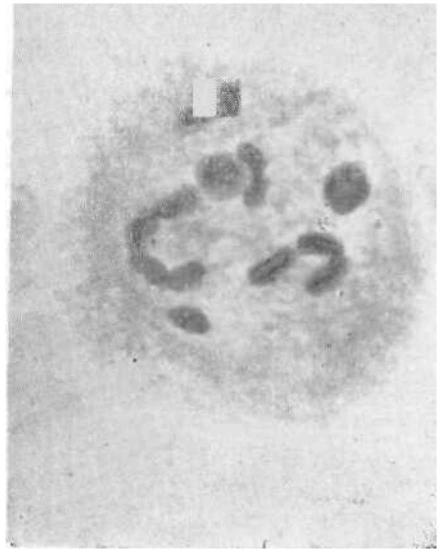

Fig. 8. Meiotic cell showing
1I + 3II + 1III

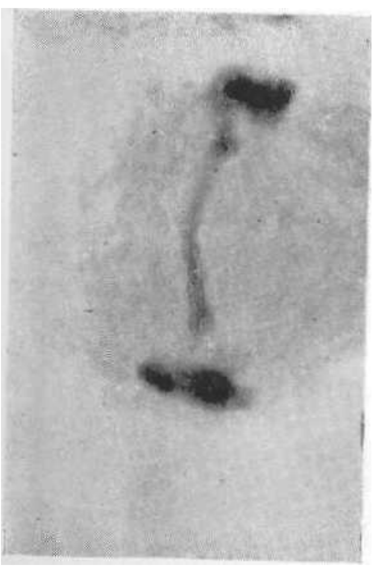

Fig. 9. Meiotic cell showing
anaphase bridge

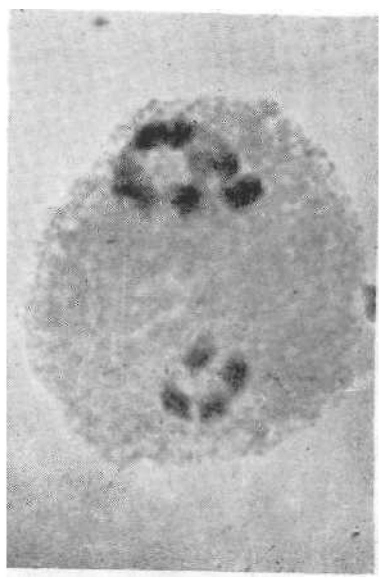

Fig. 10. Meiotic cell showing
unequal distribution of chromo-
somes at anaphase I

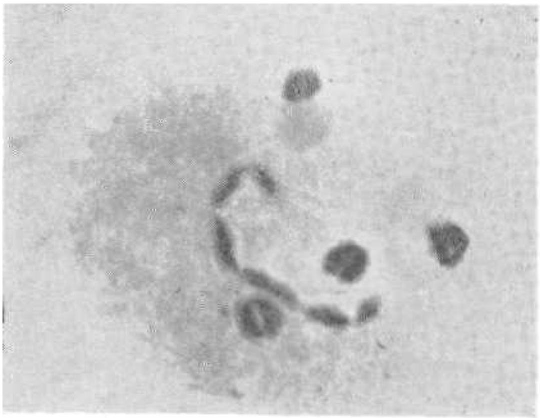

Fig. 5. Meiotic cell showing **4II** + 1VI

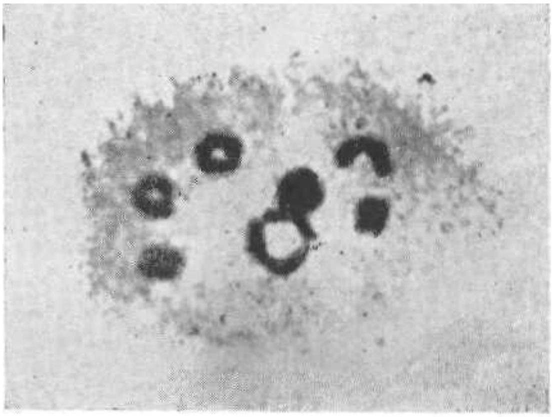

Fig. 6. Meiotic cell showing **5II** + 1IV

Fig. 1. Somatic metaphase showing fragments

Fig. 2. Somatic cell showing anaphase bridge

Fig. 3. Somatic metaphase showing ring shaped chromosomes

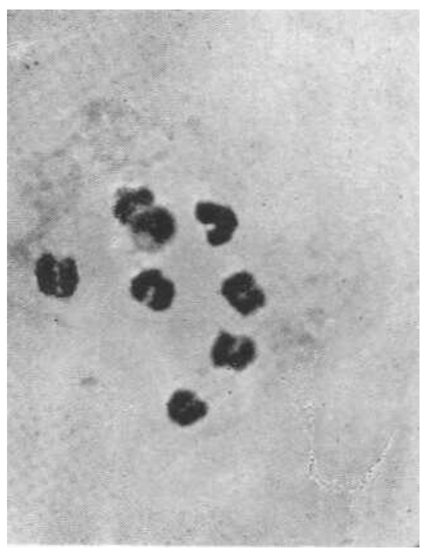

Fig. 4. Diakinesis in BII-4 showing 7II

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