CV

CITOGENÉTICA VEGETAL

PLANT CYTOGENETICS

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CV 1

PATRONES DE BANDAS C-DAPI Y DAPI/CMA3 EN ESPECIES DIPLOIDES DEL GÉNERO *Andropogon* L., GRAMINEAE

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Andropogon L. posee especies con diferentes niveles de ploidía distribuidas en América y África. En el presente trabajo se analizaron los patrones de distribución y composición de la heterocromatina de tres especies (2n=2x=20) del género: *A. selloanus* (Hack.) Hack., *A. macrothrix* Trin. y *A. gyrans* Michx. mediante bandeo C-DAPI y DAPI/CMA3. Se analizaron cromosomas provenientes de meristemas de ápices radiculares pretratados con 8-Hidroxiquinoleína durante 5 h y conservadas en solución Farmer a 5° C. Las células se observaron y fotografiaron mediante el uso de un microscopio de epifluorescencia Leica con cámara digital. Las imágenes fueron procesadas mediante Photoshop CS5. Los resultados permitieron revelar cinco patrones diferentes de distribución de la heterocromatina C-DAPI y siete patrones de composición de la heterocromatina según la distribución y el tamaño de las bandas DAPI/CMA3. Si bien los patrones son muy conservados entre estas especies, existen variaciones en la posición y número de las bandas. La heterocromatina terminal tiene aspecto de pequeñas bandas, las intersticiales y pericentroméricas algunas poseen el aspecto de bandas delgadas y otras puntiformes, las centroméricas adoptan una disposición lateralizada en la mayoría de los cromosomas. Respecto de la posición de las bandas, *A. selloanus* y *A. macrothrix* mostraron un mayor número de bandas centroméricas DAPI+/CMA+, y *A. gyrans*, DAPI+/CMA-; la presencia de bandas terminales, en su mayoría DAPI+/CMA+ (*A. selloanus* y *A. macrothrix*) y en menor número CMA+/DAPI- (*A. gyrans*). Cabe destacar que los satélites revelaron heterocromatina C+ y CMA+/ DAPI-.

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CV 2

¿EL NÚMERO CROMOSÓMICO INCREMENTA CON LA LATITUD EN PLANTAS TERRESTRES CHILENAS?

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La configuración geográfica de Chile, extendida a lo largo de un gradiente latitudinal y climático (4.200 km, 18° S-54° S), ha generado un mosaico de ambientes favorables para el crecimiento de las plantas. La flora de Chile continental incluye ca. 5.800 especies y la distribución de su diversidad genética a lo largo del gradiente no es bien conocida. Actualmente, se conoce el número cromosómico (NC) de 500 especies incluidas en Briófitos, Pteridófitos, Pinófitos y Magnoliófitos (8,6% del total). Una hipótesis citogeográfica propuesta para plantas del hemisferio norte sugiere que el número cromosómico promedio (NCP) incrementa hacia latitudes mayores y climas fríos. Esto no ha sido estudiado en especies chilenas. Con el objetivo de someter a prueba esta hipótesis, se recopilaron datos sobre número cromosómico y localización geográfica de 500 especies distribuidas en cuatro zonas macrobioclimáticas a lo largo del gradiente. Se compararon valores NCP entre especies de cada zona y los datos se representaron gráficamente. Los resultados mostraron diferencias en los NCP entre zonas, con un incremento gradual del NC y niveles de ploidía desde los 18° S hacia el sur. El mayor NCP (34,6 ± 46,7) se observó en la zona templada y sub-mediterránea de Chile (zona sur 36° S-45° S), con un rango de NC entre 8 (2x) y 480 (8x). Los valores de NCP disminuyen entre los 46° S y 54° S. Estos resultados, aún preliminares, respaldan parcialmente la hipótesis planteada para una parte del gradiente en Chile que cubre cerca de 3.700 km entre los 18° S y 45° S.

