

Chromosome numbers of *Jatropha curcas* L.: an important agrofuel plant

Nair Dahmer¹, Maria Teresa Schifino Wittmann^{1*}, and Luiz Antônio dos Santos Dias²

Received 05 June 2009

Accepted 20 September 2009

ABSTRACT - Chromosome numbers were determined in five populations of the agrofuel physic nut plant (*Jatropha curcas* L.). Somatic chromosome numbers were counted from root-tip cells of four individuals per population and all had $2n=22$ chromosomes, corresponding to the diploid level ($x=11$). The chromosomes are small (ca. 1 to 2 μm), meta or submetacentrics. The present results are in line with literature and indicate that all *J. curcas* populations examined so far are diploid. This lack of variation in chromosome number contrasts with the high variability in other characteristics. From the plant breeding viewpoint, the fact that all populations analyzed are diploid and have the same chromosome number is an advantage for planning crosses and obtaining hybrids.

Key words: *Jatropha curcas*, biofuel, cytogenetics, physic nut.

INTRODUCTION

The genus *Jatropha* (Euphorbiaceae) comprises around 160 to 175 species, typical of tropical and warm climates. The species can be monoecious or dioecious, trees, shrubs rhizomatous subshrubs, or geophytes, and herbs, including some annual taxa (Dehgan 1984, Mabberley 1987, Heller 1991) and can be used for basket making, as living fences, in folk medicine, as ornamental and the oil of some species is used for candle and soap making and as a biofuel (Mabberley 1987). Some species may be toxic to humans and animals. One of the most famous and economically important species of the *Jatropha* genus is *Jatropha curcas* L., known as physic nut ("pinhão-mansô" in Brazil), an agrofuel plant that is increasingly attracting the attention for its oil. *J. curcas* has the potential of becoming the most important biodiesel plant producer. It is a rustic plant, which is perennial, easy to manage, contains 38% of oil in its

grains, and compatible in quality with diesel oil and it is appropriate to the consortium with other crops in small properties (Dias et al. 2007, Dias et al. 2008).

Cytogenetic studies in the genus are rather restricted. Chromosome numbers are known for ca. 36 species plus several interspecific hybrids. Almost all of the species are diploid, with $2n=2x=22$ chromosomes, and the few reports on meiosis point to a regular meiotic behavior. Three species (*J. cuneata* Wiggins & Rollins, *J. dioica* Sesse and some populations of *J. heterophylla* Heyne) are tetraploid ($2n=4x=44$) and *J. tirucalli* L. has $2n=20$ chromosomes (IPCN, Fedorov 1969, Soontornchainaksaeng and Jenjittikul 2003, Carvalho et al. 2008). Most of the reported artificial interspecific hybrids are generally diploid but two triploid hybrids ($2n=3x=33$) have been recovered from crosses between the diploid *J. curcas* x *J. cathartica* Teran & Berlan and *J. curcas* x *J. podagrica* Hook (Dehgan 1984). Artificial

¹ Universidade Federal do Rio Grande do Sul (UFRGS), Departamento de Plantas Forrageiras e Agrometeorologia, Avenida Bento Gonçalves 7712, C.P. 15100, 91540-000, Porto Alegre, RS, Brazil. *E-mail: mtschif@ufrgs.br

² Universidade Federal de Viçosa (UFV), Departamento de Fitotecnia, 36570-000, Viçosa, MG, Brazil

hybrids have been produced in *Jatropha* as a way to understand phylogenetic relationships of the genus (Dehgan 1984) and also for practical uses such as to introducing a novel variation into ornamental species (Sujhata and Prabakaran 2003).

This study aimed to determine the chromosome number in plants of five populations of *J. curcas* which are part of the genebank and of the oil plant breeding program of the Federal University of Viçosa, Brazil.

MATERIAL AND METHODS

The populations evaluated were: 1) Filomena; 2) Bento; 3) Oracília; 4) Paraguassú and 5) Gonçalves, all collected by NNE Minas Agro Florestal Ltda, in Janaúba, MG (Tominaga et al. 2007).

Somatic chromosome numbers were determined in root-tip cells, following the technique normally used at our laboratory for other species (Reis et al. 2008, Guisso-Navarini et al. 2008) with some adjustments. The seeds were germinated in Petri dishes lined with moist filter paper at room temperature. When the roots were 0.8 to 1.0 cm long, they were pre-treated in a saturated solution of paradichlorobenzene at 4 °C for 24 hours, fixed in a 3:1 ethanol-acetic solution for 24 hours, and stored in 70% ethanol below 0° C until required. The roots were treated with 1N HCl at 60 °C for 12 minutes, stained by Feulgen's method for 3 hours, treated with 2% pectinase for 20 minutes and then squashed in 2% propionic carmine.

At least four seedlings (individuals) and ten cells of each were examined per population. Only intact cells with well-spread chromosomes were included in the analysis. Results were recorded by photomicrographs and digital images.

