RAQUEL BEZERRA CHIAVEGATTO

Karyotype evolution in Eleusininae Dumort. (Poaceae, Chloridoideae, Cynodonteae): a phylogenetic and cytogenetic approach focusing on the genus *Cynodon* Rich.

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APROVADA em 29 de junho de 2018

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LAVRAS-MG

2018

A todos aqueles que saíram da sua zona de conforto em busca de conhecimentos.

Dedico.

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Resumo

A subtribo Eleusininae é a maior subtribo da família Poaceae com cerca de 238 espécies distribuídas em 31 gêneros. Na subribo Eleusininae encontra-se o gênero *Cynodon*, composto por 15 espécies, algumas com potencial forrageiro. Para ambos os taxa existem lacunas de conhecimento em relação a circunscrição taxonômica. As espécies pertencentes a subtribo Eleusininae apresentam formas citológicas diploides e/ou poliploides. *Cynodon* é um gênero conhecido pela ampla variação nos níveis de ploidia de diploide (2n = 2x = 18) a hexaploide (2n = 6x = 54). Compreender os eventos que ocasionaram a poliploidização natural na subtribo Eleusininae e no gênero *Cynodon* permitem selecionar as espécies com potencial forrageiro que poderão ser utilizadas no melhoramento, além de responder lacunas sobre a evolução do grupo. Muitas das questões taxonômicas, morfológicas e citogenéticas que ainda permanecem pouco elucidadas, poderão ser avaliadas com um robusto estudo citotaxonômico. Dessa forma, o presente estudo tem como objetivo reconstruir o estado ancestral do número cromossômico da subtribo Eleusininae, a fim de elucidar a história evolutiva do grupo. Este trabalho tem como foco o gênero *Cynodon*, devido à sua ampla distribuição geográfica e ao seu potencial forrageiro. A partir de análises citomoleculares comparou-se espécies/acessos de *Cynodon*, visando à compreensão da organização genômica e cromossômica do gênero sendo geradas informações para estudos taxonômicos, evolutivos e de melhoramento genético do grupo. A evolução das espécies de ambos os taxa ocorreu por forte influência de eventos de poliploidização e disploidia. O número cromossômico ancestral da subtribo Eleusininae é $p = 6$ e do gênero *Cynodon* é $p = 10$. Contudo, o número básico para *Cynodon* é $x = 9$. O ancestral comum das espécies, *Cynodon dactylon* (L.) Pers., *Cynodon nlemfuensis* Burtt- Davy, *Cynodon incompletus* Nees e *Cynodon transvaalensis* Vanderryst, apresentava dois sítios de rDNA 5S e 35S, duas bandas de CMA, todos os cromossomos com bandas $DAPI^+$ terminais no braço longo, cinco cromossomos com cinco bandas $DAPI^+$ no braço curto e três cromossomos com bandas $DAPI^+$ na região centromérica. A diversidade cariotípica de *Cynodon* é influenciada por perdas e ganhos de sequências que formam bandas $DAPI^+$ e pela mobilidade de genes ribossomais.

Palavras-chaves: Evolução cromossômica. Estado ancestral. Gramíneas forrageiras.

Abstract

Eleusininae is the largest subtribe of the Poaceae family with about 238 species in 31 genera. Within subtribe Eleusininae there is the genus *Cynodon*, with 15 species accepted, some species with forage potential. In both taxa the taxonomic circumscription, it is not well elucidated. Species belonging to Eleusininae are diploid and/or polyploid cytological forms. *Cynodon* is a genus with a wide variation in levels ploidy from diploid (2n = 2x = 18) to hexaploid (2n = 6x = 54). Understanding the events that caused natural polyploidization in the subtribe Eleusininae and *Cynodon*, allow selecting the species with potential forage that can be used in genetic breeding. Besides, answering gaps on the evolution of the group. Many taxonomic, morphological, and cytogenetic questions which are not yet well elucidated can be evaluated with a robust cytotaxonomic study. Thus, the present study aims to reconstruct ancestral state of the chromosome number in Eleusininae, in order to elucidate the evolutionary history of the group. This work has a focus in *Cynodon* genus, due to its wide geographical distribution and forage potential. Based on cytomolecular and morphological analyzes we compared *Cynodon* species/accessions, aiming to understand the genomic and chromosomal organization of the genus and generate information for taxonomic, evolutionary and genetic breeding studies of the group. In both taxa, speciation events occurred due to high influence of polyploidization events. The chromosome number ancestral of Eleusininae is $p = 6$ and *Cynodon* is $p = 10$. However, the basic number for *Cynodon* is $x = 9$. The common ancestor of the species, *C. dactylon*, *C. nlemfuensis*, *C. incompletus*, and *C. transvaalensis*, has two 5S and 35S rDNA sites, two CMA^+ bands, all chromosomes have $DAPI^+$ terminal bands on the long arm, five chromosomes have five $DAPI^+$ bands on the short arm and three chromosomes with $DAPI^+$ bands on the centromeric region. The karyotypic diversity of *Cynodon* is influenced by losses and gains of $DAPI^+$ bands and the mobility of ribosomal genes.

Key-words: Ancestral state. Chromosomal evolution. Forage grasses.

SUMÁRIO

PRIMEIRA PARTE

1 ANTECEDENTES E JUSTIFICATIVA

A subtribo Eleusininae é a maior subtribo da família Poaceae com cerca de 238 espécies distribuídas em 31 gêneros. A circunscrição taxonômica da subtribo Eleusininae vem passando por mudanças após análises filogenéticas baseadas em espaçadores transcritos internos (ITS) e DNA nuclear e cloroplastidial. Os gêneros pertencentes às subtribos Pommereullinae e Chloridinae foram reclassificados, e atualmente, pertencem a Eleusininae (PETERSON et al., 2007; 2010; 2015; 2016).

O gênero *Cynodon* está incluído na subtribo Eleusininae (SORENG et al., 2015), é composto por espécies com potencial forrageiro e utilizadas para múltiplos fins em todos os continentes do mundo, como pastagens ou forragens armazenadas, na cobertura do solo e relvado (WU, 2011).

A maioria das espécies de *Cynodon* é originária das regiões orientais, tropicais e do sul da África, sendo introduzida nos Estados Unidos, Canadá, Nova Zelândia, Austrália e América do Sul (JEWELL et al., 2013). Caro e Sanchez (1969) relataram a ampla distribuição geográfica de *Cynodon*, sendo algumas plantas nativas, coletadas e identificadas no México, Guatemala, Panamá, Argentina, Uruguai e Brasil. *Cynodon dactylon* var. *dactylon* (L.) Pers é cosmopolita, enquanto as espécies *Cynodon incompletus* Nees, *Cynodon transvaalensis* Burtt Davy e *Cynodon nlemfuensis* Vanderyst são endêmicas do Sul da África (ASSEFA et al., 1999; WU, 2011).

Comumente, caracteres morfológicos são utilizados como critérios de classificação dentro do gênero. Baseando-se na presença/ausência de caracteres como estolões e/ou rizomas, pode-se delimitar dois grandes grupos, como as “gramas bermuda” que são caracterizadas pela presença de rizomas e estolões, como *C. dactylon*, e as “gramas estrelas”, que apresentam apenas estolões, como C*ynodon plectostachyus* (K. Schum.) Pilg, *Cynodon aethiopicus* Clayton & Harlan e *C. nlemfuensis*. Estas estruturas conferem características especiais às plantas, como por exemplo, maior resistência ao pastejo devido à capacidade de rebrota conferida pelo meristema (NASCIMENTO et al., 2002).

Apesar de caracteres morfológicos apresentarem grande valor de identificação dentro do grupo, algumas espécies apresentam variedades e/ou citótipos morfologicamente semelhantes, o

que pode ter levado a interpretações equivocadas na identificação dos indivíduos e consequentemente a classificações pouco robustas. Outros estudos como os de Harlan e colaboradores (1969), baseados na hibridização das espécies de *Cynodon*, também reforçaram a dificuldade na delimitação taxonômica do grupo. Não obstante, atualmente não há na literatura uma classificação taxonômica completamente satisfatória, no qual a complexidade está em determinar o grau de parentesco entre estes diversos táxons. Dessa forma, há um elevado número de sinonímias no grupo e o número de espécies diverge entre 9 a 15. De acordo com o banco de dados Kew's online grass database, são aceitas 15 espécies dentro do gênero *Cynodon* (CLAYTON et al., 2016).

Além das dificuldades encontradas para circunscrição taxonômica do gênero, existem lacunas de conhecimento em relação ao número básico de cromossomos, sendo encontrados relatos de espécies com o número básico de x=9 (AVDULOW, 1931) e x=10 (HUNTER, 1934). *Cynodon* apresenta variação no nível de ploidia de diploide (2n = 2x = 18) a hexaploide (2n = 6x = 54) (DHALIWAL; GUPTA, 2011). Essa amplitude indica que a diversificação das espécies de *Cynodon* foi influenciada por eventos de poliploidização.

Essas ambiguidades constituem um problema para a conservação e para o intercâmbio dos recursos genéticos, pois muitas plantas podem ser incorporadas às coleções de germoplasma ou usadas em cruzamentos de maneira equivocada. A citotaxonomia é uma excelente fonte de informação para os estudos taxonômicos e evolutivos, uma vez que o cariótipo é o próprio genoma da espécie e não depende da expressão de genes, condições ambientais, idade e fase de desenvolvimento (GUERRA, 2012).

Análises citogenéticas convencionais (CHIAVEGATTO et al., 2016) e citomoleculares via bandamento CMA e DAPI (CHAVES et al., dados não publicados) e hibridização *in situ* fluorescente (FISH), tais como os sítios de rDNA 5S e 35S (ZHI-YUN et al., 2013) já foram empregadas com sucesso no gênero *Cynodon* a fim de identificar e localizar sequências específicas nos cromossomos, facilitando a análise do cariótipo e do genoma das espécies. Essas análises têm valor não apenas na identificação de cromossomos e dos genomas como também na elucidação das relações evolutivas e taxonômicas (HESLOP-HARRISON, 2000; GUERRA, 2004; VAIO et al., 2005; COSTA et al., 2017).

Compreender os eventos envolvidos na poliploidização natural da subtribo Eleusininae e, especificamente, do gênero *Cynodon*, permite selecionar as espécies com potencial forrageiro que

poderão ser utilizadas no melhoramento, além de responder lacunas sobre a evolução do grupo. Muitas das questões taxonômicas, morfológicas e citogenéticas que ainda permanecem pouco elucidadas, poderão ser avaliadas com um robusto estudo citotaxonômico.

O presente estudo teve como objetivo reconstruir o estado ancestral do número cromossômico da subtribo Eleusininae, a fim de elucidar a história evolutiva do grupo. Este trabalho apresenta foco no gênero *Cynodon*, devido ao seu potencial forrageiro. Dessa forma, a partir de análises citomoleculares comparou-se acessos de *Cynodon*, visando à compreensão da organização genômica e cromossômica do gênero e geração de informações para estudos taxonômicos, evolutivos e de melhoramento genético do grupo. Os resultados obtidos também poderão dar suporte a possíveis revisões taxonômicas, além de gerar informações importantes para os programas de melhoramento, intercâmbios e programas de conservação de recursos genéticos.

