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Cytological Studies of Selected Medicinal Plants: *Euphorbia pulcherrima* Willd. ex Klotz., *Moringa oleifera* Lam., *Catharanthus roseus* (L.) Don., and *Chrysanthemum indicum* Linn.

Merlyn S. Mendioro*, **Maria Genaleen Q. Diaz**, **Maria Theresa B. Alcantara, Oscar J. Hilario, Patrocinio Mateo** and **Reycel D.M. Maghirang**

Genetics and Molecular Biology Division, Institute of Biological Sciences
College of Arts and Sciences, University of the Philippines
Los Baños, College, Laguna, Philippines

Meioses I and II in young flower buds of *Euphorbia pulcherrima* Willd. ex Klotz. (poinsettia), *Moringa oleifera* Lam. (malunggay), and *Catharanthus roseus* (L.) Don. (periwinkle) were studied using iron-acetocarmine squash technique. The chromosome number of *C. roseus* is 2n=16 while both *M. oleifera* and *E. pulcherrima* have 2n=28. Although late disjunction and presence of laggards were noted at Metaphase I, Anaphase I and II, Telophase I and II were 100% normal. These would indicate that lagging chromosomes were able to catch up, reached the opposite poles, and were included in daughter nuclei. Highly normal meiosis I and II resulted to high pollen fertility (90.62 to 91.91%). On the other hand, acetocarmine squash preparations of root tip cells of *Chrysanthemum indicum* Linn. (manzanilla) pre-treated with 0.5% colchicine for 2 hours revealed that the diploid chromosome number ranged from 44 to 48. The chromosomes were monocentric. Based on the position of the centromeres, the chromosomes were categorized into three groups, namely; Group I-median, Group II-submedian, and Group III-subterminal. Relative lengths of the chromosomes ranged from 0.40 to 1.00.

Key words: cytological studies, iron-acetocarmine squash technique, *Euphorbia*, *Moringa*, *Chrysanthemum*, karyotype

INTRODUCTION

Plant products are widely used in pharmaceuticals, cosmetics and food industries. Many pharmaceutical companies in the Western world depend on many plants for their medicinal properties. Pharmacopoeias have developed from ancient herbs (de Padua et al. 1977; de Padua 1996).

Euphorbia pulcherrima Willd. or poinsettia (Fig. 1a), which is commonly cultivated for ornamental purposes has curative properties, too. Decoction of the bracts and flowers are taken as galactagogue by nursing women to increase milk flow although the practice is said to be dangerous (Quisumbing 1978). The leaves are applied as poultice and used as emetocathartic causing vomiting and bowel movement.

Moringa oleifera Lam. or malunggay (Fig. 1b) is a multi-purpose crop indigenous to Northwest India. Tender pods, leaves and flowers are consumed as vegetables, the twigs and leaves as fodders, and oil extracted from the seeds is used as spice, in illumination and cosmetics (Concha 1980). The plant is used as tonic to enhance lactation, as poultice to reduce glandular swelling, and as purgative. The roots, when chewed and applied to snake bite, can prevent the poison from spreading (de Padua and Pancho 1983).

*Corresponding author: msmendioro@yahoo.com

Catharanthus roseus (L.) Don. or periwinkle (Fig. 1c), besides being cultivated as an ornamental plant, is a source of as many as 200 different alkaloids, two of which, vincristine and vinblastine, are anti-cancer drugs (Kingston and Sami 1979). These anti-cancer alkaloids are expensive (Fontanel and Tabata 1986). Vinblastine has been extensively used in combination with cis platinum and bleomycin to treat testicular and ovarian cancers (Pratt 1994).