RESULTS AND DISCUSSION

The chromosome numbers, number of individuals and cells analyzed are shown in Table 1. All the five populations have $2n=22$ chromosomes, corresponding to the diploid level. The chromosomes are small, from ca. 1 to 2 μm with median or submedian centromeres (Figure 1 A-C). Although difficult to clearly visualize, in some cells a pair of chromosomes with possibly secondary constrictions (Nucleolus Organizing Regions) were seen.

Table 1. *Jatropha curcas* populations analyzed, number of individuals and cells and chromosome number

Populations	Number of individuals and (number of cells analyzed)	Chromosome number (2n)
Filomena	5(24)	22
Bento	4(10)	22
Oracília	5(14)	22
Gonçalo	8(35)	22
Paraguassú	6(20)	22

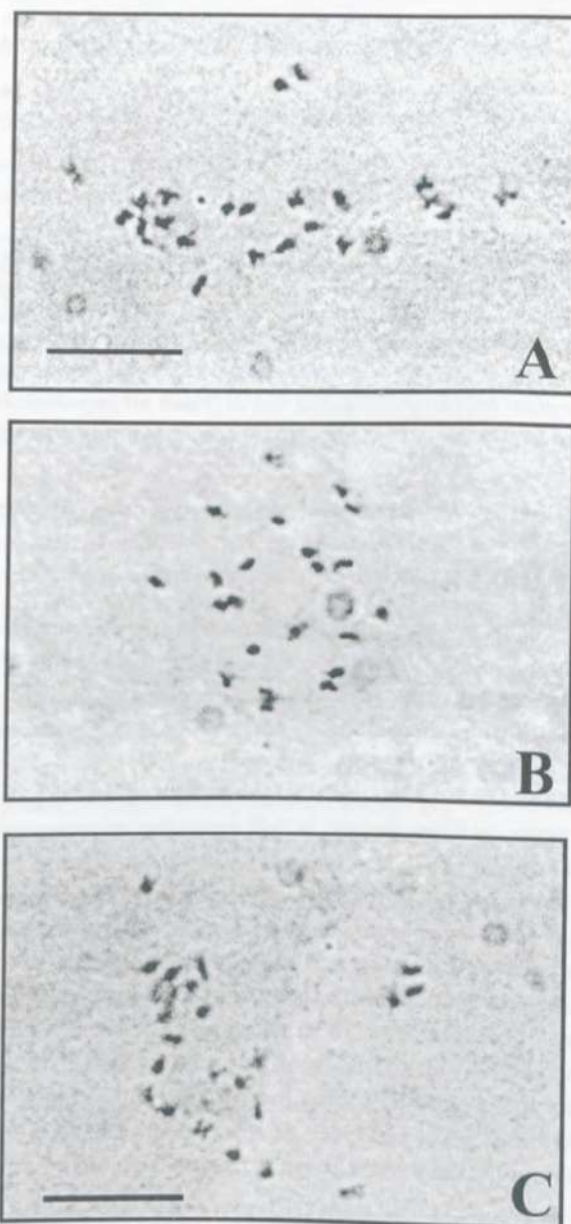


Figure 1. Somatic metaphase plates. A) Oracília, $2n=22$; B) Gonçalves, $2n=22$; C) Filomena, $2n=22$. Scale bar = 10 μm

The chromosome number of $2n=2x=22$ presented here for *J. curcas* agrees with literature data for other populations of the species (IPCN 2009, Fedorov 1969, Soontornchainaksaeng and Jenjittikul 2003, Carvalho et al. 2008). Carvalho et al. (2008) also analyzed the Gonçalves population besides determining the chromosome number, presented the genome size (0.85 pg DNA/2C), base composition (38.7% GC) and a karyotype with five metacentric and six submetacentric chromosome pairs, ranging from 1.24 to 1.71 μm . Our results are in accordance with those presented by Carvalho et al. (2008) in terms of chromosome morphology, size and number. The suggestion of Carvalho et al. (2008), based on morphometric similarities of ten heterologous pairs, that *J. curcas* is an autotetraploid species, is not supportable: first, most of the *Jatropha* species analyzed in the literature are diploid, with $2n=2x=22$ chromosomes, the basic number of the genus being $x=11$ (see the Introduction to this paper); second, for the species to be an autotetraploid, we would have to suppose a basic number of $x=5.5$. On the other hand, $x=11$ could be a basic number derived

from ancient polyploidization events in the ancestor of the *Jatropha* genus, followed by chromosomal rearrangements; however to even propose that as a suggestion would need an enormous amount of cytogenetical and phylogenetic data. At the present state, any suggestions would merely be speculative without scientific support.