2 REFERENCIAL TEÓRICO

2.1 A subtribo Eleusininae

A família Poaceae Barnhart abrange cerca de 12,074 espécies, 771 gêneros, 12 subfamilias, 51 tribos e 80 subtribos. Eleusininae é a maior subtribo da tribo Cynodonteae, com 237 espécies distribuídas em 30 gêneros (SORENG et al., 2015). Essas espécies são de regiões tropicais, distribuídas especialmente na África, Sudeste da Ásia, América e Austrália (PETERSON et al., 2010).

Apesar da sua abrangência, há poucas informações disponíveis na literatura a respeito dessa subtribo (PETERSON, 2015; SORENG et al., 2015). A subtribo Eleusininae era uma subtribo formada por apenas um gênero, *Eleusine* Gaertn. (PETERSON et al., 2007). A tribo Cynodonteae era formada por outras duas subtribos, a Chloridinae (*Chloris* Sw., *Ctenium* Panz., *Cynodon*, *Dactyloctenium* Willd., *Eleusine*, *Eustachys* Desv., *Gymnopogon* P.Beauv., *Harpochloa* Kunth, *Leptochloa* P.Beauv., *Microchloa* R.Br., *Pleuraphis* Torr., *Schoenefeldia* Kunth, *Spartina* Schreb. e Triplasis P.Beauv.) (PETERSON et al., 2010) e a subtribo Pommereullinae (*Astrebla* F.Muell., *Lintonia* Stapf e *Pommereulla* L.f.) (PETERSON et al.,

2007). Através de análises filogenéticas baseadas em DNA nuclear e cloroplastidial, Peterson et al. (2010) reorganizaram a subtribo Eleusininae, agrupando essas três subtribos.

A subtribo Eleusininae é considerada um grupo monofilético com o gênero *Dinebra* como grupo-irmão dos demais gêneros do táxon (MUCHUT et al., 2017). Os estudos filogenéticos realizados por Peterson e colaboradores (2007, 2010, 2015, 2016) trouxeram grandes conhecimentos das relações de parentesco do grupo e possibilitaram uma nova circunscrição taxonômica do taxon. Os gêneros *Brachyachne*, *Leptochloa*, *Chloris* e *Tetrapogon* foram os que mais sofreram mudanças. *Cynodon* é o gênero mais derivado da subribo e era considerado parafilético, pois algumas espécies do gênero *Brachyachne* estavam circunscritas nesse clado. Atualmente, o gênero *Brachyachne* não existe e suas espécies foram reclassificadas para o gênero *Cynodon* e *Micrachne*, este último descrito como um novo gênero. Algumas espécies do gênero *Chloris* foram reclassificadas no gênero *Stapfochloa*. Por sua vez, os gêneros *Tetrapogon* e *Leptochloa* incorporaram algumas espécies dos gêneros *Chloris* e *Enteropogon*. Apesar da nova circunscrição taxonômica, os gêneros *Coelachyrum* e *Schoenefeldia* permanecem parafiléticos.

As espécies de Eleusininae compartilham características morfológicas semelhantes, como a presença de espiguetas abrangendo pequenas flores hermafroditas, porém as espiguetas podem conter uma, duas ou múltiplas flores (MUCHUT et al., 2017). Esses autores inferiram o estado ancestral das inflorescências da subtribo Eleusininae com o objetivo de elucidar a história evolutiva das inflorescências e contribuir para compreender as relações entre tipos diferentes de inflorescências, ilustrando a ancestralidade e os processos evolutivos.

2.2 Importância, Taxonomia e morfologia do gênero *Cynodon*

Cynodon é um gênero pertencente à subfamília Chloridoideae Kunth ex Beilschm e tribo Cynodonteae Dumort e subtribo Eleusininae (SORENG et al., 2015). A maioria das espécies de *Cynodon* é originária das regiões orientais, tropicais e do sul da África, sendo introduzida nos Estados Unidos, Canadá, Nova Zelândia, Austrália e América do Sul (JEWELL et al., 2013). A espécie *C. dactylon* é cosmopolita, enquanto as espécies *C. transvaalensis* e *C. incompletus* são endêmicas do Sul da África (ASSEFA et al., 1999). Caro e Sanchez (1969) relataram a ampla distribuição geográfica de *Cynodon*, sendo algumas plantas nativas, coletadas e identificadas, no México, Guatemala, Panamá, Argentina, Uruguai e Brasil.

As espécies de *Cynodon* apresentam grande capacidade de adaptação, podendo crescer nos mais variados tipos de solo. São utilizadas como espécies forrageiras, devido ao elevado valor nutritivo, utilizadas sob pastejo ou na forma de feno, em gramados, campo de golfe e jardinagem (NASCIMENTO et al., 2002). Nas condições brasileiras, essas espécies apresentam um bom potencial forrageiro quando bem adubadas, com elevada produção e porcentagem de proteínas. Também são utilizadas na produção de feno, devido a elevada produção de massa seca por unidade de área e apresenta facilidade de rebrota e secagem (CORRÊA; SANTOS, 2003).

Durante muitas décadas, as espécies de *Cynodon* foram vistas como invasoras, devido a sua grande capacidade de adaptação a diferentes ambientes, sendo consideradas como um grande problema à agricultura americana. Em 1943, houve o lançamento da primeira cultivar melhorada, Coastal, pelo Dr. Glenn Burton do Departamento de Agricultura dos Estados Unidos (USDA-ARS). Após este lançamento iniciou-se a utilização dessas gramíneas como espécies forrageiras, representando uma revolução na pecuária dos EUA. A partir de então ocorreram diversos lançamentos de híbridos advindos de programas de melhoramento genético (PEDREIRA et al., 1998).

Os representantes do gênero *Cynodon* são divididos popularmente em dois grupos, as "gramas bermuda" que são caracterizadas pela presença de rizomas e estolões, como *C. dactylon* e as "gramas estrelas", que apresentam apenas estolões, como *C. plectostachyus*, *C. aethiopicus* e *C. nlemfuensis*. Estas estruturas conferem características especiais às plantas, como por exemplo, maior resistência ao pastejo devido a capacidade de rebrota conferida pelo meristema (NASCIMENTO et al., 2002). As gramas são bem resistentes e adaptadas aos invernos moderadamente frios, enquanto as estrelas, ainda que bem adaptadas ao frio, são menos resistentes, devido à ausência de rizomas (VILELA; ALVIM,1998). A princípio as espécies do gênero *Cynodon* foram classificadas baseadas apenas nessa morfologia do caule (NASCIMENTO et al., 2002), uma vez que a classificação de acordo com os padrões dessas variações morfológicas é relativamente simples de construir.

Para o gênero *Cynodon* não há uma classificação taxonômica favorável, além das controvérsias em relação ao número de espécies, há um grande número de espécies com sinonímias e alguns táxon que não foram completamente elucidados (CHIAVEGATTO et al., 2016).

Harlan e colaboradores (1969) classificaram as espécies de *Cynodon* de acordo com a morfologia, comportamento ecológico e distribuição geográfica. Nesta revisão taxonômica, o gênero incluiu nove espécies: *Cynodon aethiopicus* Clayton & Harlan, *Cynodon arcuatus* J. Presl, *Cynodon barberi* Rang & Tadul , *Cynodon dactylon* (L.) Pers, *Cynodon incompletes* Nees, *Cynodon nlemfuensis* Vanderyst, *Cynodon plectostachyus* (K. Schum.) Pilg, *Cynodon transvaalensis* Burtt Davy, *Cynodon* x *magennisii* Hurcombe. Além das espécies, foram descritas 10 variedades: *Cynodon dactylon* (L.) Pers var dactylon, *Cynodon dactylon var* afghanicus Harlan & de Wet, *Cynodon dactylon* var *aridus* Harlan & de Wet, *Cynodon dactylon* var coursii A. Camus, *Cynodon dactylon* var elegans Rendle, *Cynodon dactylon* var polevansii (Stent) Harlan & de Wet, *Cynodon incompletus* Nees incompletus, *Cynodon incompletus* var *hirsutus* (Stent) de Wet & Harlan, *Cynodon nlemfuensis* Vanderyst var *nlemfuensis*, *Cynodon nlemfuensis* var *robustus* de Wet & Harlan. Além disso, em *Cynodon dactylon* var. *dactylon* foram reconhecidas três raças, tropical, temperada e selêucida (WU, 2011). A raça selêucida é distribuída no Oriente Médio (HARLAN et al., 1970).

Pesquisadores liderados por Caro (CARO; SANCHEZ, 1969; 1972; CARO, 1983) estudando as características morfológicas externas e anatomia das folhas do gênero *Cynodon* do continente americano, identificaram doze novas espécies pertencentes a este táxon: *Cynodon laeviglumis* Caro e Sanchez, *Cynodon hirsutissimus* Caro e Sanchez, *Cynodon affinis* Caro e Sanchez, *Cynodon maritimus* Caro e Sanchez, *Cynodon aritistglumis* Caro e Sanchez, *Cynodon mucronatus* Caro e Sanchez, *Cynodon nitidus* Caro e Sanchez, *Cynodon aristulatus* Caro e Sanchez, *Cynodon iraquensis* Caro, *Cynodon pedicellatus* Caro, *Cynodon scabrifolius* Caro e *Cynodon umbellatus* Caro. *Cynodon erectus* Presl. e *Cynodon pascuus* Nees foram classificadas como sinônimos de *Cynodon dactylon* , devido a grande semelhança entre as espécies. Porém, Caro e Sanchez (1972), após estudos taxonômicos, observaram que se tratavam de espécies distintas.

Recentes estudos filogenéticos baseados em regiões ITS e DNA nuclear e cloroplastidial investigaram as relações parentais das espécies descritas e classificadas por Harlan et al (1970) e Caro e Sanchez (1969; 1972; 1983). Nesses trabalhos algumas espécies do gênero *Brachyachne* foram circunscritas no gênero *Cynodon* (PETERSON et al., 2015; 2016). Após os trabalhos realizados por Peterson e colaboradores (2015; 2016) ocorreu uma nova circunscrição no gênero *Cynodon*. As espécies de *Brachyachne*, circunscritas em *Cynodon*, foram reclassificadas no

gênero *Cynodon*. Contudo, as espécies identificadas como *Cynodon* por Caro e Sanchez (1969; 1972; 1983) permanecem como sinonímias de *C. dactylon* e *C. incompletus*.

Atualmente são aceitas 15 espécies de *Cynodon*: *C. aethiopicus*, *Cynodon ambiguus* (Ohwi) P.M.Peterson, *C. barberi*, *Cynodon convergens* F.Muell., Fragm., *C. coursii*, *C. dactylon*, *C. incompletus*, *C. × magennisii*, *C. nlemfuensis*, *C. plectostachyus*, *Cynodon prostratus* (C.A.Gardner & C.E.Hubb.) P.M.Peterson, *Cynodon prostratus* (C.A.Gardner & C.E.Hubb.) P.M.Peterson, *Cynodon radiatus* Roth in J.J.Roemer & J.A.Schultes, *Cynodon simonii* P.M.Peterson, *Cynodon tenellus* R.Br. e *C. transvaalensis* (CLAYTON et al., 2016).

