Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Chromosome number and cytogenetics of *Euphorbia heterophylla* L.

J.R. Aarestrup[1], D. Karam[2] and G.W. Fernandes[3]

[1]Departamento de Biologia Geral, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
[2]Laboratório de Herbicidas e Manejo de Plantas Daninhas, Empresa Brasileira de Pesquisas Agropecuárias (EMBRAPA), Sete Lagoas, MG, Brasil
[3]Departamento de Ecologia, Laboratório de Ecologia Evolutiva de Herbívoros Tropicais, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil

Corresponding author: J.R. Aarestrup
E-mail: jroriz@yahoo.com.br

Genet. Mol. Res. 7 (1): 217-222 (2008)
Received December 5, 2007
Accepted December 17, 2007
Published March 4, 2008

ABSTRACT. *Euphorbia heterophylla* L. (Euphorbiaceae) is a herbaceous species of great economic importance due to its invasive potential and consequent damage to agriculture and pasture land. For the first time, we provide information on its chromosome number, morphology, and behavior of mitotic chromosomes. Seeds were germinated and submitted to four treatments to obtain metaphases: 0.5% colchicine for 2 to 5 h, at ambient temperature; 0.5% colchicine for 16 to 24 h; 0.0029 M 8-hydroxyquinoline (8-HQ) for 2 to 5 h at ambient temperature, and 0.0029 M 8-HQ for 16 to 24 h at 4°C. The material was then fixed in methanol:acetic acid (3:1) and kept at -20°C for 24 h. Roots were macerated in the enzyme solution of Flaxzyme™ (NOVO FERMENT™)-distilled water (1:40) at 34°C for 2 h and later fixed again. Chromosome preparations were obtained by the dissociation of the apical meristems. The best chromosome preparations were obtained with the use of 8-HQ for 21 h 30 min at 4°C. *E. heterophylla* showed 2n = 28 chromosomes. The short arm of the largest pair of chromosomes of the complement (pair number 1) displayed a secondary constriction while the nucleolus was observed

in the interphasic cell. Structural rearrangements were also observed in the *E. heterophylla* L. genome. The genomic instability associated with polyploidy may be the result of selection shaped by environmental adaptations and/or human-induced manipulation through agricultural practices.

Key words: Chromosome; Cytogenetics; *Euphorbia heterophylla*; Nucleolus

INTRODUCTION

The emergence of many invasive plants has been related to human-induced selection often shaped by anthropic environments and/or by several habitat disturbances. Plants that occupy cultivated areas adapt to the new environmental conditions and become invasive, becoming undesirable for humans (Gupta and Tsuchiya, 1991).

The control of invasive plants has been done mainly through the use of herbicides due to the efficiency of such chemical products (Christoffoleti, 1994). However, the constant use of one herbicide or different herbicides with the same mechanism of action has exerted a high selective pressure, increasing the selection of resistant plants (Holt and LeBaron, 1990). This is the case of *Euphorbia heterophylla* L. (Euphorbiaceae), one of the most important invasive species of soy and many other crops in Brazil (Willard and Griffin, 1993). About ten *E. heterophylla* L. plants in one square meter can reduce grain yield of the crop by 7% (Chemale and Fleck, 1997).

Although several studies dealing with chemical control of *E. heterophylla* have been conducted, little is known about the cytogenetic aspects of this species (Costa, 1982). The species may show 2n = 14, 26, 28, 32, or 56 chromosomes (Hans, 1973; Kissmann and Groth, 1997). Suda (2001) argued that the species is a tetraploid, with a basic number of X = 7 chromosomes.

The karyotypic description, such as morphology and number of chromosomes, is of great value for the understanding of the evolutionary processes of a species (Gupta and Tsuchiya, 1991). Among the most important morphological aspects, chromosome size, localization of the primary constriction and, when present, the secondary constriction are the main ones (Sybenga, 1992).

Some mechanisms have been identified as responsible for the evolution of plants among them, structural chromosomal changes and polyploidy (Narayan, 1998). Such chromosomal modifications can occur spontaneously or by induction both in somatic cells and in the gametes (Sybenga, 1992).

Polyploidy, or genomic multiplication, is a common and continuous phenomenon in the evolution of plants (Adams and Wendel, 2005), and about 70% of the angiosperms are polyploids (Leitch and Bennet, 1997). In general, polyploids are good colonizers (De Wet, 1980) and some invasive species can be considered as such, since they exhibit fast chromosomal evolutionary events (Reznick and Ghalambor, 2001). Also, the genomic reorganization and chromosomal repatterning that occur in polyploids (Schifino-Wittmann, 2004) can modify their tolerance to the environment (Lee, 2002).