Núcleo de Estudios Ambientales (NEA-UCT)

CV 3

PREDICTION OF POLYEMBRYONY AND POLYPLOIDY USING A STOMATAL MORPHOMETRY OF POPULATIONS MONOEMBRYONICS AND POLYEMBRYONICS OF *Eriotheca gracilipes*

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Polyembryony, the presence of more than one embryo per seed, is common in Cerrado species, the brazilian savanna. *Eriotheca gracilipes*, an arboreal species widely distributed, presents distinct embryonic patterns among its population and are related to different modes of reproduction and polyploidy (basic number for *Eriotheca* x=46). However, studying the distribution of these reproductive and cytological patterns is difficult and costly, so morphological proxies that help understand such distribution would be important. We evaluated the relationship between the embryonic pattern found in populations of *E. gracilipes* and stomatal size for estimate ploidy level. Twenty-five populations of *E. gracilipes* were analyzed. The stomatal slides were prepared using the foliar decal technique using the abaxial part of the leaves and height and width of the stomata were measured. The embryonic patterns presented by the populations were established by seed dissection in a previous study. Six populations of the present study were monoembryonic, 11 polyembryonic and two had low frequencies of polyembryony. Some populations were treated as indefinite since their embryonic pattern could not be identified previously. Embryonic patterns and polyploidy showed a certain geographical division, allowing inferences on how the distribution and dispersion of these populations may have occurred. The polyembryonic pattern has shown to be more widely distributed, evidencing the dispersion and colonization ability that this characteristic can confer to individuals. All studied populations that presented high polyembryony were polyploidy (2n=6x estimated based in stomatal size) and the sexual populations were diploid (2n=2x estimated based in stomatal size).

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Plantas cultivadas - Dicotiledóneas / Crop Species - Dicotyledons

CV 4

COMPARATIVE CYTOGENOMICS OF THE GENERA *Cenostigma* AND *Libidibia* (CAESALPINIA: LEGUMINOSAE)

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Cenostigma is the most diverse genus of the Caesalpinia group in the Northeast Brazil; in contrast, *Libidibia* genus is distributed disjointly in three centers of diversity: [I] Northeast/Southwest of Brazil, [II] Chaco and [III] Mesoamerica. Cytogenetically, both genera show 2*n*=24 with heterochromatic bands in the proximal regions of the chromosomes. Genomically, this heterochromatin seems to be enrichment for retrotransposons (RTs) and DNA satellites (DNAsat). The objective of this work was to perform a comparative genomic and cytogenomic analysis in species of *Cenostigma* and *Libidibia* (including representatives from the three centers of diversity). Cytogenomic analyzes included hybridizations of repetitive elements (RTs Ty3/Tekay and Athila; DNAsat LifeSat148) previously mapped in *C. microphyllum* and *L. ferrea*. Genomes of *C. microphyllum* and *C. pyramidale* showed similar repeats composition/abundance. Furthermore, RTs mapping in the proximal region of the *C. pluviosum* and *C. pyramidale* chromosomes was similar observed in *C. microphyllum*. Nevertheless, the genomes of *L. ferrea* and *L. coriaria* showed differences in the repeat's composition/abundance. The Athila and DNAsat LifeSat148 signals were located in the proximal region of the chromosomes. However, absence of Tekay signals in *L. coriaria* and *L. punctata* karyotypes suggest species-specific lineages. This results suggest a greater degree of genomic divergence in *Libidibia* than in *Cenostigma*. These distinct trends may be related to the different ages of the genera *Libidibia* (27 Mya) and *Cenostigma* (15 Mya). Additionally, it is possible that differentiated ecological niches of *Libidibia* species with disjoint distribution have an impact on the divergence of their genomes.