Apesar de Caro e Sanchez (1983) mostrarem evidências que os gêneros *Cynodon* e *Brachyachne* são gêneros sinôminos, apenas com as investigações de Soreng et al. (2015) essas sinonímia foi aceita. As espécies do gênero *Brachyachne* foram reagrupadas nos gêneros *Cynodon* e no novo gênero *Micrachne* P.M.Peterson, Romasch. & Y.Herrera.

2.3 Aspectos citogenéticos do gênero *Cynodon*

Cada cromossomo ou par de cromossomos representa um papel fundamental no desenvolvimento de um indivíduo. O número de cromossomos e as variantes que cada um deles apresenta dentro de uma espécie são dados importantes para a determinação da posição filogenética e taxonômica das espécies. O número cromossômico é um dos parâmetros mais utilizados para a caracterização citológica de uma espécie que, aliado à outros caracteres citológicos, fornece informações para a compreensão das alterações genéticas envolvidas (GUERRA, 1988).

O gênero *Cynodon* apresenta espécies com diferentes níveis de ploidia, variando de diploide a hexaploide (DHALIWAL; GUPTA, 2011), com número básico de cromossomos de $x = 9$ (AVDULOW, 1931), porém há relatos para $x = 10$ (HUNTER, 1934) e $x = 8$ (DHALIWAL; GUPTA, 2011).

Hurcombe (1947) investigou o número de cromossomos de um híbrido e de três espécies de *Cynodon*: *Cynodon bradleyi* Stent (*C. incompletus* Nees var. *incompletus* x *C. incompletus* var. *hirsutus* (Stent) de Wet), *C. transvaalensis*, *C. dactylon*, e *C. magennisii* e observou 18, 20, 30 e 40 cromossomos, respectivamente. Devido a esses resultados a autora propôs o número básico $x = 10$ e sugeriu que *C. bradleyi* é um aneuploide com $2n = 2x = 18$.

Forbes e Burton (1963) descreveram a ocorrência de espécies diploides (2*n* = 2*x* = 18), como *C. bradleyi, C. incompletus, C. plectostachyus* e *C. transvaalensis,* espécie triploide (2*n* = 3*x* = 27), como *C. magennisii*, enquanto *C. dactylon* apresentou citótipos tanto diploides (2*n* = 2*x* = 18) quanto tetraploides (2*n* = 4*x* = 36). Dessa forma, consideraram o número básico *x* = 9. Durante esse estudo, em algumas espécies/acessos, os autores visualizaram "fragmentos" de cromossomos de vários tamanhos, sendo considerados, por estes autores, como satélites desprendidos do cromossomo que continha a região organizadora do nucléolo. Devido à baixa visibilidade das constrições secundárias ou quebra das mesmas durante a manipulação citológica, estes satélites estavam sujeitos a erros de interpretações. A maioria desses "fragmentos" apresentava quase a metade do tamanho de todo o cromossomo portador da região organizadora do nucléolo.

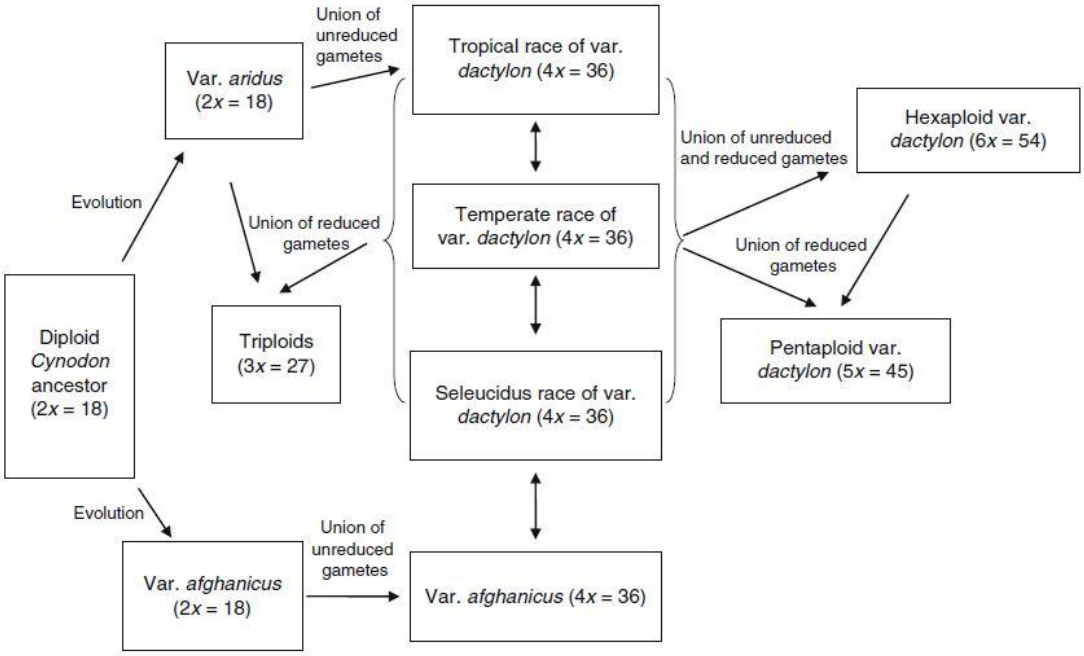
Assefa e colaboradores (1999) estudaram o número cromossômico de oito espécies e sete variedades de *Cynodon* baseado no trabalho descrito por Harlan et al (1970) totalizando 62 acessos. Observaram acessos diploides (2*n* = 2*x* = 18) e tetraploides (2*n* = 4*x* = 36) para *C. aethiopicus, C. dactylon* var. *afghanicus, C. incompletus* var. *incompletus, C. nlemfuensis* var. *robustus* e *C. plectostachyus*. Enquanto *C. arcuatus, C. barberi, C. dactylon* var. *dactylon, C. dactylon* var. *coursii, C. dactylon* var. *elegans* e *C. transvaalensis* eram tetraploides com 2*n* = 4*x* = 36. *Cynodon dactylon* var. *aridus* foi a única variedade com todos os acessos diploides (2*n* = 2*x* = 18).

Cynodon dactylon apresenta cinco citótipos, sendo estes diploides (2*n* = 2*x* = 18), triploides (2*n* = 3*x* = 27), tetraploides (2*n* = 4*x* = 36), pentaploides (2*n* = 5*x* = 45) e hexaploides (2*n* = 6*x* = 54) (DHALIWAL; GUPTA, 2011). Em sua revisão a respeito do gênero *Cynodon*, Wu (2011) descreveu a origem da grande variabilidade genética de *C. dactylon*. Segundo essa proposta, os citótipos poliploides tiveram origem por meio de diversos eventos de poliploidização/hibridização, conforme a figura 1.

Chiavegatto e colaboradores (2016) realizaram uma investigação sobre a assimetria cariotípica em acessos diploides e poliploides de *Cynodon*. A espécie diploide *C. nlemfuensis* apresentou o cariótipo mais simétrico, ou seja, cariótipo mais primitivo. Por sua vez, o diploide *C. incompletus* apresentou o cariótipo mais derivado, ou seja, é a espécie com cariótipo mais assimétrico. Os acessos poliploides são citótipos de *C. dactylon* e formam um grupo com assimetria cariotípica intermediária entre as duas espécies diploides. Ainda nesse estudo, os

autores observaram que a fórmula cariotípica mais frequente é 9m, com exceção da espécie *C. incompletus* que apresentou apenas cromossomos submetacentricos e fórmula cariotípica de 9sm.

Figura 1 Origem da variabilidade genética de *Cynodon dactylon.*



Fonte: Wu (2011).

Em *Cynodon* (Bermudagrass), até o momento, somente um trabalho utilizou técnicas de citogenética molecular para análise dos cromossomos (ZHI-YUN et al., 2013). Embora não tenham proposto um mapa físico dos sítios de rDNA nos cromossomos, Zhi-Yun et al. (2013) relataram que há variações nos números de sítios de rDNA 35S e 5S nos dois acessos tetraploides avaliados. Para o rDNA 35S, o acesso C121 apresentou três sinais de hibridização na região centromérica, com diferente intensidade. Com as sondas para marcar o rDNA 5S, foram observados três sinais com diferentes intensidades. No acesso C29 foram observados quatro sinais de hibridização para o rDNA 35S, nas regiões centroméricas, dois fortes e dois fracos. O mesmo foi observado para o rDNA 5S com dois sinais fortes e dois fracos.

Chaves et al. (dados não publicados) investigaram o padrão de bandas heterocromáticas CMA e DAPI em citótipos de *C. dactylon*, variando de diploide ($2n = 2x = 18$) a pentaploide ($2n = 5x = 45$). Os citótipos apresentaram diferentes padrões cariotípicos evidenciando variabilidade

intraespecífica para número, tamanho, posição e intensidade de bandas heterocromáticas. Com isso, os autores relacionaram ao processo de reorganização genômica, após o processo de poliploidização, visando a diploidização no comportamento meiótico. Em todos os acessos estudados, o primeiro par cromossômico apresentava a região pericentromérica corada com cromomicina A. As bandas $DAPI^+$ foram associadas às regiões terminais dos cromossomos. A maioria dos acessos apresentaram apenas cromossomos metacêntricos, consequentemente a fórmula cariotípica foi de 9m. Apenas o acesso PI 291966-27 ($2n = 4x = 36$) apresentou um cromossomo submetacêntrico com a fórmula cariotípica de 35m+1sm.

Thomas e Murray (1978) estudaram sete acessos de *C. dactylon*, variando de diploide ($2n = 2x = 18$) a pentaploide ($2n = 5x = 45$) e observaram que os cariótipos são formados de pequenos cromossomos metacêntricos e submetacêntricos (1 a 2 µm). Não foram identificados cromossomos com satélites e, pelo menos um par de cromossomos em cada nível de ploidia apresentou regiões heterocromáticas distintas. Além disso, para esses autores, os citótipos diploides e tetraploides apresentam apenas bivalentes na meiose, enquanto nos citótipos triploides foram observados univalentes, bivalentes e trivalentes na meiose I.

Outros autores descreveram irregularidades no comportamento meiótico de alguns genótipos de *C. dactylon*. Forbes e Burton (1963) reportaram um acesso de *C. dactylon* com pareamento cromossômico estreitamente irregular (Is) com média de 1.6. Malik e Tripathi (1968) observaram a formação de 18 bivalentes, entretanto um ou dois quadrivalentes foram encontrados em *C. dactylon* tetraploides. Hanna e Burton (1977) reportaram a citogenética e a fertilidade de quatro cultivares de *C. dactylon*. A cultivar Coastcross-1 apresentou meiose muito irregular com associação média de 5.07 I + 13.94 II + 0.41 III + 0.51 IV por célula.

Um trabalho recente (SILVA et al., 2018) sobre o comportamento meiótico em *Cynodon* mostrou que acessos diploides de *C. nlemfuensis* apresentaram a configuração de 9 bivalentes durante a formação dos gametas e, aproximadamente, 1,38% de anormalidades. Os acessos tetraploides de *C. dactylon*, por sua vez, apresentaram a configuração de univalentes, bivalentes e tetravalentes e cerca de 2.04% de anormalidades.