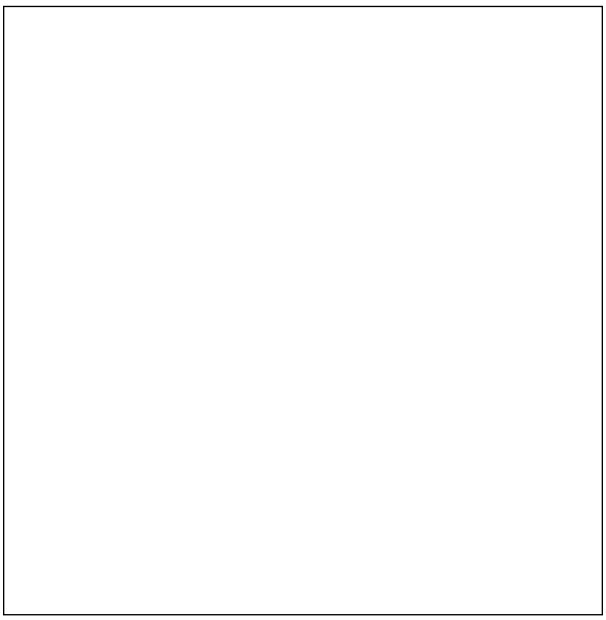

Figure 1. Pictures of selected medicinal plants used in the study. (a) *Euphorbia pulcherrima* Willd. (poinsettia); (b) *Moringa oleifera* Lam. (malungay); (c) *Catharanthus roseus* (L) Don. (periwinkle); (d) *Chrysanthemum indicum* Linn. (manzanilla)

Another medicinal plant native to China and Japan is *Chrysanthemum indicum* L. or manzanilla (Fig. 1d.). Its infusion is a remedy for intermittent fever, hysteria, and monthly irregularities. It is also used as carminative, tonic sedative, and for hypertension. Leaf decoction is a remedy for colds, headache, bronchitis, rheumatism, swellings, and boils (de Padua et al. 1977).

There is a resurgent interest in the use of medicinal plants. Researches were geared towards morphological and pharmaceutical importance. But little is done on cytological characterization, which is important in species identification, diversity studies, and plant improvement. This study was therefore conducted to determine the chromosome numbers, meiotic chromosome behavior, and pollen fertility of *E. pulcherrima*, *M. oleifera* and *C. roseus*, and to characterize the mitotic chromosomes of *C. indicum* through karyotype analysis.

MATERIALS AND METHODS

For meiotic studies, young flower buds of *E. pulcherrima*, *M. oleifera* and *C. roseus* were collected between 10:00 a.m. to 2:00 p.m. and fixed in freshly prepared Farmer's solution (3 parts 95% ethyl alcohol : 1 part 100 % glacial acetic acid) with a few drops of ferric chloride. After 1 h in fixative, the flower buds were transferred to 70% ethyl alcohol. Meiotic cells were obtained using iron acetocarmine squash technique. At least 100 cells per meiotic stage were scored. Pollen fertility was obtained by staining the pollens with 2% iodine potassium iodide solution. Fertile pollen absorbs the stain but the sterile pollen does not. At least 500 pollen grains were counted. Pollen fertility was based on the number of fertile pollen out of the total number of pollen counted.

For karyotype analysis, new roots of *C. indicum* grown to about 8 mm were placed in cold 0.5% colchicine for 2 h then it was fixed in freshly prepared Farmer's solution. A good metaphase spread was prepared using iron-acetocarmine squash technique. Photomicrographs of C metaphase spread were taken and the following data were obtained:

a. Chromosome number

b. Relative length = $\frac{\text{chromosome length}}{\text{length of the longest chromosome}} \times 100$

c. Centromeric index = $\frac{\text{length of short arm}}{\text{chromosome length}} \times 100$

The chromosomes were arranged and grouped according to Levan et al. (1964) classification.

RESULTS AND DISCUSSION

Meiotic Studies on Selected Medicinal Plants

Poinsettia, *Euphorbia pulcherrima* Willd..ex Klotz

Cells at Diakinesis revealed the chromosome number of *E. pulcherrima* as 2n=26 and 2n=28 (Figs. 2a and b). However, the modal number is 2n=28 (14II) with an average mean of 77.09%. This confirms earlier studies on the chromosome count of *E. pulcherrima* as 2n=28 (Moyer 1934; Ewart and Walker 1960). The high frequency of bivalent formation is due to the balanced number of homologous set of chromosomes favoring preferential pairing. Some species would really show variation in