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CV 5

ANÁLISIS CITOMOLECULAR DEL EFECTO DE LA ALOPOLIPLOIDÍA SOBRE EL ADNr EN EL MANÍ CULTIVADO Y SU ANTECESOR TETRAPLOIDE SILVESTRE

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Las unidades repetitivas de los genes ribosomales no evolucionan de forma independiente dentro de un mismo genoma, sino que lo hacen de manera concertada, homogeneizando las distintas copias. Sin embargo, estos genes presentan variaciones interespecíficas, más significativas a mayor distancia filogenética. En alopoliploides, las variantes parentales del ADNr pueden permanecer intactas, estar sujetas a conversión génica o presentar distintos grados de modificación epigenética, lo que, en su conjunto, determina el perfil de expresión de los mismos. En algunos casos, como en *Arachis,* sólo los genes de un genoma se expresan, fenómeno denominado anfiplastia. El objetivo de este trabajo fue investigar el efecto de la alopoliploidía sobre los ADNr en *Arachis* a nivel citomolecular e inferir las causas de la anfiplastia, usando como modelo al maní cultivado, *A. hypogaea*, y su antecesor silvestre, *A. monticola*, ambos alopoliploides AABB. En base a las diferencias detectadas previamente por nuestro grupo en los ADNr de las especies parentales diploides, *A. duranensis* (AA) y *A. ipaënsis* (BB), se diseñó una sonda de 120pb específica para el IGS del genoma B. Las hibridaciones *in situ* fluorescentes revelaron señales en los tres loci ribosomales de *A. ipaënsis* pero en ninguno de los de *A. duranensis*. En los alotetraploides, sólo se observaron señales de hibridación sobre los tres loci ribosomales del genoma B. Estos resultados evidenciaron que no habría ocurrido conversión génica intergenómica masiva desde el evento de alopoliploidízación ocurrido hace unos 10.000 años. Se postula que la dominancia nucleolar en híbridos AABB de *Arachis* estaría determinada principalmente por interacciones epigenéticas.

CV 6

CYTOGENETICS AND IDENTIFICATION OF THE NUCLEOLAR ORGANIZING REGION (NOR) IN CAROLINA REAPER (*Capsicum chinense*)

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Among *Capsicum chinense* Jacq. cultivars, Carolina reaper variety has the highest level of pungency. This is a hybrid obtained from the crossing between 'Habanero' (*C. chinense*) and 'Naga Bhut Jolokia' (hybrid between *C. chinense* and *C. frutescens*). The aim of this study was to characterize the karyotype of Carolina reaper for plant breeding. The meristematic tissue from roots was pre-treated with 0.05% (w/v) of colchicine for six hours at 18º C, and fixed in Carnoy solution for 12 hours. For the preparation of the slides, the roots were washed with distilled water and dipped in HCl 2M at 37° C for 20 minutes for acid hydrolysis, dissected in acetic acid (45% v/v), and stained with Giemsa 10%. For the detection of nucleoli, cells were impregnated with silver nitrate. Chromosomal observations were made with a binocular optical microscope (Leica DM 750). Analysis of metaphasic cells revealed a chromosome number of $2n=24$, with a karyotypic formula of 11 metacentric chromosomes and 1 submetacentric chromosome. When stained with silver nitrate the cells evidenced one or two nucleoli marks, suggesting that the species is a simple NOR carrier. We observed that the occurrence of single nucleolus (69.5%) was predominant over the occurrence of two nucleoli (30.5%), and that heteromorphic nucleoli (72%) were more frequent than homomorphic ones (28%). The karyotype analysis is an important parameter to follow the genetic stability and to be used in plant breeding.