Considering the economic loss that *E. heterophylla* imparts on cultivated plants, the scarce data referring to its mitotic cell cycle and the need to gain genetic knowledge that may help in its reproductive control, the objective of the present study was to determine the number and morphology of chromosomes, as well as the behavior of the mitotic chromosomes of this species.

MATERIAL AND METHODS

Seeds of *E. heterophylla* L. were collected from 40 plants, from different cultivated areas of Embrapa Milho e Sorgo, Sete Lagoas, Minas Gerais, Brazil. About 500 seeds were randomly selected from the original pools of seeds and germinated in gerbox, containing germtest paper moistened with distilled water. The seeds that had 0.5 to 1.0 cm long roots were submitted to four types of pretreatments: 1) 0.5% colchicine for 2 to 5 h, at room temperature; 2) 0.5% colchicine for 16 to 24 h, at 4°C; 3) 0.0029 M 8-hydroxyquinoline (8-HQ) for 2 to 5 h, at room temperature, and 4) 0.0029 M 8-HQ for 16 to 24 h, at 4°C. Next, roots were washed in distilled water for 5 min. They were immediately fixed in a solution previously cooled at -20°C. The fixative was a methanol-acetic acid solution mixed in a 3:1 (v/v) proportion. Roots were stored in the fixative at -20°C for 24 h.

The enzymatic maceration was carried out in 1.5-mL Eppendorf™ tubes containing a solution of the enzymatic reagent Flaxzyme™ (NOVO FERMENT™) and distilled water 1:40 (v/v) for 2 h at 34°C. Roots were washed for removal of the enzymatic solution, and the digested material was stored at -20°C for 24 h in Eppendorf™ tubes containing the fixative.

Roots were transferred to a glass plate containing 1 mL fixative and the apical meristem of each root was extracted and fragmented on a glass slide at an angle of approximately 30°, with the use of a surgical knife and a stereomicroscope. Chromosomal preparations were made by cell dissociation of apical meristems, by adding three to five drops of fixative at -20°C. The slides were then air dried quickly and then allowed to dry on a hot plate at 50°C. Staining of the slides was done with a 5% Giemsa solution, in phosphate buffer, pH 6.8, for 15 min at room temperature. Finally, the slides were washed in distilled water and dried on a hot plate at 50°C.

RESULTS AND DISCUSSION

The analysis of 500 somatic cells of *E. heterophylla* indicated that the species exhibits 2n = 28 chromosomes (Figure 1). This same chromosomal number has been also cited by Hans (1973) and Kissmann and Groth (1997). X = 7 is one of the basic chromosome numbers found in the genus *Euphorbia* (Löve, 1984), suggesting that the species is indeed tetraploid (Suda, 2001).

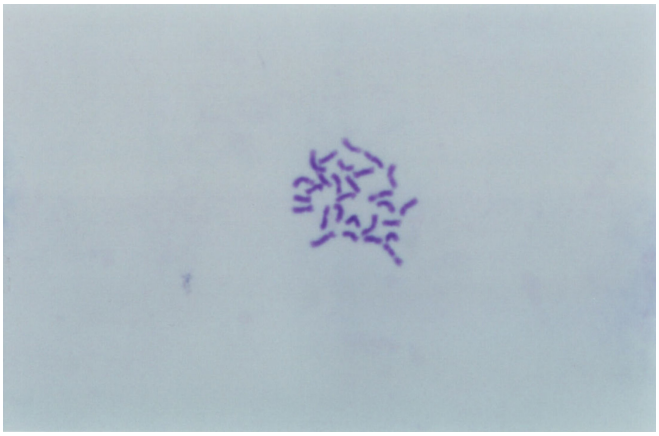

Figure 1. Metaphasic chromosomes of *Euphorbia heterophylla* L. treated with 0.0029 M 8-hydroxyquinoline for 21 h 30 min at 4°C.

In metaphasic cells, it was observed that the largest chromosome pair of the complement (pair number 1) exhibits a secondary constriction or nucleolar organizing region (NOR), located on the short arm (Figure 1), and during interphase, one nucleolus was identified per cell (Figure 2). The number of NORs and nucleoli found in *E. heterophylla* can be considered characteristic of polyploidy. Generally, polyploids show less visible NORs in the chromosome set than expected by the sum of the diploid genomes (Vaughan et al., 1993). A reduction in the number of NORs and nucleoli during polyploidization of *Scilla autumnalis* was reported by Vaughan et al. (1993) who also argued that this reduction in the expression of NORs may be the result of nucleolar suppression.

