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Cytology studies in germplasm of Jatropha (*Jatropha curcas* L.)

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ABSTRACT: Chromosomes numbers and pollen studies were done in 27 germplasm accessions of *Jatropha curcas*. The analysis of gametic cells indicated that the species exhibits 2n=22 chromosomes. The chromosomes were paired in bivalents at first metaphase. At late pachytene, just before the transition to diplotene, chromosomes disjoin from their typical pericentromere clustering at zygotene and early pachytene, which makes this an optimal stage for meiotic chromosome pairing studies. Metaphase I was regular as expected, showing eleven bivalents with clear, stretched centromeres facing the poles, with each bivalent containing one chiasmata. Anaphase I/II showed balanced segregation with chromosome chromatid numbers of 11+11. The normal pollen mother cell formation ranged from 90.32-100%. Consequently, pollen fertility was also very high and ranged from 95-100%. The results suggest that the species is highly fertile and stable in its natural habitat. Accessions namely NRCJ-1, NRCJ-2, NRCJ-3, NRCJ-8, NRCJ-23 and NRCJ-25 showed up to 99% fertility. The pollen viability of accessions ranged from 92-96%. Accession NRCJ-7 was recorded for 98% pollen viability. These results suggest that pollen viability is not a limitation for seed development in *Jatropha curcas*. Cytological studies suggested that *Jatropha curcas* is fully fertile and stable at various geographical ranges where it has been naturalized.

Key words: Chromosomes, *Jatropha curcas* L., meiosis, pollen fertility, pollen viability.

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1. INTRODUCTION

Energy independence is one of the vital areas to make India a developed nation. Among different sources of energy, bioenergy has to play a great role as the end of fossil fuel age has already begun (Singh *et al.*, 2006). Among the different sources of bioenergy, plant route is considered most promising because of its renewable nature. Many developed countries are using edible oil-seed crops such as soybean, rapeseed, groundnut, sunflower for production of biodiesel. However, developing countries like India, having dearth of huge quantity of edible oil (6.31 million tonnes) for consumption, cannot afford to use edible oils for bio-diesel production and hence non-edible oil seeds such as Pongamia (*Pongamia pinnata*) and Jatropha (*Jatropha curcas* L.) are explored along with meeting additional criteria of greening the wastelands without compromising the food, fodder security and improve livelihoods (Reddy *et al.,* 2008).

Commonly known as Physic nut, *J. curcas* is a multipurpose shrub tree of significant economic importance. It is known by nearly 200 different names such as Chandrajot, Jungli arandi, Kala arand, Safed arand, Pahari arand etc., indicating its significance and various possible uses. Its capacity to rehabilitate degraded or dry lands makes it suitable for up gradation of land resources. Like all trees, *J. curcas* removes carbon from the atmosphere, stores it in the woody tissues and assists in the buildup of the soil carbon. With the combination of oil production and erosion control and the ability to grow in marginal areas with poor soil and low rainfall, this species has great potential in rural development as a source of house hold income and at the same time creating environmental benefits (Singh *et al.,* 2006). Cytological information from chromosomes is of great value to those researchers aiming to conserve genetic resources and biodiversity. Since *Jatropha curcas* can grow in extreme environmental conditions the cytological studies may lead to identify accessions having distinct characters & knowledge of the pollen viability will help in understanding the pollination and fertilization of the plant. Therefore present study was taken to investigate basic

chromosomes number pairing pattern, pollen viability and fertility in *Jatropha curcas* L. germplasm.

2. MATERIALS AND METHODS

The plant material (floral buds) for cytogenetic studies was collected from 27 accessions of *Jatropha curcas* growing at the experimental farm of National Research Centre for Agroforestry, Jhansi. These accessions were collected from different part of India *viz*. Madhya Pradesh, Gujarat, Rajasthan, Maharashtra, Uttar Pradesh, Andhra Pradesh, and Chhattisgarh. Young buds were collected between 6.00h to 9.00h each day and fixed in 5ml viols containing cornoy's fluid (3:1 ethanol and acetic acid) for at least 12 hours. The next day, the anther were washed three times in distilled water and stored in 70% ethyl alcohol. Squash preparations were made in 1% acetocarmine (Sharma and Sharma, 1980). The acetocarmine stain prepared from carmine. Carmine is a basic dye that is prepared from the insect *Coccus cacti*. For meiotic studies, 10 g carmine was dissolved in 1 L of 45% glacial acetic acid, boiled, and refluxed for 24 h. The solution was then filtered into dark bottles and stored at 4°C. This solution can be stored for a long time. To intensify the staining, 5 mL of a 10 % ferric chloride ($FeCl_2 \cdot 6H_2O$) solution per 100 mL of % acetocarmine was added.