FAPEMIG

CV 7

REVISITING THE CHROMOSOME EVOLUTION OF *Phaseolus* L. (FABACEAE) USING OLIGO-FISH

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Beans are well-known for their nutritional and economic potentials, with five domesticated species belonging to *Phaseolus* L. (Fabaceae). The genus comprises about 75 species, mostly diploids with 2n=22, but also a diploid group of species (Leptostachyus, 2n=20), which experienced structural rearrangements. In this study, we investigated ten accessions through oligo-FISH barcode and painting, to compare the rate of chromosome evolution between Leptostachyus and the remaining species. Oligo probes were hybridized to identify the ortholog chromosomes. The identified rearrangements were indicated in a dated phylogenetic tree and chromosome evolution rates (number of rearrangements/million years) were estimated. The synteny of orthologs 2 and 3 were conserved for most species, except for the Filiformis and Leptostachyus groups, which presented different translocations. The barcode was efficient to identify all chromosome pairs in the analysed species. Changes in the position of signals in few orthologs of *P. microcarpus*, Acutifolius and Lunatus groups revealed breaks of collinearity due to inversions. The *P. leptostachyus* genome was more rearranged than predicted and, due to this, the barcode was less efficient to identify its orthologs. Nevertheless, it was possible to demonstrate that *P. leptostachyus* presented the highest chromosome evolutions rate (6.82 rearrangements per My) in comparison to its sister species *P. macvaughii* (1.82) and other species, such as Acutifolius (0.43), Filiformis (0.63), Lunatus (0.37), *P. microcarpus* (0.7), and Vulgaris (1.67). Our analysis indicated an increased chromosome evolution in the Leptostachyus group and the necessity of further approaches for understanding the causes of *P. leptostachyus* genomic repatterning.

Plantas cultivadas - Monocotiledóneas / Crop Species - Monocotyledons

CV 8

COMPARATIVE ANALYSIS OF THE GENOME REPETITIVE FRACTION OF *Cenchrus purpureus* AND *Cenchrus americanus*

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Cenchrus L. is an important genus of Poaceae that comprises several agricultural species, like *Cenchrus purpureus* (elephant grass) and *Cenchrus americanus* (pearl millet). They are of great interest either for breeding, due to their economical relevance, or for evolutionary genomic studies, due to their genome complexity. The use of next-generation sequencing (NGS) data combined with pipelines developed for the characterization of the repetitive genomic fraction is an interesting approach for comparative genomics studies. The objective of the present work was the comparative analysis of the repetitive genome fraction of *C. purpureus* and *C. americanus*. For that, the genomic DNA of both species was sequenced on the Illumina HiSeq™ 4000 platform. The individual and comparative analysis took place on the *RepeatExplorer* pipeline with the standard setup and on the Geneious Prime 2020.1.1. The repetitive fraction of the genome of *C. purpureus* and *C. americanus* corresponded to 52.23 and 76.82%, respectively. The most abundant repetitive elements in both species were the LTRs retrotransposons. Satellite DNA sequences were also identified in both genomes and corresponded to 2.55 and 4.17% of the genome of each species, respectively. The ancestral relationship and the polyploidization-diploidization cycles played a fundamental role in the composition of their repetitive fraction. These cycles led *C. americanus*, a possible paleopolyploid, to have a greater abundance of transposable elements when compared to *C. purpureus*, a recent allopolyploid. The satellite DNA concerted evolution process is the basis for the differentiation and amplification of those sequences between species.

CAPES, CNPq, FAPEMIG, Oswaldo Cruz Institute

CV 9

CARACTERÍSTICAS CROMOSÓMICAS REFLEJAN EL ORIGEN DE VARIEDADES CRIOLLAS DE MAÍZ DE LAS TIERRAS BAJAS

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Los *knobs* cromosómicos son bloques de heterocromatina que se tiñen diferencialmente y han sido utilizados para caracterizar citogenéticamente diferentes razas de maíz. El objetivo del trabajo fue caracterizar citogenéticamente y relacionar variedades criollas y razas de maíces de Uruguay y Brasil por características cromosómicas, en base al estudio del número y largo total de *knobs* cromosómicos, largo de complemento total, % de heterocromatina y presencia de cromosomas supernumerarios B. Se caracterizaron citogenéticamente 39 accesiones de variedades criollas pertenecientes a 21 razas de maíz, colectadas en Brasil y Uruguay, 21 y 18 accesiones, respectivamente. El análisis de número y largo de *knobs* se realizó mediante tinción con fluorocromo DAPI y se llevó a cabo un análisis de agrupamiento por el método de Ward . Se observó una clara separación en dos grupos correspondientes a ambos países, debido a diferencias en todas las características evaluadas, y reflejando los diferentes orígenes. En los resultados obtenidos se destacó la diferencia en la frecuencia de cromosomas B, de 0,250 para las variedades de Uruguay y 0,041 para las de Brasil. Los cromosomas B son comunes en razas andinas, lo que permite asumir que estas diferencias están asociadas con la ruta de dispersión hasta las tierras bajas.