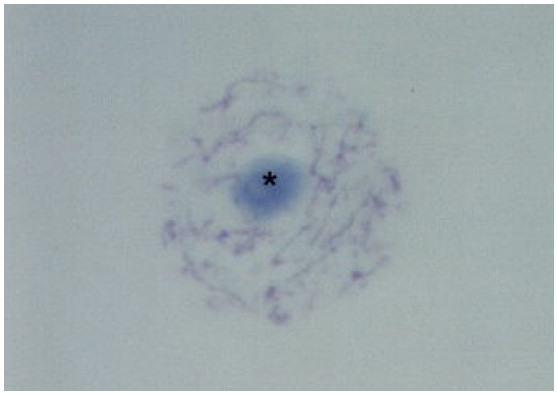

Figure 2. Interphasic cell of *Euphorbia heterophylla* L. Note nucleolus distinguished with Giemsa (asterisk).

The root meristematic cells of *E. heterophylla* L. submitted to 0.5% colchicine and 0.0029 M 8-HQ for 21 h and 30 min at 4°C allowed us to obtain mitotic metaphasic chromosomes. However, with the 8-HQ solution, better metaphases were seen in relation to those with colchicine, since chromosomes pretreated with this drug were more compacted (Figure 3) than those treated with 8-HQ (Figure 1). Although colchicine is extensively utilized in cytogenetic studies (e.g., Hadlaczky et al., 1983; Sharma and Sharma, 1999), the drug made the identification of the chromosomes' secondary constrictions difficult.

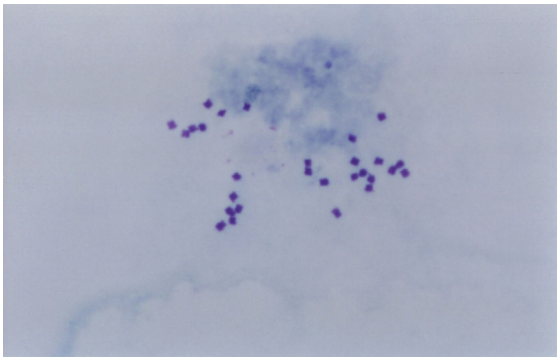

Figure 3. Metaphasic chromosomes of *Euphorbia heterophylla* L. treated with 0.5% colchicine for 21 h 30 min at 4°C.

According to Singh (1993), 8-HQ is very useful in chromosomal analyses because it allows a good visualization of the secondary constrictions. In addition to allowing a better packing of chromosomes, colchicine also tends to induce chromosomal agglomerations, which is very pronounced after longer treatments (Wang et al., 1986). Such agglomerative effect has not been observed in *E. heterophylla*. Nonetheless, the absence of superposed chromosomes can be the result of the cell dissociation techniques and air-drying, since slides with well-spread chromosomes can be obtained with both techniques (see Carvalho and Saraiva, 1997).

In about 1% of the metaphases analyzed, chromosomal figures suggestive of structural rearrangements in the genome of *E. heterophylla* L. were observed (Figure 4). Since the species is considered tetraploid, chromosomal rearrangements can arise as a consequence of polyploidization (Schifino-Wittmann, 2004). However, the karyotype dynamics hypothesis in *E. heterophylla* due to the use of herbicides cannot be discarded, since, according to Christoffoleti et al. (1994), disturbances caused by humans in agriculture can trigger the evolution of invasive plants.

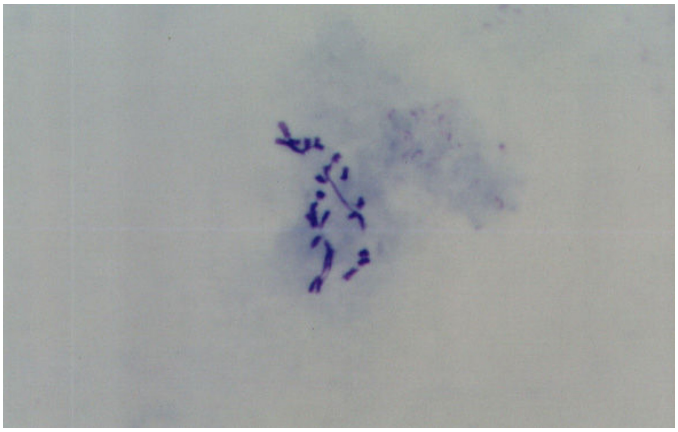

Figure 4. Metaphasic chromosomes of *Euphorbia heterophylla* L. treated with 0.0029 M for 21 h 30 min at 4°C.

Pagliarini (2000) analyzed gametic cells of several economically important plants including *E. heterophylla* but did not report any chromosomal rearrangement event in this species. The differences between the results on chromosomal rearrangements presented in this study and those of Pagliarini (2000) may have occurred due to the analysis of different populations of *E. heterophylla*.

