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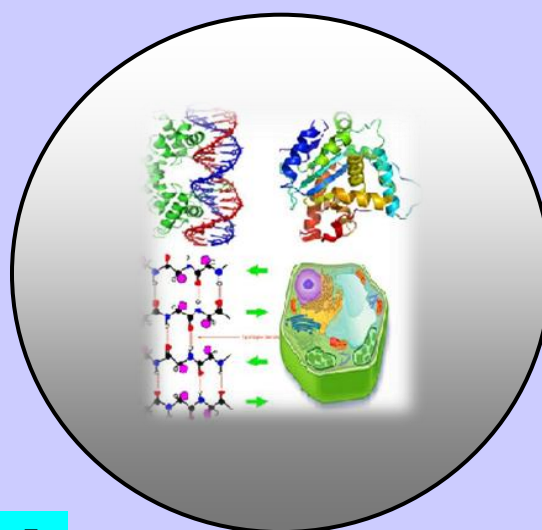
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Effects of Gamma Rays on the Root Tip Mitosis of *Jatropha curcas* L.

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ABSTRACT

Jatropha curcas L. is a well-established petro crop. The seed oil of *Jatropha curcas* known as 'curcas oil' can be used as substitute for diesel engine fuel. Under the crop improvement program, the seeds of *J. curcas* were treated with different doses of gamma rays. Due to the presence of high concentration of latex it's very difficult to carry out the studies on root tip mitosis of *Jatropha curcas*. In order to get the optimum fixation time the mitotic index of *J. curcas* was determined starting from 6am to 6 pm at 2 hrs interval. As per the mitotic index study, the most appropriate time for fixation of the *Jatropha curcas* root is 8 am. After gamma ray treatment various type of chromosomal aberrations were observed. The maximum chromosomal aberration was observed in 18 Kr gamma ray treated population.

Keywords: *Jatropha curcas* L., Gamma Rays, Mitosis and Chromosomal Aberration.

INTRODUCTION

Jatropha curcas L., a tropical species has naturalized in India in several areas and is generally cultivated as a hedge around cultivated field in a semi-wild condition. It was introduced in India by Portuguese as an oil yielding plant. It is known as physic nut and purging nut in English. Different names are given in different states of India and in different countries. It is a shrub or small tree with stem up to 20 cm. diameter and 6 meter height. Branches are stout, fleshy and glabrous. Bark is smooth, pale greenish or light ash-color, wood soft and spongy. Leaves are alternate, petiolate, ovate-rounded, widely cordate at the base, five-angled and smooth. Flowers are protogyne i.e. female flowers mature first than male flowers. Flowers in loose panicles of auxiliary cymes, yellowish green, peduncles and pedicels more or less tomentose. The male flowers at the extremities of the ramifications, on short, articulated pedicels and the female ones in their divisions, with their pedicels not

articulated. Female flowers are hypogynous, ovary tri-carpillary, syncarpous, one anatropous ovule in each locule with axile placentation. Pollination is entomophilous type. Capsules ellipsoid, scarcely lobed. Fruit is long, dull dark brown or black, ovoid-oblong, breaking up into three two-valved cocci, 2.4-2.7 cm. long, seeds are normally three ovoid-oblong, 0.5- 0.6 gm. in weight, 1.6-1.7 cm in length and 1.0-1.1 cm. in diameter. The chromosome number of *J. curcas* is $2x = 22$. No regular seed collection has been done for any such uses in our country. The seed oil productivity is very low at present since it has never been improved for oil crops and the basic knowledge for its domestication is quite limited. Systematic efforts have not been made up till now for better cultivation and for increase in production by using present day knowledge of plant breeding and increasing agronomic experience. The characterization of 'curcas oil' and the utility research to decide on its alternative industrial application, and the overall plant productivity and yield figures to develop economically feasible husbandry of *Jatropha* has not received due attention.

Tests were made with diesel engines using *Jatropha curcas* L. oil as part of a research project carried out with the co-operation of Yanmar (Thailand) Co. Ltd. and very satisfactory results were obtained. The engine performance and fuel consumption compared favourably with running the engines on normal diesel engine oil (Forson *et al.* 2004, Takeda 1982). Utilization of *Jatropha curcas* oil as a new source of oil for diesel engine is well established and a lot of published literatures are available to answer the many specific questions about its production and commercialization.