chromosome number such as observed in this study, and this phenomenon is said to be genotypically determined (Sinha and Acharia 1975). Metaphase I (Table 1) shows non-congression (25.45%), early disjunction (14.67%), and presence of laggards (14.91%). Both normal and abnormal Metaphase I cells exhibited stickiness and clumping of chromosomes. Laggards resulted from late migration of bivalent at the equatorial plane. Non-congression may be due to early disjunction of bivalents. The frequency of normal Metaphase I (Fig. 2c) is only 44.95%. The frequency of normal Anaphase I cells (Fig.2d) is also low at 38.42%. Laggards (Fig. 2e) at 43.16% and late disjunction at 18.42% were also observed. At Telophase I (Fig. 2f), all the cells exhibited normal behavior. This could mean that the observed laggards at Anaphase I were able to migrate to the poles, caught up with the other chromosomes, and were included in the daughter nuclei. Although Metaphase II (Fig. 2g) and Anaphase II showed abnormal behavior such as presence of laggards, non-congression of the chromosomes, early and late disjunction, these abnormalities were absent at telophase II (Fig. 2h).

Table 1. Frequencies (%) of occurrence of different meiotic chromosome configurations and behavior in *Euphorbia pulcherrima* Willd. (poinsettia)

Meiotic Stages	Total no. of Cells Observed	Frequency of Occurrence	Average Frequency (%)
Meiosis			
Diakinesis	275		
14 II		212	77.09
13 II		63	22.90
Metaphase I	436		
Normal		196	44.95
Laggards		65	14.91
Non-congression		111	25.45
Early Disjunction		64	14.67
Anaphase I	380		
Normal		146	38.42
Laggards		164	43.16
Late Disjunction		70	18.42
Telophase I			
Normal	434	434	100.00
Meiosis II			
Metaphase II	340		
Normal		143	42.06
Laggards		86	25.29
Non-congression		75	22.06
Early Disjunction		36	10.59
Anaphase II	293		
Normal		182	62.12
Laggards		24	8.19
Non-congression		33	11.26
Early Disjunction		27	9.21
Asynchronous separation		27	9.21
Telophase II	761		
Normal		761	100.00

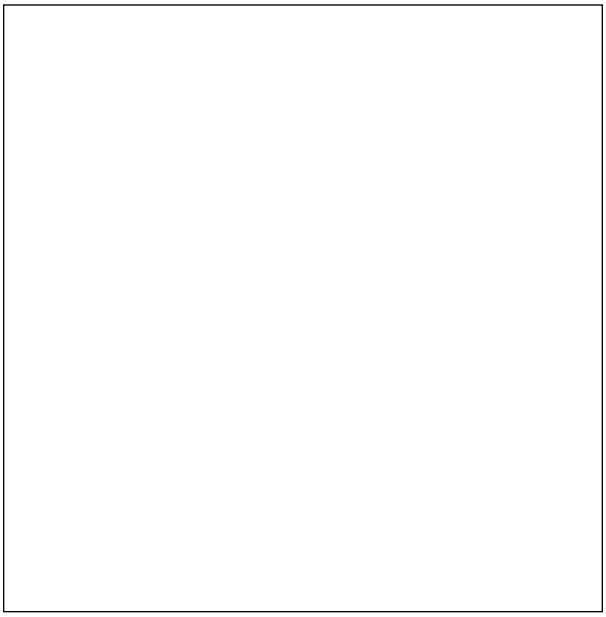

Figure 2. Photomicrographs (400X) of Meiosis I and II in *Euphorbia pulcherrima* Willd. (poinsettia). (a) Diakinesis with 12 II + 4 I; (b) interpretative drawing of Fig. 2a; (c) normal Metaphase I; (d) normal Anaphase I; (e) laggards at anaphase I; (f) normal Telophase I; (g) normal Metaphase II; (h) normal Telophase II

Malunggay, *Moringa oleifera* Lam.