In summary, the 0.0029 M 8-HQ solution for 21 h and 30 min was efficient for obtaining *E. heterophylla* metaphases, since it allowed the visualization of less compacted metaphasic chromosomes and their secondary constrictions. The species exhibits 2n = 28 chromosomes, a chromosome pair, number 1, with a secondary constriction in metaphase, and a nucleolus in each interphasic cell. The observation of chromosomal structural rearrangements allowed us to evaluate the importance of these events in the natural evolution of *E. heterophylla*, or as a consequence of the selective pressure because of the use of herbicides. Future studies will clarify the evolutionary aspects of the karyotype of this species.

ACKNOWLEDGMENTS

We thank A.V.B.F. Vilas Boas and anonymous reviewers for suggestions on earlier versions of the manuscript. We thank E.J.A. Corrêa for statistical support, and Embrapa Milho e Sorgo, Brazil, for logistical support. This study was in partial fulfillment of the requirements for a PhD in Genetics at UFMG for J.R. Aarestrup.

REFERENCES

Adams KL and Wendel JF (2005). Polyploidy and genome evolution in plants. *Curr. Opin. Plant Biol.* 8: 135-141.

Carvalho CR and Saraiva LS (1997). High-resolution HKG-banding in maize mitotic chromosomes. *J. Plant Res.* 110: 417-420.

Chemale VM and Fleck NG (1997). Avaliação de cultivares de soja (*Glycine max* (L.) Merrill) em competição com *Euphorbia heterophylla* L. sob três densidades e dois períodos de ocorrência. *Planta Daninha* 5: 36-45.

Christoffoleti PJ, Victória Filho R and Da Silva CB (1994). Resistência de plantas daninhas a herbicidas. *Planta Daninha* 12: 13-20.

Costa OMM (1982). Morfologia e desenvolvimento de *Euphorbia heterophylla*. *Agric. Sulriogr.* 18: 59-66.

De Wet JMJ (1980). Origins of polyploids. In: Polyploidy: biological relevance (Lewis WH, ed.). Plenum, New York, 3-15.

Gupta PK and Tsuchiya T (Editors) (1991). Chromosome manipulations in higher plants - an overview. In: Chromosome engineering in plants: genetic, breeding, evolution. Elsevier Science Publishers, Amsterdam, 149.

Hadlaczky G, Bisztray G, Praznovszky T and Dudits D (1983). Mass isolation of plant chromosomes and nuclei. *Planta* 157: 278-285.

Hans AS (1973). Chromosomal conspectus of the Euphorbiaceae. *Taxon* 22: 591-636.

Holt J and LeBaron HM (1990). Significance and distribution of herbicide resistance. *Weed Technol.* 4: 141-149.

Kissmann KG and Groth D (1997). Plantas infestantes e nocivas. BASF Brasileira, São Paulo.

Lee CE (2002). Evolutionary genetics of invasive species. *Trends Ecol. Evol.* 17: 386-391.

Leitch IJ and Bennet MD (1997). Polyploidy in angiosperm. *Trends Plant Sci.* 2: 470-476.

Löve EA (1984). Chromosome number reports. *Taxon* 35: 611-613.

Narayan RK (1998). The role of genomic constraints upon evolutionary changes in genome size and chromosome organization. *Ann. Bot.* 82: 57-66.

Pagliarini MS (2000). Meiotic behavior of economically important plant species: the relationship between fertility and male sterility. *Genet. Mol. Biol.* 23: 997-1002.

Reznick DN and Ghalambor CK (2001). The population ecology of contemporary adaptations: what empirical studies reveal about the conditions that promote adaptive evolution. *Genetica* 112-113: 183-198.

Schifino-Wittmann MT (2004). Poliploidia e seu impacto na origem e evolução das plantas silvestres e cultivadas. *Rev. Bras. Agrocienc.* 10: 151-157.

Sharma AK and Sharma A (1999). Preparation of material for analysis of chromosome at structural fluorochromes. Plant Chromosomes HAP, Amsterdam.

Singh RJ (1993). Plant Cytogenetics. CRC Pres, Boca Raton.

Suda CNK (2001). Hidrolases da parede celular em sementes de *Euphorbia heterophylla* L. durante a germinação e desenvolvimento inicial da plântula. Doctoral thesis, Departamento de Bioquímica e Imunologia, USP, São Paulo.

Sybenga J (1992). Cytogenetics is plant breeding. Springer-Verlag, Berlin.

Vaughan HE, Jamilena M, Rejón CR, Parker JS, et al. (1993). Loss of nucleolar-organizer regions during polyploid evolution in *Scilla autumnalis*. *Heredity* 71: 574-580.

Wang AS, Phillips RL and Mi CC (1986). Cell cycle parameters and accumulation of metaphase cells in maize suspension cultures. *Plant Sci.* 46: 53-61.

Willard TS and Griffin JL (1993). Soybean (*Glycine max*) yield and quality responses associated with poinsettia (*Euphorbia heterophylla*) control programs. *Weed Technol.* 7: 118-122