Mitosis is a process of nuclear division in eukaryotic cells that occurs when a parent cell divides to produce two identical daughter cells. During cell division, mitosis refers specifically to the separation of the duplicated genetic material carried in the nucleus. The mitotic index is simply a measurement to determine the percentage of cells undergoing mitosis. Mitosis may also be defined as division of somatic cells when genetic information from one single cell is equally dispersed into two daughter cells. Durations of the cell cycle and mitosis vary in different cell types. An elevated mitotic index indicates more cells are dividing, and thus obvious in cancer cells. The mitotic index may be elevated during necessary processes to life, such as the normal growth of plants or animals, as well as cellular repair at the site of an injury.

MATERIAL AND METHODS

Dry seeds with pre-determined moisture content were treated with 6, 12 and 18 Krad gamma rays (^{60}Co radiation source) with a dose rate of 14.7 second/ Krad in National Botanical Research Institute, Lucknow. Cytological preparation of *Jatropha curcas* is difficult due to presence of mucilaginous substance and latex on the surface of root tips. Attempts were made to standardize the method for staining chromosome in root tip cells. 1.5-2.0 cm long root tips were cut from the roots and the mucilaginous substance was removed by rubbing with the finger and thoroughly washed with water. Then the root tips were kept in mouth for bathing in saliva for 5 minutes. The root tips were washed again and pre-treated in a mixture of saturated aqueous solution of para-di-chloro benzene and asclic acid (1:1 v/v) for 12 minutes in an ice chamber and then at 4°C for 2 hrs. The root tips were then fixed in modified Carnoy's fluid (Chloroform: Acetic acid: Alcohol: 1:1:3, v/v) for 12 hrs (Sharma and Sharma 1965). Root tips were washed and hydrolyzed in 1N HCL at 60°C for 14 minutes and stained in leucobasic fuchsin for 2 hrs.

In order to determine the mitotic index, root tips were fixed between 6 am to 6 pm at the interval of 2 hours. The temporary preparations for cytological studies were made permanent, whenever required. The cover glass was removed and mounted in Canada balsam following dehydration steps with n-butyl alcohol.

Mitotic index was calculated by following formula-

$$\text{Mitotic Index} = (P+M+A+T/N) \times 100$$

(P+M+A+T) — The sum of all cells in phase as prophase, metaphase, anaphase and telophase, respectively; N — total number of cells.

RESULT AND DISCUSSIONS

Somatic chromosome number of *J. curcas* was found to be $2n=22$ in root tip cells. The chromosomes were very small (Fig-1). The interphase nuclear volume and interphase chromosomal volume were $427.27 \mu\text{m}^3$ and $19.42 \mu\text{m}^3$ respectively.

After gamma irradiation different type of chromosomal abnormalities like early- and late separation, bridges, clumping and laggards were recorded during root tip mitosis (fig-2). The percentage of cells with chromosomal aberrations increased with increase in gamma ray doses (Table-2). There was no cell with change in ploidy level after exposure to gamma rays. Radiation induced chromosomal studied carried out extensively by large number of workers in different crop plants (Datta *et al.* 1991, Eroglu *et al.* 2007, Kumar and Srivastava 2010, Rahimi and Bahrani 2011). Radiation induced chromosomal aberration aspect is known for long time but present study on chromosomal aspects after treatment with gamma rays and colchicine was for the first time and has been considered to be necessary for including *J. curcas* as a radiation genetic programme with the aim to increase its genetic variability by mutation.