Os dados cariológicos e do comportamento meiótico, sugerem a origem alopoliploide de *C. dactylon*. Guo et al. (2015), estudando marcadores genéticos em *C. dactylon*, observaram que essa espécie é formada por dois subgenomas e também sugerem a origem de alopoliploide segmental para esta espécie.

Em relação ao conteúdo de DNA nuclear para estimar o nível de ploidia dos acessos de *Cynodon,* Wu e colaboradores (2006) quantificaram o DNA nuclear e realizaram a contagem cromossômica de 132 acessos de *Cynodon* e observaram os acessos triploides (6 acessos), tetraploides (116 acessos), pentaploide (3 acessos) e hexaploide (7 acessos), com valores entre 1,55 – 1,65; 1,96 – 2,30; 2,37 – 2,49 e 2,90 – 3,13 pg/2C, respectivamente. Em outro estudo, Kang et al. (2008), por meio de citometria de fluxo, juntamente com contagem cromossômica, avaliaram 43 acessos de *Cynodon* e para os acessos triploides, tetraploides, pentaploides e hexaploides, observaram 1,42−1,56; 1,94−2,19; 2.54 e 2,77−2,85 pg/2C, respectivamente. Gulsen et al (2009) também realizou estudos com acessos de *Cynodon* e obtiveram o nível de ploidia, via conteúdo de DNA nuclear e contagem cromossômica, de 182 acessos de *Cynodon*, no qual haviam diploides (6 acessos), triploides (3 acessos), tetraploides (123 acessos), pentaploides (36) e hexaploides (14) com DNA nuclear com 1,07-1,26; 1,36-1,74; 1,87-2,29; 2,47-2,79 e 3-3,6 pg/2C, respectivamente. Pang e colaboradores (2010) quantificaram o DNA nuclear, juntamente com contagem cromossômica de 51 acessos de *Cynodon* obtendo como resultados 1 acesso diploide com 1,17 pg/2C, 21 acessos triploide com 1,38-161 pg/2C, 25 acessos tetraploide com 1,94-2,24 pg/2C, 1 acesso pentaploide com 2,47 pg/2C e 3 acessos hexaploide com 2,64-2,75 pg/2C.

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SEGUNDA PARTE - ARTIGOS

ARTIGO 1 - RECONSTRUCTING ANCESTRAL CHROMOSOME NUMBERS IN Eleusininae Dumort. (Poaceae, Chloridoideae, Cynodonteae)

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Abstract

The chromosome number in Poaceae has been changing widely over 77 million years ago of polyploidization and evolution. Chromosome number changes can display an elevated rate of diversification and evolutionary novelties, and such change can contribute to speciation. Despite this, chromosome numbers alone do not allow to trace the evolutionary history of a group. Combined phylogenetic and karyological analyses can clarify evolutionary history of taxa and infer the taxonomic relationships and hierarchical levels. The subtribe Eleusininae is the largest subtribe of the subfamily Chloridoideae, comprising species that are very diverse morphologically. Therefore, we investigated the chromosome number evolution in Eleusininae by ChromEvol 2.0 software. Chromosome number evolution in Eleusininae was mainly influenced by polyploid events. The ancestral basic chromosome number for Eleusininae was p = 6, but the most recent common ancestor showed p_1 = 10. In addition, some derived basic chromosome numbers, such as x = 9, arose through dysploidy, whereas x = 20 was generated via polyploidy.

Key words: Basic chromosome number; Chromosome evolution; Dysploidy; Polyploidy

Abbreviations

Mya	million years ago
MRCA	most recent common ancestor

1 INTRODUCTION

Poaceae Barnh. is a large angiosperm family (SHCHAPOVA, 2012) covering approximately 20% of the earth land surface (KELLOGG, 2001; GUANT, 2002). Currently, over 12,074 species, 771 genera, 12 subfamilies, 6 supertribes, 51 tribes and 80 subtribes are included into this family (SORENG et al., 2015). Many species are used in human or animal food and, for this reason, they show high economic importance (STEBBINS, 1956; KELLOGG, 2001).

Within the tribe Cynodonteae of Chloridoideae, there is the subtribe Eleusininae (PETERSON et al., 2015; SORENG et al., 2015; PETERSON et al., 2016; MUCHUT et al., 2017), which is the largest subtribe in this subfamily (PETERSON et al., 2016), including 237 species in 30 genera (SORENG et al., 2015). In spite of most Eleusininae species are characterized by racemose syninflorescences, this group is very diverse morphologically. They occur primarily at low latitudes in Africa, Asia, Australia, and in the Americas (PETERSON et al., 2015; PETERSON et al., 2010).

Eleusininae is supported as monophyletic by phylogenetic studies based on plastid and nuclear DNA analyses, but some genera are polyphyletic (PETERSON et al., 2007; PETERSON et al., 2010; PETERSON et al., 2012; PETERSON et al., 2015; PETERSON et al., 2016; Muchut et al., 2017). Initially, Eleusininae was considered a subtribe including the sole genus *Eleusine* (PETERSON et al., 2007). Later, Peterson et al (2010) reorganized two subtribes, namely Pommereullinae and Chloridinae within Eleusininae. Peterson et al (2015) also provided new taxonomic insights, especially concerning *Cynodon*, *Brachyachne*, and *Micrachne.*

The evolutionary history of Poaceae has been extensively investigated and there are many hypotheses about its chromosome number evolution (AVDULOV, 1931; FLOVIK, 1938; STEBBINS, 1956; SHARMA, 1979; STEBBINS, 1985; KELLOGG, 2001; GAUT, 2002; HILU, 2004; SHCHAPOVA, 2012). Avdulov (1931) considered $x = 7$ as the basic chromosome number, evolved from an ancestral basic chromosome number $p = 12$ (for the difference between x and p, see PERUZZI, 2013). Flovik (1938) proposed $p = 5$, whereas Sharma (1979) suggested $p = 6$ and finally Stebbins (1985) $p = 5$, 6, and 7. According to Gaut (2002), the chromosome number in Poaceae has been changing widely over 77 million years ago (Mya) of evolution and polyploidization would account for most of this variation since 44% of the Poaceae species are polyploids. In Chloridoideae the ancestral basic chromosome number is $p = 10$ (PETERSON et

al., 2007). Basic chromosome numbers x = 9 and x = 10 were both reported in Eleusininae (AVDULOV, 1931; HIREMATH; CHENNAVEERAIAH, 1982; HARLAN et al., 1970; ASSEFA et al., 1999; ROODT; SPIES 2003).

Chromosome number changes can display an elevated rate of diversification and evolutionary novelties (GUERRA, 2008, GUERRA, 2012), and such changes can contribute to speciation (CUSIMANO et al., 2012). Despite this, chromosome numbers alone do not allow to trace the evolutionary history of a group (SCHUBERT; LYSÁK, 2011). Combined phylogenetic and karyological analyses can clarify evolutionary history of taxa (MURRAY, 2002; DOBIGNY et al., 2004; PERUZZI et al., 2009; ASTUTI et al., 2017; PERUZZI et al., 2017). Such approach allows identifying sister-group relationships among taxa, and character-states that are shared due to common ancestry (DOBIGNY et al., 2004). Moreover, it allows identifying the independent origin of polyploidy as well as the point that a gross reduction in chromosome number can mask the polyploid nature of the species (MURRAY, 2002).

This study aims to reconstruct the chromosome number evolution in Eleusininae subtribe, in order to clarify the direction of chromosome number change, besides polyploidy and dysploidy events. Moreover, those results may assist in the taxonomic circumscription of this subtribe.

2 MATERIALS AND METHODS

2.1 Chromosome number evolution

Recently, new tools for inferring chromosome number evolution have been proposed. Glick and Mayrose (2014) developed a probabilistic model to infer the likely direction of chromosome number change, as well as number and order the chromosome rearrangements along a phylogenetic tree. According to this approach, ancestral chromosome numbers are reconstructed for each clade and the chromosome number inferred from the most recent common ancestor (MRCA) of the group examined is designated as ancestral basic chromosome number.

To investigate the chromosome number evolution in Eleusininae, we used the approach of Glick & Mayrose (2014). The method assess the fit of several models of chromosome number change along a phylogeny by using different combinations of the following of parameters: (1) duplication, (2) demi-duplication, (3) gain or (4) loss of a single chromosome pairs, (5) gain or

(6) loss of a single chromosome pairs starting from the current chromosome number, (7) starting from a specified chromosome number characterizing a phylogenetic group, and (8) rate for transitions by basic chromosome number (MAYROSE et al., 2010; GLICK; MAYROSE, 2014). Reconstructions of chromosome number changes are based upon the best-fitted model that was selected using Akaike information criterion (AIC) (BURNHAM; ANDERSON, 2004).

We first collected haploid chromosome number from Eleusininae species from the Chromosome Counts Database (http://ccdb.tau.ac.il/) and from the Index to Plant Chromosome Number database (http://www.tropicos.org/Project/IPCN) (Table 1). Then we used a consensus phylogenetic tree of Eleusininae proposed by Muchut et al. (2017) as a backbone to plot chromosome number of the species. We used the software ChromEvol 2.0 (GLICK; MAYROSE, 2014) to run the analyses and perform model selection. We included in our analysis 55 species and 18 genera of subtribe Eleusininae (ca. 64% of the subtribe), and two species from different genera as outgroup (Table 1).

Many cytotypes can be found within Eleusininae, both in diploid and polyploid species (ROODT; SPIES, 2002; ROODT; SPIES, 2003). All the chromosome numbers found in literature were plotted on phylogenetic tree and their frequencies were studied by means of ChromEvol 2.0

Table 1. Chromosome numbers in Eleusininae.