M. oleifera has a chromosome number of 2n=28 (Table 2.). Diakinesis shows 14 II (Figs. 3 a and b). The observed chromosome number confirms the entry for *M. oleifera* in Darlington and Wylie's (1945) listing of chromosome numbers. The frequency of cells with normal Metaphase I (Fig. 3c) is only 76.33%. Laggards (4.55%), non-congression (1.41%), and early disjunction (17.71%) were observed. Non-congression could be due to an abnormality in the spindle fiber formation or late alignment of the alignment of chromosomes at the equatorial plane (Escote 1978; Tarar 1980). The lagging chromosomes at Metaphase I were able to catch up at Anaphase I. This is the reason why the frequency of cells with normal

Table 2. Frequencies (%) of occurrence of different meiotic chromosome configurations and behavior in *Moringa oleifera* Lam. (malunggay)

Meiotic Stages	Total no. of Cells Observed	Frequency of Occurrence	Average Frequency (%)
Meiosis			
Diakinesis	321		
14 II		321	100.00
Metaphase I	638		
Normal		487	76.33
Laggards		29	4.55
Non-congression		9	1.41
Early Disjunction		113	17.71
Anaphase I	260		
Normal		236	90.77
Laggards		14	5.38
Bridge		7	2.69
Late Disjunction		3	1.15
Telophase I	416		
Normal		416	100.00
Metaphase II	497		
Normal		468	94.16
Laggards		21	4.23
Non-congression		8	1.61
Anaphase II	260		
Normal		229	88.08
Laggards		31	11.92
Telophase II	1253		
Normal		1253	100.00

Anaphase I (Fig. 3d) is 90.77%. Although laggards, bridge and late disjunction were observed at Anaphase I, the Telophase I (Figs. 3f to h) is highly normal (100%). Cells at Meiosis II showed normal behavior especially at Telophase II (100%). The high frequencies of normal cells in Meiosis I and II would explain why pollen fertility is high (90.62%).

Periwinkle, *Catharanthus roseus* Don.

The chromosome number of *C. roseus* is 2n=16. It regularly formed 8 II (Table 3.) (Figs. 4a & b). A high frequency (95.97%) of normal Metaphase I cells (Fig. 4c) was noted. Cells with laggards were observed at a very low frequency of 1.34% as well as cells showing non-congression of chromosomes at 2.68% (Fig. 4d). Anaphase I and Telophase I cells are highly normal with

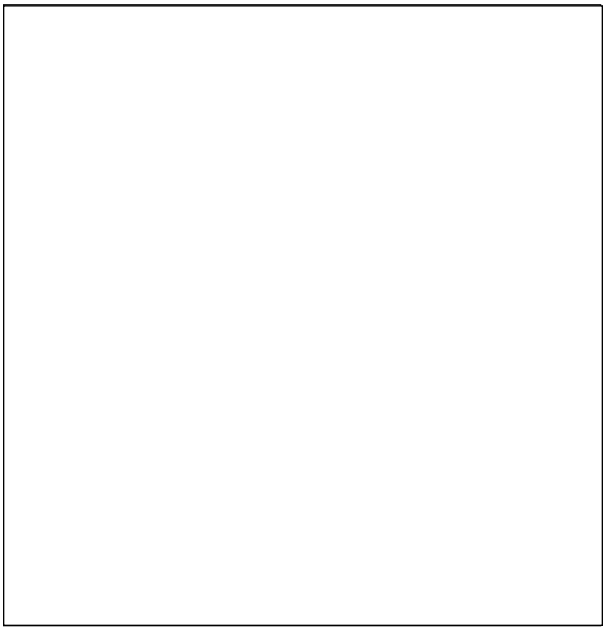

Figure 3. Photomicrograps (400X) of meiosis I and II in *Moringa oleifera* Lam. ("malunggay"). (a) Diakinesis with 14 II; (b) interpretative drawing of Fig. 3a; (c) normal Metaphase I; (d) normal Anaphase I; (e) normal Telophase I; (f) normal Metaphase II; (g) normal Anaphase II; (h) normal Telophase II