In an approach to plant breeding, the lack of chromosome number variability in *J. curcas* may be an advantage when crosses are planned due to regular meiosis. Finally, the lack of variation in chromosome numbers in *J. curcas* contrasts its variability in terms of seed traits in oil content (Kaushik et al. 2007) as well as in terms of phorbol ester content and DNA molecular profiles (Basha et al. 2009).

ACKNOWLEDGEMENTS

We thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and FAPEMIG (Fundação de Amparo a Pesquisa do Estado de Minas Gerais) for their financial support.

Números cromossômicos *Jatropha curcas* L.: uma importante espécie para agrocombustível

RESUMO - Os números cromossômicos de cinco populações de pinhão manso (*Jatropha curcas*) – uma importante oleaginosa para agrocombustível – foram determinados. O número de cromossomos somáticos foi obtido por contagem em células de pontas de raiz, em quatro indivíduos por população. Todos eles apresentaram $2n=22$ cromossomos, correspondendo ao nível diplóide ($x=11$). Os cromossomos são pequenos (1 a 2 μm), meta ou submetacêntricos. Esses resultados estão em concordância com a literatura e indicam que todas as populações de *J. curcas* examinadas até momento são diplóides. A ausência de variação no número cromossômico contrasta com a alta variabilidade em outras características. Do ponto de vista do melhoramento, o fato de todas as populações analisadas serem diplóides e terem o mesmo número cromossômico constitui vantagem no planejamento de cruzamentos e na obtenção de híbridos.

Palavras-chave: *Jatropha curcas*, agrocombustível, citogenética, pinhão manso.

REFERENCES

- Basha SD, Franis G, Makkar HPS, Becker K and Sujatha M (2009) A comparative study of biochemical traits and molecular markers for assessment of genetic relationships between *Jatropha curcas* L. germplasm from different countries. **Plant Science** 176: 812-823.
- Carvalho CR, Clarindo WR, Praça MM, Araújo FS and Carels N (2008) Genome size, base composition and karyotype of *Jatropha curcas* L., an important biofuel plant. **Plant Science** 174: 613-617.
- Dias LAS, Leme LP, Laviola BG, Pallini A, Pereira OL, Dias DCFS, Carvalho M, Manfio CE, Souza LCA, Marra A and Pretti LA (2007) **Cultivo de pinhão manso (*Jatropha curcas* L.) para produção de óleo combustível**. UFV, Viçosa, 40p.
- Dias LAS, Muller M and Freire E (2008) Potencial do uso de oleaginosas arbóreas em sistemas silvipastoris. In: Fernandes EM, Paciullo DSC, Castro CRT, Muller MD, Arcuri PB, and Carneiro JC (eds.), **Sistemas Agrossilvipastoris na América do Sul: desafios e potencialidades**. Embrapa Gado de Leite, Juiz de Fora, p. 283-314.

- Dehgan B (1984) Phylogenetic significance of interspecific hybridization in *Jatropha* (Euphorbiaceae). **Systematic Botany** **94**: 467-478.
- Fedorov An A (1969) **Chromosome numbers of flowering plants**. Academy of Sciences of the USSR, Leningrad, 926p.
- Guisso-Navarini AP, Schifino-Wittmann MT, Barros IBI, and Almeida D (2008) Cytogenetics of *Hypericum caprifoliatum* Cham. & Schltdl. (Clusiaceae) populations and other species of the genus. **Crop Breeding and Applied Biotechnology** **8**: 337-345.
- Heller J (1996) **Physic nut (*Jatropha curcas* L.): promoting the conservation and use of underutilized and neglected crops 1**. Roma, IBPGR, 66p. (IBPGR 161)
- IPCN (2009) **Index to plant chromosome numbers**. Available in: <http://mobot.mobot.org/W3T/Search/ipcn.html>. Assessed on May 2009.
- Kaushik N, Kumar K, Kumar S, Kaushik N and Roy S (2007) Genetic variability and divergence studies in seed traits and oil content of *Jatropha* (*Jatropha curcas* L.) accessions. **Biomass and Bioenergy** **3**: 497-502.
- Mabberley DJ (1997) **The plant book**. Cambridge University Press, Cambridge, 858p.
- Reis CAO, Schifino-Wittmann MT and Dall'Agnol M (2008) Cytogenetic characterization of a collection of *Paspalum nicorae* Parodi accessions. **Crop Breeding and Applied Biotechnology** **8**: 212-218.
- Soontornchainaksaeng P and Jenjittikul T (2003) Karyology of *Jatropha* (Euphorbiaceae) in Thailand. **Thai Forest Bulletin (Botany)** **31**: 105-112.
- Sujatha M and Prabakaran AJ (2003) New ornamental *Jatropha* hybrids through interspecific hybridization. **Genetic Resources and Crop Evolution** **50**: 75-82.
- Tominaga N, Kakida J and Yasuda EK (2007) **Cultivo de pinhão manso para produção de biodiesel**. CPT, Viçosa, 220p. (Série Agroindústria).