Taxa	**2*n***	**Reference(s)**
Outgrup		
Chasmanthium latifolium (Michx.) H.O.Yates	24	Delay, 1947; Yates, 1966
	48	Abayjiob 1928; Brown, 1950; Tateoka, 1955; Yates, 1963
Danthonia compressa Austin	36	de Wet, 1953; Myers, 1947
Eleusininae		
Astrebla lappacea (Lindl.) Domin	40	Brown, 1950; Cave,1957
Astrebla pectinata (Lindl.) F.Muell. ex Benth.	40	Cave,1957
Chloris barbata Sw.	20	Fedorov, 1974; Gould, 1966; Moinuddin, Vahidy and Ali, 1994; Parodi, 1946
	40	Chen and Hsu 1962; Davidse and Pohl, 1972; Davidse and Pohl, 1974; Gould and Soderstrom, 1970; Krishnaswamy 1941; Mary and Rao, 1988; Sharma, Behera and Dash, 1978;
	56	Rao and Mwasumbi, 1981
Chloris cucullata Bisch.	40	Fedorov, 1974; Anderson, 1965; Avdulov, 1928; Brown, 1950; Gould, 1966; Gould, 1968; Nielsen, 1939

Chloris gayana Kunth	10	Ochoa de Suárez, Zumeizú and Bressano, 1983; Spies and Jonker, 1987
	20	Avdulov, 1928; Pritchard and Gould, 1964; Singh and Godward 1963; Abiiyjiob 1928; Bennett and Smith, 1976; Bir and Sahni, 1986; Brown, 1950; Gould, 1968; Hunter 1934; Kumar, 1987; Ochoa de Suárez and Zumeizú, 1983; Parodi 1946; Sinha and Jha, 1972; Strydom and Spies, 1994
		Pritchard and Gould, 1964
	30	Sinha and Jha, 1972; Tateoka 1965; Pritchard and Gould,
	40	1964; Rao and Mwasumbi, 1981; Singh and Godward 1963; Tateoka, 1965;
Chloris halophila Parodi	80	Bowden and Senn, 1962
Chloris pycnothrix Trin.	36	Thomas,1955
	40	Dujardin 1978; Dujardin, 1979; Moffett and Hurcombe 1949; Tateoka 1965
	60	De Wet, 1954
Chloris submutica Kunth	80	Gould, 1965; Singh and Godward, 1960; Reeder, 1971
Chloris truncata R.Br.	40	Abiiyjiob 1928; Krishnaswamy 1941; Darlington, 1955
Chloris virgata Sw.	14	Nielsen and Humphary, 1937
	20	Sahni and Bir, 1985; Spies and Jonker. 1987; Strydom and Spies. 1994; Brown, 1950; Davidse and Pohl, 1972; Gould 1966; Gould, 1968; Gupta, 1971; Mesicek and Sojak, 1972; Moffett;Hurcombe, 1949; Pritchard and Gould, 1964; Probatova, Rudyka and Sokolovskaya, 1996; Rao and Mwasumbi, 1981; Roodt and Spies, 2002; Roodt and Spies, 2003; Singh and Godward, 1960; Yan et al., 1989
	26	Thomas, 1955
	30	Krishnaswamy, 1941
	36	Rao and Mwasumbi, 1981
	40	Krishnaswamy, 1941
Chrysochloa hindsii C.E.Hubb.	14	Renard et al., 1983
Coelachyrum lagopoides (Burm.f.) Senaratna	18	Hiremath, 1980
	36	Krishnaswamy and Ayyangar, 1935; Krishnaswamy, 1951
Cynodon arcuatus J.Presl	36	Nowack, 1992; Harlan et al.,1970; Assefa et al., 1999
Cynodon dactylon (L.) Pers.	18	Harlan et al., 1970; Brilman, Kneebone and Endrizzi, 1982; Rawal and Chheda, 1971; Devesa et al., 1991; Silva and Sanydon, 1995; Forbes and Burton, 1963; Assefa et al., 1999
	27	Guptaa and Srivastavaa, 1968
	36	Harlan et al.,1970; Chiavegatto et al., 2016; Silva and Sanydon, 1995; Forbes and Burton, 1963; Assefa et al., 1999
	45	Harlan et al.,1970; Chiavegatto et al., 2016
	54	Harlan et al.,1970
Cynodon incompletus Nees	18	Assefa et al., 1999; Forbes and Burton, 1963; Harlan et al., 1970; Hurcombe 1946
	36	Assefa et al., 1999
Cynodon incompletus var. *hirsutus* (Stent) de Wet & Harlan	18	Covas, 1949; Harlan et al., 1970; Chiavegatto et al., 2016
	36	Harlan et al,1970
Cynodon nlemfuensis Vanderyst	18	Harlan et al,, 1970; Chedda; and Rawal,1971; Chiavegatto et al., 2016)

		Chedda and Rawal,1971
	36	
Cynodon plectostachyus (K.Schum.) Pilg.	18	Forbes and Burton, 1963; Harlan et al.,1970; Assefa et al., 1999; Sampath and Ramanathan, 1949; Moffett, Hurcombe, 1949
	36	Assefa et al., 1999
Cynodon tenellus R.Br.	18	Moffett, 1949; Pritchard and Gould, 1964; Spies, Merwe, Plessis and Saayman, 1991; Tateoka 1965
	36	Pritchard and Gould, 1964
Cynodon transvaalensis Burtt Davy	18	Forbes and Burton, 1963; Harlan et al.,1970; Assefa et al., 1999
	36	Chiavegatto et al., 2016
Dinebra coerulescens (Steud.) P.M.Peterson & N.Snow	20	Dujardin 1978; Olorode 1975
Dinebra neesii (Thwaites) P.M.Peterson & N.Snow	20	Abayjiob, 1928; Avdulov 1928
	40	Gould and Soderstrom, 1974
Dinebra panicea var. *mucronata* (Michx.) P.M.Peterson & N.Snow	20	Bhattacharyya, 1973; Brown, 1950; Gould, 1968; Olorode, 1975
Dinebra panicoides (J.Presl) P.M.Peterson & N.Snow	20	Sherif and Smith; Hornberger, 1983
Dinebra retroflexa (Vahl) Panz.	20	Bir; Sahni, 1986; de Wet and Anderson, 1956; Moinuddin, Vahidy and Ali, 1994; Shanthamma, et al. 1976; Abayjiob, 1928; Avdulov, 1931; Krishnaswamy, N. 1941
	40	Bir and Sahni, 1986
Dinebra scabra (Nees) P.M.Peterson & N.Snow	60	Davidse and Pohl. 1972; Davidse and Pohl. 1974
Diplachne fusca subsp. *uninervia* (J.Presl) P.M.Peterson & N.Snow	20	Gould, 1968; Gould, 1958
Disakisperma dubium (Kunth) P.M.Peterson & N.Snow	40	Covas 1949; Gould, 1966; Gould, 1968
	60	Brown 1950; Reeder, 1984
	80	Gould, 1965
Disakisperma obtusiflorum (Hochst.) P.M.Peterson & N.Snow	20	Janaki, 1955
Eleusine africana Kenn.-O'Byrne	18	Roodt and Spies, 2003; Renard et al., 1983; Roodt and Spies, 2002; Singh and Godward 1960
	36	Roodt and Spies, 2002; Roodt and Spies, 2003; Singh and Godward, 1960; Bisht and Mukai, 2000; Bisht and Mukai, 2001; Chennaveeraiah and Hiremath, 1974; Dixit and Vishnu-Mittre, 1987; Hiremath and Chennaveeraiah, 1982; Hiremath and Salimath, 1991; Mallikharjun, 2005; Mehra, 1963; Tateoka, 1965; Werth, Zeng and Baird, 1997
Eleusine coracana (L.) Gaertn.	18	Mehra, 1982
	27	Chennaveeraiah and HJremath, 1974
	36	Kalia, 1978; Bhattacharyya, 1973; Bisht and Mukai, 2000; Christopher and Abraham. 1974; Devarumath et al., 2005; Gupta, 1969; Gupta, 1971, Hiremath and Chennaveeraiah, 1982; Hiremath and Devarumath, 1995; Hiremath and Salimath, 1991; Kempanna and Setty, 1969; Mallikharjun, 2005; Mehra, 1963; Mysore and Baird, 1997; Singh and

	40	Godward 1960; Sinha, Bhardwaj and Singh, 1990 Bennett and Smith, 1976
Eleusine floccifolia Spreng.	18	Bisht and Mukai, 2000; Bisht and Mukai, 2001; Chennaveeraiah and Hiremath, 1973; Devarumath, et al., 2005; Hiremath and Chennaveeraiah, 1982; Hiremath and Salimath, 1991; Mallikharjun, 2005; Mysore and Baird, 1997; Salimath and Hiremath; Murthy, 1995
	36	Hiremath and Salimath, 1991
Eleusine indica (L.) Gaertn.	18	Baquar and Saeed, 1969; Bhattacharyya, 1973; Bir and Chauhan. 1990; Bir and Sahni, 1986; Bisht and Mukai, 2000; Bisht and Mukai, 2001; Christopher, Abraham; Devarumath et al., 2005; Dujardin, 1979; Faruqi, Quraish and Inamuddin, 1987; Hiremath and Salimath, 1991; Singh 1965; Gupta, 1971; Gould and Soderstrom, 1967; Hiremath and Chennaveeraiah, 1982; Koul and Gohil, 1988; Larsen 1963; Llauradó Miravall, 1984; Malik and Mary, 1970; Mallikharjun, 2005; Mehra, 1982; Mehra and Sharma, 1975; Moinuddin and Vahidy; Ali, 1994; Mysore and Baird, 1997; Olorode, 1974; Rao and Sindhe, 1977; Renard et al., 1983; Salimath, Hiremath and Murthy, 1995; Sarkar et al., 1976; Sarkar et al., 1978; Singh and Godward 1960; Sinha and Bhardwaj; Singh, 1990; Sinha and Jha, 1972; Yang, 1998
	20	Bennett and Smith, 1976
	27	Mehra, 1982
	28	Kaur, Gupta and Kumari, 2011
	36	Bir and Chauhan, 1990; Dujardin, 1979; Faruqi, Quraish and Inamuddin, 1987; Gould and Soderstrom, 1967; Moffett and Hurcombe, 1949; Moinuddin and Vahidy; Ali, 1994;
	54	Subramanyam and Kamble, 1967; De Wet, 1954 Dujardin, 1979; Faruqi and Quraish; Inamuddin, 1987
Eleusine jaegeri Pilg.	20	Hiremath and Salimath, 1991; Hiremath and Chennaveeraiah, 1982; Hiremath and Salimath, 1991; Mysore and Baird, 1997
Eleusine multiflora Hochst. ex A.Rich.	16	Bisht and Mukai, 2000; Hiremath and Chennaveeraiah, 1982; Hiremath and Salimath, 1991; Mysore and Baird, 1997; Salimath and Hiremath; Murthy, 1995
	18	Reeder, 1984
Eleusine tristachya (Lam.) Lam.	16	Reeder, 1968
	18	Bisht and Mukai, 2000; Bisht and Mukai, 2001; Cesmedziev, 1976; Chennaveeraiah and Hiremath, 1973; Devarumath et al, 2005; Hiremath and Chennaveeraiah, 1982; Hiremath and Salimath, 1991; Krishnaswamy, 1940; Llaurad Miravall, 1984; Mallikharjun. 2005; Mysore and Baird, 1997; Salimath and Hiremath; Murthy, 1995; Singh and Godward, 1960; Devesa, Luque and Gómez, 1990
Enteropogon dolichostachyus (Lag.) Keng	20	Mehra et al. 1968; Sahni and Bir. 1985; Saxena and Gupta, 1969
	40	Mehra and Kohli, 1966; Gould and Soderstrom, 1974; Mehra and Sharma, 1975; Muniyamma 1976; Sahni and Bir. 1985; Saxena and Gupta. 1970; Christopher and Abraham, 1974; Gould and Soderstrom, 1974; Mehra and Sharma,
	60	1975; Saxena and Gupta, 1970; Saxena and Gupta, 1969