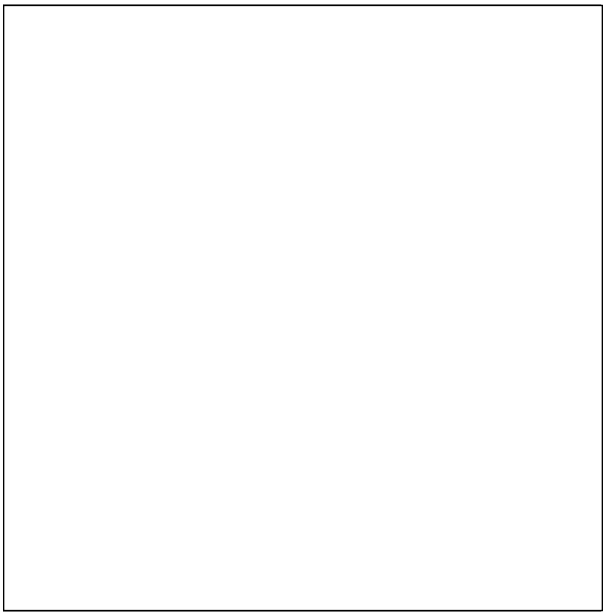

Figure 4. Photomicrographs (400X) of meiosis I and II in *Catharanthus roseus* (L.) Don. (periwinkle). (a) Diakinesis; (b) normal Metaphase I; (c) non-congression at Metaphase I; (d) non-congression at Anaphase I; (e) normal Telophase I; (f) normal Metaphase II; (g) normal Anaphase II; (h) and (i) normal Telophase II

Table 3. Frequencies (%) of occurrence of different meiotic chromosome configurations and behavior in *Catharanthus roseus* (L.) Don.(periwinkle)

Meiotic Stages	Total no. of Cells Observed	Frequency of Occurrence	Average Frequency (%)
Meiosis			
Diakinesis	128		
8 II		126	98.44
7 II + 21		2	1.56
Metaphase I	149		
Normal		143	95.97
Laggards		2	1.34
Non-congression		4	2.68
Anaphase I	103		
Normal		99	96.11
Laggards		3	2.91
Telophase I	163		
Normal		162	99.39
Laggards		1	0.61
Meiosis II			
Metaphase II	97		
Normal		87	90.63
Laggards		4	4.17
Early Disjunction		5	5.20
Anaphase II	109		
Normal		107	98.17
Laggards		2	1.83
Telophase II	165		
Normal		163	98.79
Laggards		2	1.21

frequencies of 96.11% and 99.39%, respectively. Non-congression (Fig. 4e) at Anaphase I was observed at a very low frequency. A photomicrograph of normal Telophase I is shown in Fig. 4f. Meiosis II is normal (Figs. 4g to i) as well. Although laggards were observed, their frequency is very low. The normal occurrence of meioses I and II in *C. roseus* would explain why pollen fertility in this species is high (94.96%).

Karyotype Analysis in *Chrysanthemum indicum* Linn.

The chromosome number of *C. indicum* was established using somatic root tip cells. Diploid chromosome number ranged from 44 to 48. Variation in chromosome counts could be due to bud mutations (Darlington 1963). Chromosomes are lost or gained at mitosis during the ordinary course of growth. Layers of tissue arise with different chromosome