CSIC I+D UDELAR (Uruguay), CNPq, FAPESP (Brasil)

CV 10

COMPARATIVE CHROMOSOMAL BEHAVIOR DURING MICROSPOROGENESIS OF SUGARCANE

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The modern varieties of sugarcane arose through interspecific hybridizations between the domesticated species *Saccharum officinarum* and the wild species *Saccharum spontaneum*. The goal was then the selection of superior progenies toward the ideotype of *S. officinarum* through the nobilization process, establishing the genetic basis of modern cultivars, which have a complex and polyploid genome. However, there are few studies on meiotic chromosomal behavior involving the parental species and modern varieties. In this study, the microsporogenesis of one clone of *S. officinarum* (Caiana Fita, 2n=80), one clone of *S. spontaneum* (SES205, 2n=64) and the Brazilian variety SP80-3280 (2n=112) were analyzed. Chromosomal abnormalities were documented in about 200 pollen mother cells from all genotypes. The variety SP80-3280 showed abnormalities in 77.5% of the cells; this result was similar to that found in clone SES205, in which 50.3% of the cells presented abnormalities, including several irregularities, mainly delayed chromosomes. In the Caiana Fita clone, on the other hand, a low number of irregularities (5.7%) was found, showing normal meiotic behavior. The association of chromosomes at diakinesis was analyzed using the FISH technique with centromeric probes. This analysis allowed us to infer that chromosomal pairing in all the genotypes is predominantly bivalent. This is an extremely important fact, as it implies that there is coordinated segregation of chromosomes, regardless of their origin, suggesting the existence of genetic control that regulates sugarcane meiosis, probably inherited from *S. officinarum*, since it has regular meiosis.

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CV 11

CHROMOSOMAL COMPOSITION AND TRANSMISSION IN A BRAZILIAN SUGARCANE VARIETY

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Sugarcane is of high industrial importance because 80% of the world's sugar is provided by this crop. Modern varieties of sugarcane are originated from interspecific hybridizations between *Saccharum officinarum* (known as "noble cane", widely cultivated due to its high sucrose content) with the wild species *Saccharum spontaneum*, which was used for incorporating resistance, vigor, and stress tolerance to clones of *S. officinarum*. During the nobilization process, hybrids were backcrossed with *S. officinarum* with the transmission of non-reduced (2n) gametes in the F_1 and RC_1 generations by *S. officinarum* (female). As a result of domestication and selection processes, modern varieties of sugarcane have a complex "artificial" genome that is polyploid and aneuploid. In this study, we demonstrate the chromosomal constitution of the Brazilian variety SP80-3280 (2n=112) using the GISH technique with nick translation-labeled probes. Caiana Fita - *S. officinarum* (2n=80) - was labelled by digoxigenin and SES205A – *S. spontaneum* (2n=64) - by biotin. About 12% of the chromosomes of the SP80-3280 variety were inherited from *S. spontaneum*, 9% are recombinant and the rest were inherited from *S. officinarum*. These results corroborate the recombinants chromosomes percentage found in modern sugarcane varieties (originated from homeological exchanges - interspecific recombination, between the genitors *S. spontaneum* and *S. officinarum*) and provide insights for future studies of chromosome behavior, genomics, and cytogenetics in Brazilians varieties sugarcane.

FAPESP / CNPq, Brazil