		Sahni and Bir, 1985
Enteropogon macrostachyus (A.Rich.) Munro ex Benth.	40	Roodt and Spies, 2003
Eustachys distichophylla (Lag.) Nees	20	Krishnaswamy, 1940; Krishnaswamy, 1941; Delay, 1947.
	40	Parodi, 1946; Huynh, 1965; Singh and Godward, 1960.
Eustachys paspaloides (Vahl) Lanza & Mattei	40	Strydom and Spies,1994
Eustachys petraea (Sw.) Desv.	40	Brown, 1950; Davidse; Pohl, 1978; Gould 1958; Gould,1968; Hunter 1934; Pohl and Davidse, 1971; Singh and Godward, 1960
Eustachys uliginosa (Hack.) Herter	40	Parodi 1946
Harpochloa falx (L.f.) Kuntze	36	Roodt and Spies, 2002; Roodt and Spies, 2003
	40	Spies et al., 1991; Strydom and Spies, 1994
	50	Strydom and Spies, 1994
	54	Roodt and Spies, 2002; Roodt and Spies, 2003
	60	Strydom and Spies, 1994
Leptochloa crinite (Lag.) P.M.Peterson & N.Snow	40	Bowden and Senn, 1962
Leptochloa pluriflora (E.Fourn.) P.M.Peterson & N.Snow	60	Brown, 1951
	80	Gould, 1968
Leptochloa virgata (L.) P.Beauv.	30	Krishnaswamy, 1940
	40	Gould and Soderstrom, 1967; Parodi, 1946; Pohl and Davidse, 1971
Lepturus radicans (Steud.) A.Camus	36	Gould; Soderstrom, 1974; Tateoka, 1958
Lepturus repens (J.R.Forst.) R.Br.	42	Hsu, 1972
	52	Dawson, 2013
	54	Crang and Dean, 1971; Dawson, 2013; Tateoka, 1958
Microchloa caffra Nees	20	Cave, 1956
Microchloa indica (L.f.) P.Beauv.	24	Norrmann, Quarín and Killeen, 1994; Davidse and Pohl, 1974; Kammacher et al. 1973
	48	
Microchloa kunthii Desv.	24	Kalia, 1978; Mehra, 1982; Mehra and Kalia 1976
	40	Gould, 1965; Moffett and Hurcombe 1949; Reeder, 1967
	44	Davidse and Pohl, 1972
Oxychloris scariosa (F.Muell.) Lazarides	40	Singh and Godward, 1960
Rendlia cupricola J.Duvign.	20	Strydom and Spies,1994
Tetrapogon cenchriformis (A.Rich.) Clayton	18	Singh and Godward, 1960
Tetrapogon villosus Desf.	10	Chopanov and Yurtsev, 1973
	20	Borgen, 1970; Moinuddin, Vahidy and Ali, 1994

3 RESULTS

3.1 Pattern of chromosome number evolution

The best-fitting model of chromosome number evolution for Eleusininae is the model 10 (BASE_NUM_DUPL), according to the AIC = 239.6 and log-likelihood -115.4 (Table 2). This model considers four parameters of pattern of chromosome number changes: (1) gain or (2) loss of only one chromosome pair, (3) whole genome duplications (WGD) and (4) transitions in basic number. The model did not assume any event of demiduplication. The two worst-fitting model are the model 4 (CONST_RATE_NO_DUPL) and the model 8 (LINEAR_RATE_NO_DUPL) with AIC = 392.8 and 374.8, respectively (Table 2).

It was possible to infer 40 events of chromosome rearrangements along the evolutionary history of Eleusininae, according to the probabilistic model used. In general, duplication rates were estimated to be more frequent than dysploidy (Table 2).

The rate of gain of single chromosome pairs was detected six times (Figure 1) along the chromosome transitions, with an expectation ranging from 0.751 to 0.995. Dysploidy events are more important during the diversification of *Microclhoa* and *Eleusine*, due to a high frequency of chromosome gain in this clade. Chromosome loss events (Figure 1) were observed in 10 clades, with an expectation ranging from 0.518 to 2.099. However, chromosome gain events were more important in a specific clade, while chromosome number loss events have resulted from the ancestral basic chromosome numbers, and along lineages of the genera *Eleusine*, *Chrysochloa*, and *Cynodon*.

The rate of whole genome duplication (polyploidy) (Figure 1) is inferred in 20 events (expectation ranging from 0.539 to 1.57) along of the phylogenetic tree. This kind of chromosome number change is the most important and has been a key factor in the speciation of Eleusininae. Transitions in basic number are observed in three times (Figure 1) in this reconstruction, such as *Dinebra scabra* (expectation 0.999), *Chloris submutica* and *Chloris halophila* (expectation 0.999).

In general, it is likely that ascending dysploidy events occurred firstly, followed by duplication events, and then dysploidy events (gain or loss chromosome pairs), with the exception of genera *Microchloa* and *Lepturus*.

Table 2 Log-likelihood and Akaike information criterion (AIC) score estimates, and events number for the dataset analyzed by ChromEvol.

Model	Log-likelihood	AIC	Gain	Loss	Polyploidy	Basic number
CONST_RATE	-122.5	251 (4)				
CONST_RATE_DEMI	-122.7	251.5 (5)				
CONST_RATE_DEMI_EST	-115.8	239.6 (2)				
CONST_RATE_NO_DUPL	-194.4	392.8 (10)**				
LINEAR_RATE	-123.3	256.6 (7)				
LINEAR_RATE_DEMI	-123.1	256.2 (6)				
LINEAR_RATE_DEMI_EST	-116.4	244.9 (3)				
LINEAR_RATE_NO_DUPL	-183.4	374.8 (9)				
BASE_NUM	-131	270.1 (8)				
BASE_NUM_DUPL	-115.4	240.8 (1)*	6	11	20	3

The best-fitting (*) and the worst-fitting (**) models of chromosome number evolution for subtribe Eleusininae.

3.2 Reconstructing ancestral chromosomes numbers in Eleusininae

We inferred that the ancestral basic chromosome number in Eleusininae is $p = 6$, according to the fit of the best model. From this basic ancestral chromosome number, the loss of one chromosome pair took place ($p = 6 \rightarrow p = 5$), followed by a whole genome duplication of the basic chromosome number transition ($p = 5 \rightarrow p_1 = 10$). In addition, some derived basic chromosome numbers, such as $x = 9$, arose through dysploidy, whereas $x = 20$ was generated via polyploidy (Figure 1).

The basic chromosome numbers in Eleusininae are homogeneous ($x = 9$, $x = 10$, and $x = 20$), a high diversity of cytotypes is found in this subtribe, with 54% having polyploid and/or haploid cytotypes in our dataset.

The ancestral basic chromosome number ($p_1 = 10$) was maintained in the genera *Astrebla*, *Chloris*, *Dinebra*, *Disakisperma*, *Enteropogon*, *Eustachys*, *Harpochloa*, *Leptochoa*, and *Microchloa*. Despite of the maintenance of ancestral basic chromosome number $p_1 = 10$, variations in chromosome number can be found in the genus *Microchloa*, from $x = 10$ to $x = 12$ by ascending dysploidy events. The ancestral basic chromosome number $p_2 = 9$ evolved independently three times, in *Eleusine*, *Cynodon*, and *Tetrapogon cenchriformis*.

4 DISCUSSION

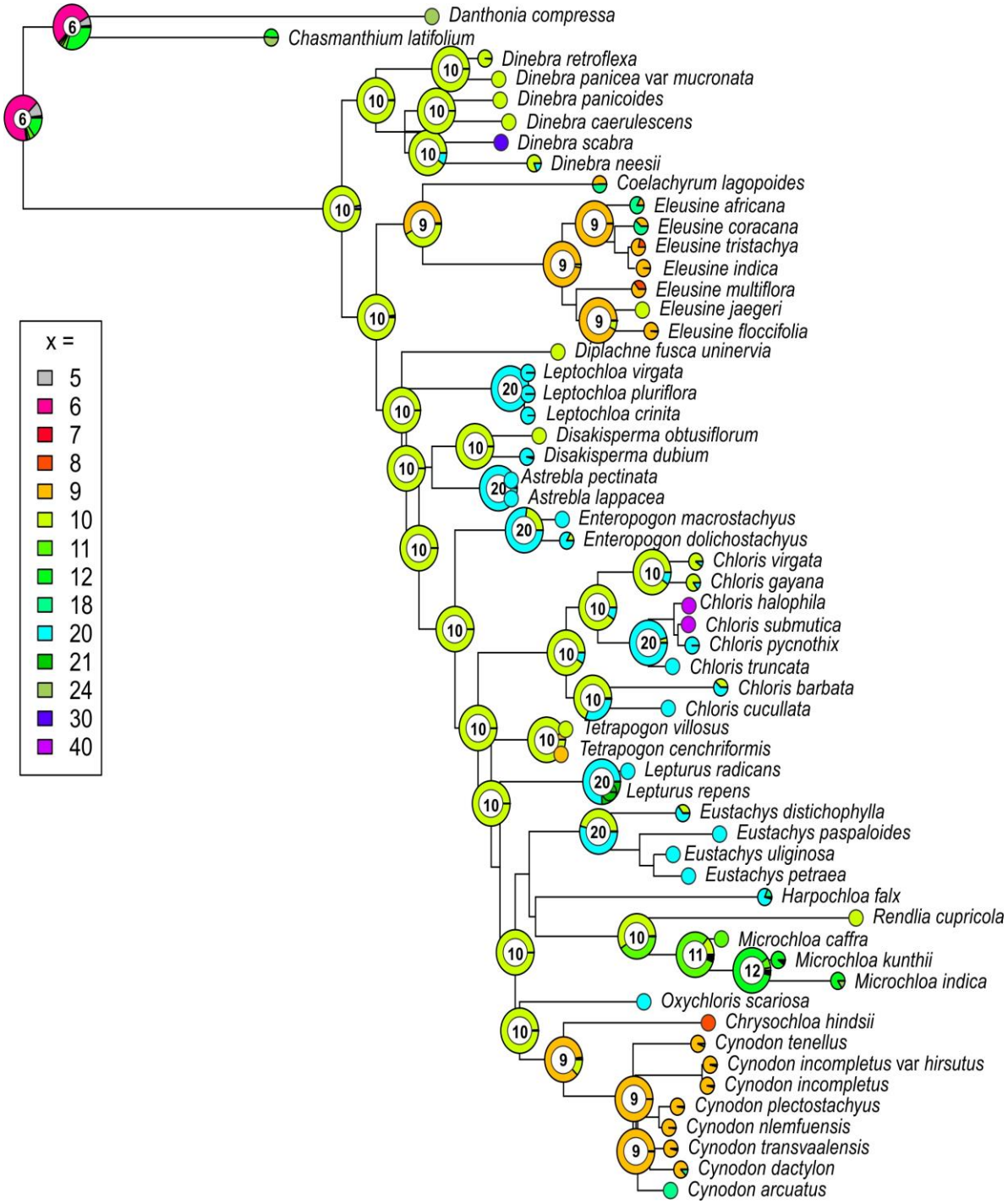
4.1 Patterns of chromosome number evolution in Eleusininae

The chromosome number evolution in Poaceae has been described with a set of events. First, Poaceae haploid genomes underwent polyploidization process, followed by structural rearrangements of chromosomes and dysploidy. Finally, a subsequent ancestral diploidization in Poaceae occurred and this event shaped the genomes of the family (PATERSON et al.; 2004; SHCHAPOVA, 2012). According to Stebbins (1985), the high percentage of polyploids in Poaceae is a consequence of these ancestral events. Moreover, it is estimated that the percentage of polyploids species was higher somewhere in the evolutionary history of this family. Genome duplications in Poaceae evolution are also supported by means of identification of the duplicate gene sequences (see more in PATERSON et al., 2004; SWIGONOVÁ, 2004; SALSE et al., 2009; MURAT et al., 2010; SHCHAPOVA, 2012).