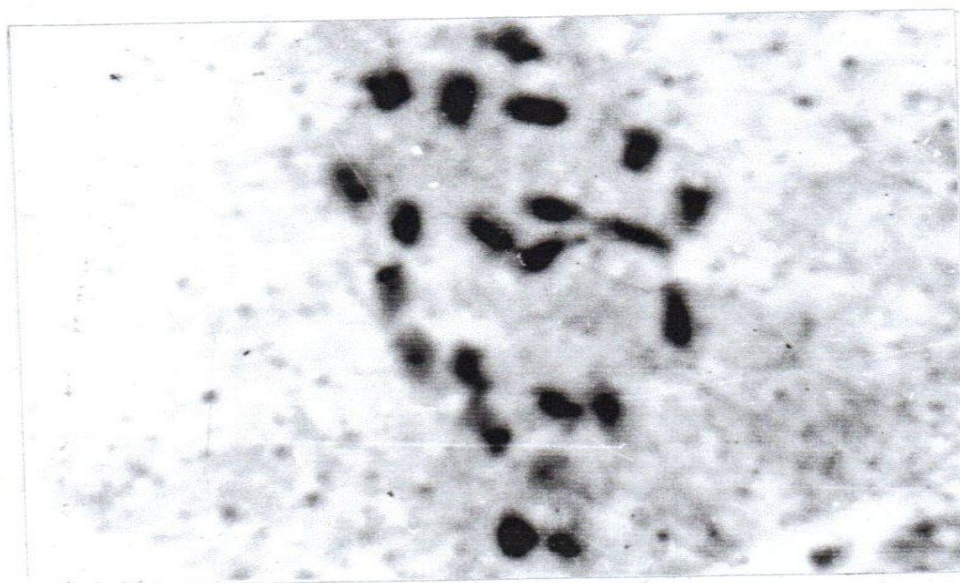


Fig 1. Chromosomes of *Jatropha curcas*

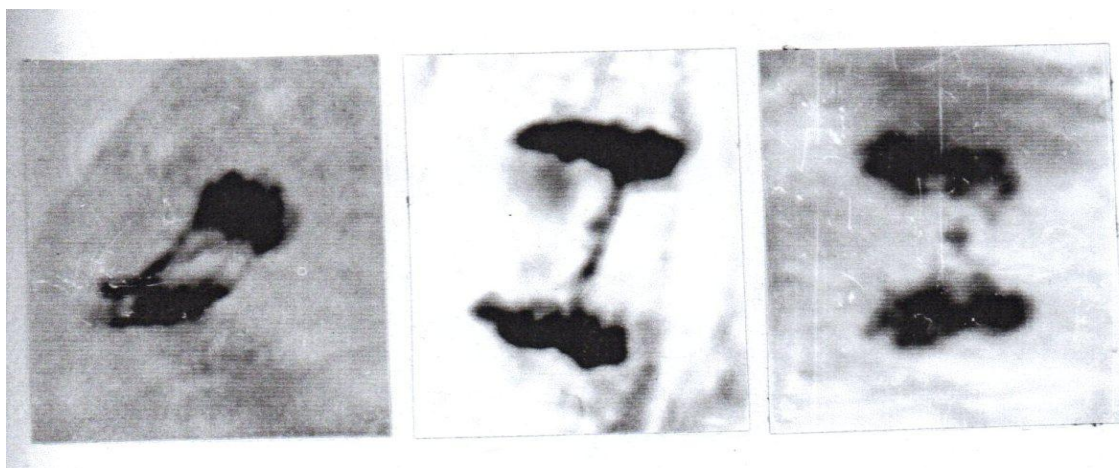


Fig 2. Chromosomal aberrations during root tip mitosis in *Jatropha curcas*.

Table 1. Mitotic Index, during root tip mitosis of *Jatropha curcas* at different fixation time.

Time	Total cells studied	%Dividing cells				Mitotic Index
		Prophase	Metaphase	Anaphase	Telophase	
6 hrs.	1190	5.13	2.35	0.76	0.00	8.24
8 hrs.	1137	12.67	3.25	1.23	0.17	17.32
10 hrs.	1100	2.81	7.72	1.09	0.18	11.81
12 hrs.	1158	0.34	2.16	2.50	1.38	6.39
14 hrs.	1232	0.16	1.21	0.32	1.29	2.99
16 hrs.	1293	0.00	2.17	0.85	0.93	3.94
18 hrs.	1242	0.00	2.09	0.56	0.64	3.30

Table 2. Effect of gamma rays during root tip mitosis of *Jatropha curcas*.

Chromosomal aberrations (%)	0 (Control)	Gamma rays (Krad)		
		6	12	18
Early separation	0	0.31	0.69	0.50
Late separation	0	0.00	0.00	0.17
Bridges	0	0.12	0.28	0.68
Clumping	0	0.06	0.97	0.84
Laggards	0	0.12	0.00	0.08
Total	0	0.63 ±0.21	1.94 ±0.34	2.29 ±0.43
Total cells studied	1380	1580	720	1181

Increase in chromosomal aberrations with increase in doses of gamma rays has already been reported by Kumar and Srivastava 2010 in safflower. In this experiment maximum chromosomal abnormality was observed 2.29% in 18 Krad gamma ray treated set. The most frequent chromosomal aberrations encountered were clumping. Due to the presence of latex and mucilaginous substances cytological studies in *Jatropha curcas* not easy hence an attempt was being made to study the mitotic index of *Jatropha curcas* from 6 am to 6 pm with interval of 2 hrs. Mitotic index was maximum in the roots fixed at 8 am and was minimum in the root tips fixed at 2 pm (Table 1). By the above experiment it is clear that most appropriate fixation time for root tip mitotic studies of *Jatropha curcas* is 8 am morning.

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