For staining of chromosome 1 % solution of acetocarmine was used. Freshly fixed pollen mother cells (24-48 hours) were transferred into 1% acetocarmine for at least 30 minutes and then analyzed by the squash method. Preserved material for a long time required a long staining time (up to several days) to reach good contrast. Excess fixative was drained and the material was kept in 1% acetocarmine for 1 to 3 h. It was heated until the acetocarmine began to boil. Anthers were squeezed out on a slide and tapped to bring out pollens mother cells and then a covered with cover slip. Chromosomes were observed under the microscope. At the diakinesis stage of pollen mother cells, the slides were covered with cover slip and were gently heated and the cover slip was pressed down to spread the chromosome. Suitable cells were photographed using the Olympus trinocular microscope.

Pollen studies were done on collected pollen. Pollens were collected in early hours of morning by shaking an inflorescence on a clean sheet of paper, pouring the dry pollen into a 1.5 ml micro centrifuge tube and covering the pollen with 0.5ml 70% ethanol. Samples were stored in a refrigerator at 4°C. Data was recorded for following traits:

The collected pollens were suspended in the ethanol solution by shaking the micro centrifuge tube. 10 µl of the acetocarmine solution was added to the 5 µl of pollen suspension and mixed. The stained suspension was placed on a microscope slide and examined at 40X with a high resolution-dissecting microscope. Pollen staining red were scored as fertile and that staining light was scored as non-fertile.

Freshly collected mature pollen grains were dusted on a drop of 0.5% TTC or TTZ/TZ (2, 3, 5 triphenyltetrazolium chloride) in sucrose solution and incubated in a humidity chamber at room temperature in dark for 30 minutes. Then they were observed under the microscope and pollen grains which stained red were scored as viable. The viable pollen grains which fluoresced were counted and the pollen viability percentage was calculated by the formula given below.

Pollen viability percentage = Number of viable pollen x 100/ Total number of pollen

3. RESULTS AND DISCUSSION

All the accessions were found to have the same base number of X=11. The size of the chromosomes varies from 1-4µm. The same chromosomal number has been also reported by Soontornchainaksaeng and Jenjittikul (2003) and Carvalho *et al.,* (2008). Eleven numbre is one of the basic chromosome numbers found in the genus Jatropha (Soontornchaineksang and Jenjittikul, 2003). In a preparation containing the pollen mother cells of a single anther, meiocytes ranging from the early prophase- I to anaphase- II stages could be found and focus was made on the following three stages for analyses: i) late pachytene for assessing chromosome pairing; ii) diakinesis / metaphase I to verify chiasma formation and orientation of the bivalents / univalents in the equatorial plane; iii) anaphase I / II for the segregation of the chromosomes to the poles. For the interpretations of meiotic stages, observations were compared with the morphology of meiotic stages of Jatropha as described by Soontornchaineksang and Chaiyasut (1999). Furthermore, pollen grains were analyzed to assess

uniformity, size and viability of the male gametes. The chromosomes were paired in bivalents at first metaphase. At late pachytene, just before the transition to diplotene, chromosomes disjoin from their typical pericentromere. Clustering at zygotene and early pachytene makes this an optimal stage for meiotic chromosome pairing studies. Metaphase I was regular as expected, showing eleven bivalents with clear, stretched centromeres facing the poles, with each bivalent containing one chiasmata. Anaphase I/II showed balanced segregation with chromosome chromatid numbers of 11+11. Meiotic configuration of *Jatropha curcas* is 7ringII + 4rodII. The meiotic chromosomes of *Jatropha curcas* are very small. The bivalent length ranges from 1-4 µm. The lack of information on chromosomal morphology in Jatropha could be attributed to the small chromosome size. The 27 accessions of Jatropha had a low frequency of meiotic abnormalities. The normal PMC formation ranged from 90.32-100%.