The number of chromosome loss events is higher than that of chromosome number gain events. This agrees with Guerra (2012), who argues that during the process of chromosome number evolution, descending dysploidy is more common than ascending dysploidy. According to Luo et al. (2009) the common ancestor of *Triticeae, rice*, and *Sorghum* had the basic chromosome number $x = 12$. By means of recognition of chromosome rearrangement sites, such as pericentric and paracentric inversions, translocations and loss of centromeric regions, descending dysploidy events mechanisms were identified in this group. However, Triticeae genomes ($x = 7$) and rice genome ($x= 12$) appear more related to each other than each is to the sorghum genome ($x = 10$).

In monocots, similarly to Poaceae, also Iridaceae show a large variability in chromosome number. By employing the same methodology, Moraes et al (2015) suggested that polyploidization and dysploidization events drove the chromosome number evolution in this family.

Figure 1. Basic chromosome number (*x*) evolution in Eleusininae, based on the phylogenetic hypothesis of Muchut et al. (2017). Reconstructions of ancestral character states are inferring for each clade. The ancestral basic chromosome number for Eleusininae was $p = 6$ and the most recent common ancestor showed $p_1 = 10$ (green). The derived basic chromosome numbers, such as $x = 9$ (orange), arose through dysploidy, whereas $x = 20$ (blue) generated via polyploidy.



Fonte: Do Autor (2018).

Our results suggest that polyploidy, followed by dysploidy events, determined chromosome number variation in Eleusininae. In addition, dysploidy, or more precisely, chromosome number loss, seems to have played an important role in the transition of ancestral basic chromosome numbers in all genera of the Eleusininae. The ancestral basic chromosome number, $p = 6$, is indeed considered as a plesiomorphic character-state for the whole subfamily Chloridoideae (PETERSON et al., 2007).

4.2 Basic chromosome number transition in Eleusininae

Eleusine was subjected to many modifications in chromosome number along its evolutionary history. In the literature, there are three reports about basic chromosome number for this genus, $x = 8$, $x = 9$, and $x = 10$ (BISHT; MUKA, 2002; LIU et al., 2011; AGRAWAL; MAHESHWARI, 2016). Our results suggest that the ancestral basic chromosome number was $p_2 = 9$, derived from a descending dysploidy event ($p_1 = 10 \rightarrow p_2 = 9$). Actually, $x = 8$ and $x = 10$ were basic chromosome number derived from $x = 9$ by dysploidy events.Duplicate genes are crucial for adaptation in stressful environmental conditions and ecological niche expansion, since these genes may evolve and quickly gain new functions (MADLUNG, 2013; ALIX et al., 2017). Consequently, polyploidy may lead to gene diversification and to an increase in genetic polymorphism (GULSEN; AHMET, 2011). For this reason polyploid Eleusininae species are likely able to quickly adapt and have a large geographic distribution, such as *Cynodon dactylon*, which is a cosmopolitan species (WU, 2011).

Several cytological mechanisms are known to induce polyploidy in plants, but the main are somatic doubling and unreduced gametes (RAMSEY; SCHEMSKE, 1998). According to Escudero et al (2014) demiduplication occurs through the crossing of gametes of different ploidy levels, so that only one homoeologous set of chromosomes is duplicated. Moreover, demiduplication can occur in an already-established polyploid. Ramsey and Schemske (1998) suggested that polyploids originating from the union of reduced and unreduced gametes generally show euploid series, as observed in *Eleusine indica* and *C. dactylon*. In addition, several authors describe the presence of unreduced gametes in *Cynodon* and suggested which polyploids were originated from their hybridization (WU; TALIAFERRO, 2009; HARLAN; de WET, 1969; WU et al., 2006; GULSEN et al., 2009; WU, 2011).

Similarly to *Eleusine* polyploids, *C. dactylon* originated from hybridization between two diploid species; consequently, there are also two subgenomes (GUO et al., 2015). Our results indicate that triploid, pentaploid and hexaploid cytotypes of *C. Dactylon*, may not have a strong influence on the chromosome number transition. Triploids in *C. dactylon* can occur naturally, but they have an abnormal meiotic behavior, with nine bivalents and nine univalents, as a consequence they form unviable gametes (GUPTA; SRIVASTAVA, 1970). However, triploids are known to have a high impact on the evolution of a taxon. Triploid lineages can establish reproductive barriers between old progenitors species and new polyploids, due to problems with chromosome pairing/segregation during the meiosis process and the production of aneuploid gametes. This way, triploids prevent gene flow, leading to speciation (RAMSEY; SCHEMSKE, 1998; MCGRATH; LYNCH, 2012; MADLUNG, 2013). *Cynodon dactylon* triploid condition could be maintained, as a result of a real efficient vegetative propagation by means of underground runners (GUPTA; SRIVASTAVA, 1970).

According to Gulsen et al. (2009), the cytotypes of *C. dactylon* are virtually morphologically indistinguishable, regardless of the ploidy level. These authors suggested that diversifications of the morphological structures were not influenced by extra genes copies in polyploids, but rather by environmental factors and epigenetic interactions, which are slow events.

5 CONCLUSION

Chromosome number evolution in Eleusininae was mainly influenced by polyploid and dysploidy events. The ancestral basic chromosome number for Eleusininae was $p = 6$, but the most recent common ancestor (MRCA) showed $p_1 = 10$.

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ARTIGO 2 - HETEROCHROMATIN BANDS AND RDNA SITES EVOLUTION IN POLYPLOIDIZATION EVENTS IN *Cynodon* Rich. (Poaceae)

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Abstract

Cynodon is a genus with a wide distribution around the world in the tropical and subtropical grass species. Ploidy levels in the genus range from diploid to hexaploid. The polyploidy is the most important event responsible for the variation of chromosome number. In general, after polyploidization occur structural rearrangements, such as deletions, insertion or duplications of DNA sequences, allowing evolution of chromosome size and chromosome number. These events might result in a wide diversity of karyotypes contribute to reproductive isolation, and consequently to speciation. In this study, we investigate the karyotype variation of *Cynodon* based on comparative cytogenetic analyses. We conducted chromosome counts, DNA quantification, CMA/DAPI double staining, FISH 5S and 35S rDNA sites. Cytomolecular data were analyzed in a phylogenetic framework in order to trace the evolutionary history of the karyotype variation considering polyploidy events in this group. *Cynodon* have different ploidy level and a high frequency of karyotype variation. Our results indicate that the most recent common ancestor of *Cynodon* had two 35S and 5S rDNA sites, two CMA bands, nine $DAPI^+$ bands on the long arm, five $DAPI^+$ bands on the short arm and three $DAPI^+$ bands on the pericentromeric region. During the events of polyploidization of the species there were losses and gains of heterochromatic sequences mainly on the short arm and centromeric regions, which were responsible by the karyotype variation in *Cynodon*.

key words Chromosome Evolution; Forage Grass; Karyotype Variation; Ploidy Level

1 INTRODUCTION

Cynodon is a genus of tropical and subtropical grass species with a wide distribution around the world. Most *Cynodon* species are considered native to Africa and were then introduced in the United States, Canada, New Zealand and Australia (HARLAN et al., 1969; JEWELL et al., 2013). Differently, Caro and Sanchez (1969) suggested that some species are native from South America. Several of the species are used as forage, so they are economically important as pasture or stored forages and for soil stabilization, soil cover and sward, on golf courses, and in gardens (WU, 2011; JEWELL et al., 2013). Currently, 15 species are included within this genus (CLAYTON et al., 2016).

The origin of polyploids *Cynodon* is largely unknown, but evidences suggest both autopolyploid (GULSEN, 2009; GULSEN, 2011) and allopolyploid (HARLAN et al., 1969; HARLAN; de WET, 1969, GUPTA; SRIVASTAVA, 1970; GUO et al., 2015; CHIAVEGATTO et al., 2016; GUO et al., 2017; SILVA et al. unpublished data; CHAVES et al. unpublished data). However, Guo et al (2015) suggested that *Cynodon dactylon* (L.) Pers. tetraploid is allopolyploid, but was originated from the same parental diploid species with different subgenomes.

The polyploidy is the most important event responsible for the variation on chromosome number and DNA content and species. In general, after polyploidization structural rearrangements occurs, such as deletions, insertion or duplications of DNA sequences, allowing evolution of chromosome size and chromosome number. These events might result in a wide diversity of karyotypes, contributing to reproductive isolation, and consequently to speciation (SCHUBERT, 2007; SCHUBERT; LYSÁK, 2011; GUERRA, 2012; LYSÁK; SCHUBERT, 2013).

Both 5S and 35S rDNA sites are present in the genome as a tandem repeat. Despite this, there are regions highly conserved in plants and are often found to be arranged as blocks during the evolutionary process (HESLOP-HARRISON, 2000; GARNATJE et al., 2012; SILJAK-YAKOVLEV, 2017). Ribossomal DNA arrays present a high potential intragenomic mobility together with the action of tranposable elements. So, it is possible to identify chromosomal rearrangements (SCHUBERT, 2007; LYSÁK; SCHUBERT, 2013). This way, comparative analyses of the numbers and positions of the DNA sequences have value not only on the identification of chromosomes and genomes, but also in the elucidation of evolutionary and

taxonomic relationships. For this reason, rDNA sites are often used in cytotaxonomic studies (HESLOP-HARRISON; SCHWARZACHER, 2001; GUERRA, 2012).

In this study, we investigate the karyotype variation of *Cynodon* based on comparative cytogenetic analyses. We conducted chromosome counts, DNA quantification, CMA/DAPI double staining, FISH of 5S and 35S rDNA sites. Cytomolecular data were analyzed in a phylogenetic framework, previously published by Muchut et al (2017), in order to trace the evolutionary history of the karyotype variation considering polyploidy events in this group.

2 MATERIALS AND METHODS

2.1 Plant material

We analyzed seven *Cynodon* species/accessions that were donated by Embrapa Gado de Leite, Juiz de Fora, Minas Gerais State, Brazil (Table 1). These accessions were obtained from the Active Germplasm Bank, United States Department of Agriculture, Tifton, GA, USA, except the genotype ERX7.

Table 1 Identification of accessions/species of the genus *Cynodon*.

Accession/Genotype	Chromosome number	cDNA	Identification
PI 291169-04	*	*	*Cynodon dactylon* (L.) Pers.
PI 502336-23	*	*	*Cynodon dactylon* (L.) Pers.
PI 291175 PL 12	*	*	*Cynodon dactylon* (L.) Pers.
PI 289750-10	2n = 5x = 45**	2.55 ± 0.098 pg **	*Cynodon dactylon* (L.) Pers.
PI 29175301 PL 15	2n = 2x = 18**	1.17 ± 0.036 pg**	*Cynodon incompletus* Nees
ERX-7* **	2n = 2x = 18**	1.08 ± 0.094 pg **	*Cynodon nlemfuensis* Vanderryst
PI 289923-09	2n = 4x = 36**	1.95 ± 0.086 pg**	*Cynodon transvaalensis* Burtt-Davy

* First chromosome count and DNA quantification for the accessions/genotypes
** Chiavegatto et al (2016)
***Self-fertilization purple star grass

2.2 Chromosome preparation

For chromosome preparations, root tips were pre-treated with cycloheximide (0.025%) at room temperature for 2h. The roots were then fixed in Carnoy's solution (3 ethanol: 1 acetic acid) and stored at -20°C until slide preparation. The roots were subjected to enzymatic digestion with an enzyme mix containing Onozuka R10 cellulase (0.7%), cellulase (0.7%) (Sigma-Aldrich), pectolyase (1%) (Sigma-Aldrich), and cytohelicase (1%) (Sigma- Aldrich) at 37°C for 60 min. Slides were prepared by acetic acid dissociation and air-dried technique (CARVALHO; SARAIVA 1993). The slides were kept at 45% acetic acid at room temperature for 3 days, in order to remove the cytoplasm.