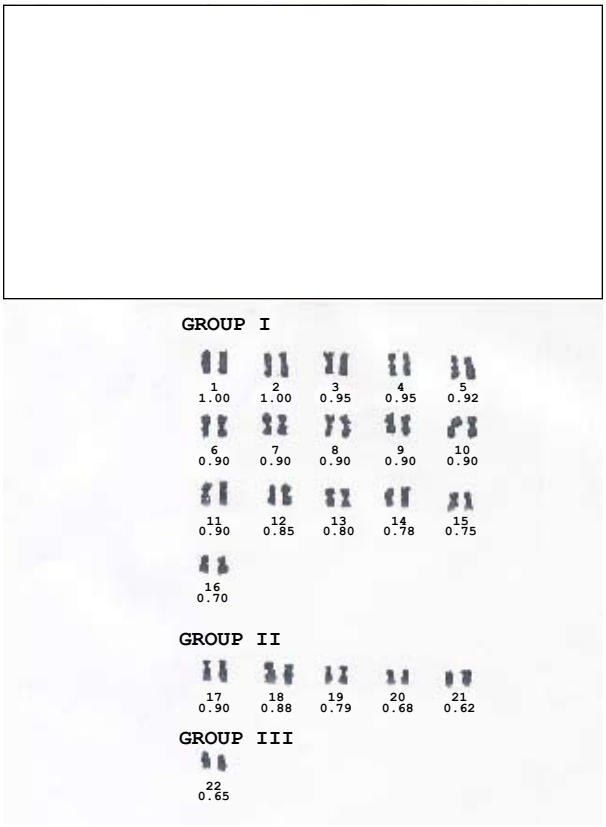

Figure 5. Cells at C-metaphase (400 X) (above) and karyogram(below) of *Chrysanthemum indicum* Linn. (manzanilla) with 2n = 44

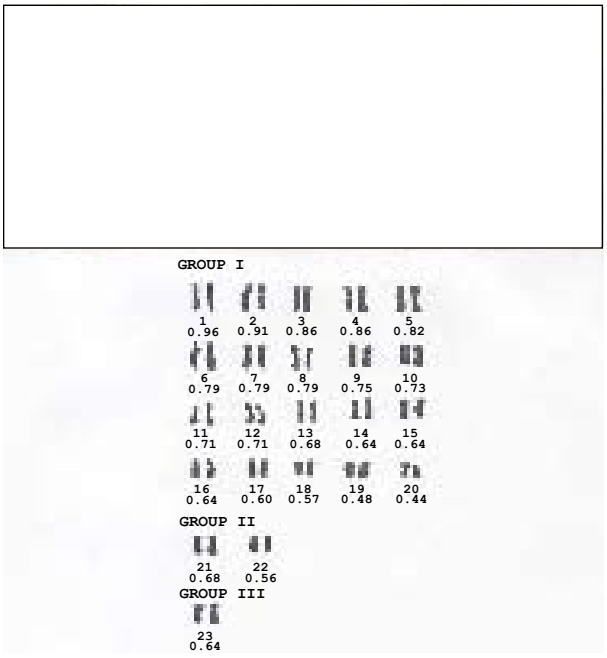

Figure 6. Cells at C-metaphase (400X) (above) and karyogram(below) of *Chrysanthemum indicum* Linn. (manzanilla) with 2n = 46

Figure 7. Cell at C-metaphase (400X) (above) and karyogram (below) of *Chrysanthemum indicum* Linn. (manzanilla) with 2n = 48

numbers, variety becomes a chimera which eventually, may grow wholly to a new type.

Karyograms of plants with 2n=44, 46 and 48 are shown in Figs.5, 6, and 7, respectively. Based on Levan's chromosome nomenclature (1964), three groups of chromosomes were found, namely:

Group I consists of median chromosomes with an average relative length ranging from 0.65 to 1.0. Arm ratios ranged from 1.17 to 1.34.

Group II consists of submedian chromosomes with an average relative length ranging from 0.52 to 0.82. Arm ratios ranged from 1.96 to 2.30.

Group III consists of chromosomes with subterminal centromeric positions. Relative lengths ranged from 0.52 to 0.67. Arm ratios ranged from 3.33 to 7.0.

Variabilities in chromosome numbers and characteristics exist in *C. indicum*. Sharma and Sharma (1959) claimed that plants grown vegetatively through cuttings like manzanilla develop different chromosomal biotypes by bud mutations.

Implications in species identification, diversity studies, and plant improvement

Information on the cytogenetic system operating in a species can serve as the bases for conclusions on taxonomy and genetic diversity. The chromosome number is known to characterize a species or a group of species and is specific for a given species. The observed chromosome numbers of the four studied species collected in the Philippines, confirmed the previous reports done on foreign populations of the species. Therefore, the established chromosome number of *E. pulcherrima* is 2n=28, *M. oleifera* is 2n=28, and *C. roseus* is 2n=16. In the case of *C. indicum*, chromosome mosaicism was observed with 2n=44 to 48. The chromosome morphology of *C. indicum* can be used to compare it with other species of the chrysanthemum family and, from there, the relationships between species can be deduced.