Pollen was regularly shaped with almost 100% viability (Table.1) and pollen fertility was also very high. The results suggest that the species is highly fertile and stable in its natural habitat. Pollen fertility test was done in 1% acetocarmine solution. Mean germination percentage was 97.3%. Accessions namely NRCJ-1, NRCJ-2, NRCJ-3, NRCJ-8, NRCJ-23 and NRCJ-25 showed up to 99% fertility (Table.1). Pollen viability test was carried out under laboratory conditions with TTC salt (2, 3, 5 triphenyl tetrazolium chloride) in sucrose solution. The viability of *J.curcas* accessions ranged from 92-96%. Accession NRCJ-7 was recorded for 98% pollen viability. These results suggest that pollen viability is not a limitation for seed development in *J.curcas*. Adhikari and Campbell (1998) reported that pollen viability is strongly influenced by temperature, moisture, genotypic differences, plant vigor and physiological stage, and flower age. Here, the differential responses of pollen viability can be ascribed to the genotype, since temperature and moisture conditions were controlled and the male inflorescences were sampled at the same physiological stage.

Table1: Cytological observations in pollen mother cells of *Jatropha curcas* L.

S.No.	Accession No.	No. of PMCs analyzed	Normal PMCs/cell			Mean	Pollen fertility (%)	Pollen Viability (%)
1	NRCJ 1	1200	100.0	100.00	99.0	99.67	99%	97%
2	NRCJ 2	1150	100.0	98.11	99.07	99.06	99%	96%
3	NRCJ 3	1380	99.20	100.00	98.06	99.08	99%	96%
4	NRCJ 4	1023	97.21	98.38	99.11	98.23	98%	97%
5	NRCJ 5	1036	99.20	98.88	97.76	98.61	98%	96%
6	NRCJ 6	1123	98.20	98.97	96.53	97.9	96%	97%
7	NRCJ 7	1242	97.51	95.99	95.26	96.25	95%	98%
8	NRCJ 8	1022	99.51	99.19	100.0	99.57	99%	96%
9	NRCJ 9	1123	93.62	95.02	95.28	94.64	95%	96%
10	NRCJ 10	1221	90.32	91.23	92.24	91.26	93%	95%
11	NRCJ 11	880	99.65	99.65	99.26	99.52	99%	97%
12	NRCJ 12	1288	97.32	96.25	96.81	96.79	97%	96%
13	NRCJ 13	1128	98.21	98.63	98.97	98.60	98%	95%
14	NRCJ 14	1067	96.52	96.51	98.72	97.25	97%	95%
15	NRCJ 15	1196	99.31	99.23	97.59	98.71	97%	97%
16	NRCJ 16	1096	97.22	96.92	97.89	97.34	96%	96%
17	NRCJ 17	1472	98.22	98.18	98.81	98.40	98%	95%
18	NRCJ 18	1313	100.0	100.0	97.52	99.17	98%	97%
19	NRCJ 19	1461	96.49	96.53	98.16	97.06	98%	96%
20	NRCJ 20	1334	95.25	92.16	93.79	93.73	95%	95%
21	NRCJ 21	1300	95.31	95.26	96.01	95.57	96%	94%
22	NRCJ 22	1008	97.62	97.12	97.2	97.31	97%	91%
23	NRCJ 23	1206	100.0	99.91	98.99	99.63	99%	92%
24	NRCJ 24	1121	98.11	98.36	98.23	98.23	98%	93%
25	NRCJ 25	1420	99.12	99.29	99.20	99.20	99%	93%
26	NRCJ 26	1321	98.63	98.12	98.10	98.28	98%	96%
27	NRCJ 27	1023	98.32	98.63	99.01	98.65	98%	92%
Mean		1190.89	97.78	97.65	97.65	97.69	97.3%	95.3%

Cytology is one of the most important branches of biology as it deals with study of cells. Meiotic studies are relevant, since they focus on details of pairing behaviour of chromosomes, recombination frequencies and their pattern of disjunction during anaphase I and II which are not deducible from mitotic studies (John and Lewis, 1965). Meiosis is an integrated process consisting of a series of events which have been shown to be under genetic control (Golublovskaya, 1979). Normal meiosis leads to the formation of functional gametes, where as an irregular meiotic division leads to abnormalities and thereby to sterility (Del-Duca, 1976; Fernandes, 1982). Considering the economic importance that *Jatropha curcas L.* promises as a biodiesel plant, the scarce data referring to its meiotic cell cycle and the need to gain genetic knowledge becomes as very important aspect. Data of pollen viability, fertility and meiotic behaviour are part of reproductive biology and breeding, ensure to produce quality crosses in order to develop new hybrids in this crop.

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