The observed regularity in the meiotic chromosome behavior of *E. pulcherrima*, *M. oleifera* and *C. roseus* means a high degree of fertility in each of the species. This information can help in the prediction of the possible outcome of hybridization with related species and in evaluating the possible gene transfer from one species to another. For example, chromosome pairing or chiasma frequency measures the potential for intrachromosomal recombination. Furthermore, aberrant chromosome behavior during meiosis may cause sterility and therefore prevent the recovery of recombinants.

SUMMARY AND CONCLUSION

Meiotic studies were done on *E. pulcherrima*, *M. oleifera* and *C. roseus*. The chromosome number of *C. roseus* is 2n=16 while both *M. oleifera* and *E. pulcherrima* have 2n=28. Meioses I and II in C. roseus are normal. Metaphase and Anaphase I and II of *M. oleifera* and *E. pulcherrima* are characterized by late disjunction and presence of lagging chromosomes. But these chromosomes were able to catch up since at Telophase I and II, all cells were normal. The highly normal meioses I and II of these three species would lead to a high degree of fertility, which makes possible the improvement of the species through conventional hybridization programs.

C-Metaphase of root tip cells of *C. indicum* revealed a diploid chromosome number of 44, 46 and 48. Based on the positions of the centromeres, the chromosomes were categorized into Group I (median), Group II (submedian), and Group III (subterminal). Relative lengths ranged from 0.40 to 1.0.

Variability of karyotypes may be attributed to bud mutations occurring in vegetatively reproducing plants like *C. indicum*. These mutations if stable can be a good source of genetic variability.

REFERENCES

CONCHA JA. 1980. Phil. National Formulary. 1st ed. National Science Development Board Taguig, Metro Manila. 17 p.

DARLINGTON CD. 1963. Chromosome Botany and the Origin of Cultivated Plants. New York: Hafner Pub. Co. 201 p.

DARLINGTON and WYLIE. 1945. Chromosome Atlas of Flowering Plants. London: George Allen and Co. Inc., Ltd. p. 37

DE PADUA LS, LUGOD and PANCHO JV 1977. Handbook of Philippine Medicinal Plants. Vol. 1. Laguna, Philippines: UPLB Documentation and Information Section. 39 p.

DE PADUA LS and PANCHO JV 1983. Handbook on Phil.Medicinal Plants Vol. 4. Laguna, Philippines: University of the Philippines, Los Baños Information Section. 68 p.

DE PADUA LS 1996. Medicinal Plants. Philippines: Philippines Island Pub. House Inc. 54 p.

ESCOTE LJ 1978. Cytological and morphological study of *Saccharum spontaneum* and related genera in the Philippines and *S. officinarum* hybrids. [M.S. Thesis] Laguna, Philippines: University of the Philippines Los Baños 154 p.

EWART MS and WALKER DE. 1960. Chromosome numbers of poinsettia, *Euphorbia pulcherrima* Klotsch. J Heredity 51:203-208.

FONTANEL A and TABATA AM. 1986. Production of Secondary Metabolites by Plant Tissue and Cell Cultures: Present Aspects and Prospects. Lausanne, Switzerland: Nestle Research News. 65 p.

KINGSTON DC and SAMI A. 1979. Cytotoxicity of modified indole alkaloids. J Pharm Sci 66(1): 1483.

LEVAN A, FREDGA K and SANDBERG AA. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52:201-218.

MOYER LS. 1934. Electrophoresis of latex and chromosome numbers of poinsettias. Bot Gaz 95:678-685.

PRATT WB. 1994. The Anticancer Drugs. 2nd ed. New York: Oxford Press. Inc. p. 189-191.

QUISUMBING E. 1978. Medicinal Plants of the Philippines. Manila: Katha Pub. Co. Inc. 507 p.

SHARMA AK and SHARMA A. 1959. Chromosomal alterations in relation to speciation. Bot Rev 25(3):514-535.

SINHA SS and ACHARIA A. 1975. Meiotic analysis in some varieties of *Lens culinaris*. Cytologia 40:269.

TARAR JL. 1980. Comparison of ethyl-methyl sulfonate and radiation induced meiotic abnormalities in *Turnera ulmifolia* L. var. angustifolia Wild. Cytologia 45:221-223.