2.3 FISH analysis

The methodology used for FISH was based on Ma et al. (2010) and Rocha et al. (2016) using 35S rDNA sequences (clone pTa71 of *Triticum aestivum* L.) and 5S rDNA (clone pTa794 of *Triticum aestivum*). The 5S and 35S probes were labeled, respectively, with digoxigenin and biotin, using nick-translation reaction. For this, slides were denatured in 70% formamide in 2× SSC for 1 min and 30s at 85 °C and then dehydrated in alcoholic series. The mixture containing the probe (50% formamide, 2× SSC, 10% dextran sulfate, and about 80ng) was denatured at 95°C for 8 min. Hybridization was performed at 37°C in a humidified chamber for at least 48h. On the second day, the stringency wash was conducted at 42°C temperature for 10 min. Detection of 5S and e 35S probes was performed with fluorescein isothiocyanate (FITC)-conjugated and anti-digoxigenin (DIG) and avidin-conjugated tetramethyl rhodamine isothiocyanate (TRITC) in tris/ sodium chloride buffer (TNB) for 1 h at 37°C in a moist chamber. Chromosome preparations were counterstained with DAPI in Vectashield and evaluated on epifluorescence microscope. The images were scanned with a cooled monochromatic camera (Axio Cam HRM, Zeiss) and processed using the AxioVision Release 4.8.2 software (Zeiss).

2.4 CMA/DAPI staining

The CMA/DAPI staining was applied on the same metaphases selected through FISH techniques and were adapted from Guerra and Souza (2002). Slides after fluorescence *in situ* hybridization were washed with McIlvaine buffer (pH 7.0) for 20 min, dried and exposed to 0.5 mg mL^{-1} chromomycin A (CMA) solution for 1h, rinsed in McIlvaine buffer, and mounted on Vectashield Antifade with 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI). After 3 days, photomicrographs of the metaphases were taken with a fluorescence microscope Olympus BX60 with an image-capture camera (QImaging RetigaEXi CCD), and analyzed using the AxioVision 4.8.2 software.

2.5 DNA quantification

To estimate the DNA content, three samples of each accession evaluated (Table 1) were quantified. Young *Cynodon* leaf tissue (20-30 mg) was macerated with the same amount of material from *Pisum sativum* (internal reference standard, 2C DNA content = 9.09 pg) on a Petri dish containing 1 mL cold buffer (MARIE; BROWN, 1993) to obtain a nuclear suspension (DOLEZEL, 1997). To this nuclear suspension, we added 25 μL propidium iodide, and for each sample we quantitated at least 10,000 nuclei.

The analysis was performed on a FACSCalibur™ cytometer (BD Biosciences), and histograms were obtained using the CellQuest™ software and analyzed in WinMDI 2.9. The nuclear DNA content of the plants was estimated by comparison with the G1 peak position of the internal reference standard.

2.6 Karyotyping

For the physical map construction of the chromosomes, we used the software KaryoType (ALTINORDU et al., 2016). Ten metaphases were measured per accession and we calculated the total length of the chromosome (TLC), the total length of the haploid lot (TLHL), and the relative length of each chromosome, by means of the short arm lengths (c) and the long arm lengths (l). The morphological classification of the chromosomes was based on the ratio of arms proposed by

Levan et al. (1964). The $DAPI^+$ bands were measured and their percentages of each metaphase were plotted on a scatter plot compared to the total length of the haploid lot (TLHL). The position of the 5S and 35 rDNA were characterized based on Roa and Guerra (2012) criteria. These information were used for assembling karyograms of the *Cynodon* accessions with Adobe Photoshop.

2.7 Ancestral karyotype state reconstruction

We used the phytools package (REVEL, 2012) and the R environment (R DEVELOPMENT CORE TEAM, 2008) to perform ancestral karyotype state reconstructions among diploid *C. dactylon*, *C. incompletus*, *C. nlemfuensis*, and *C. transvaalensis*. We used a consensus phylogenetic tree of Eleusininae proposed by Muchut et al., (2017). The ancestral 35S rDNA and 5S rDNA sites were reconstructed under maximum likelihood with the fastAnc, ace and contMap commands.

We used two species from *Eleusine* genus as outgroup. *Eleusine Africana* (Kenn.-O'Byrne) S.M.Phillips (2n = 4x = 36) with 4 sites of 35S and 5S rDNA and *Eleusine floccifolia* (Forssk.) Spreng (2n = 2x = 18) with 4 sites of 35S and 2 sites of 5S rDNA (BISHT; MUKAI, 2000).

3 RESULTS

3.1 Karyotype analysis

Chromosome number and estimated nuclear DNA content confirmed that *C. dactylon* (PI 291169-04) is a diploid with 2n = 2x = 18 and 1.22±0.067 pg. *Cynodon dactylon* (PI 502336-23) have 2n = 3x = 27 and 2.07± 0.019 pg whereas *C. dactylon* (PI 291175 PL 12) is a tetraploid with 2n = 4x = 36 and 2.303± 0.111 pg (Figure 1, 2, and 3; Table 2). These results confirm the basic chromosome number *x* = 9. All species/accessions showed only metacentric chromosomes (m) with a formula karyotype 9m (Table 2).

In diploid *C. dactylon*, the length of the shortest and longest chromosome pairs are respectively 7.92% and 14.21% of the total length of the haploid set. Interstitial-terminal 5S

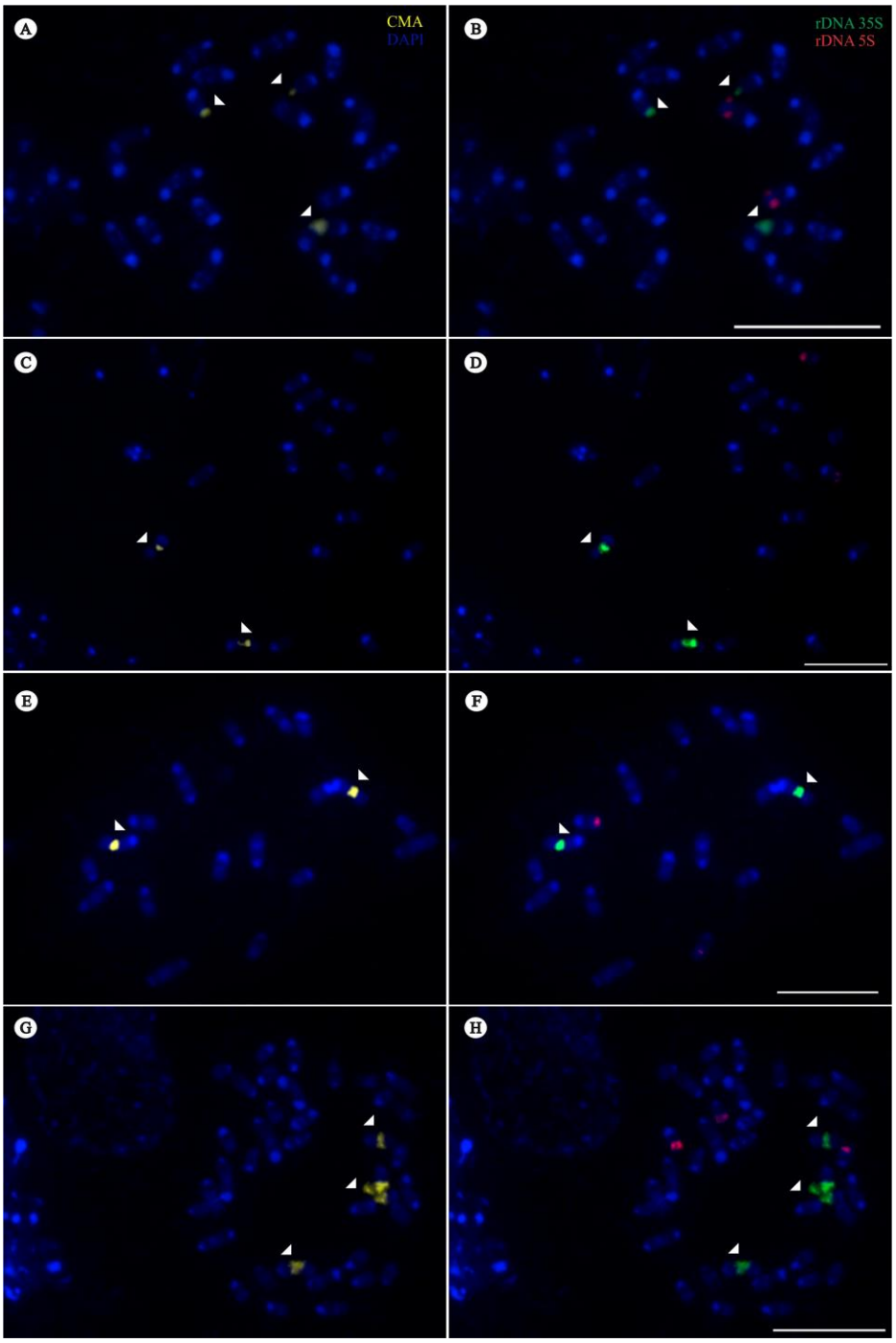
rDNA signal were found on chromosome pair 6 on short arm with 0.33 µm average size and 0.38 µm average distance from the centromere. On chromosome pair 2, 35S rDNA sites were observed on pericentromeric position, with 0.80 µm average size (Figure 1 and 3; Table 2).

For triploid *C. dactylon*, the lengths of the largest and smallest chromosome pairs corresponded to 16.25% and 7.58%, respectively, of the TLHL. Hemizygous state of chromosome pair 5 in relation to 5S rDNA sites was observed. Interstitial-terminal signals of 5S rDNA were found on the short arm of chromosomes 13 and 14, with 0.46 µm average size and 0.50 µm average distance from the centromere. On pericentromeric region, 35S rDNA sites were found of chromosomes 7, 8 and 9, with 0.85 µm average size (Figure 2 and 3; Table 2).

In the tetraploid *C. dactylon*, the relative length of the longest chromosome pair corresponds to 13.91% of the total length of the haploid set whereas the shortest corresponds to 7.98 %. In only this accession, interstitial- proximal 5S rDNA signal occurs on chromosome 19 and 20 on short arm with 0.35 µm average size and 0.12 µm average distance from the centromere. On chromosome pairs 2 and 2’ were observed on the pericentromeric position 35S rDNA sites, with 0.94 µm average size (Figure 2 and 3; Table 2).

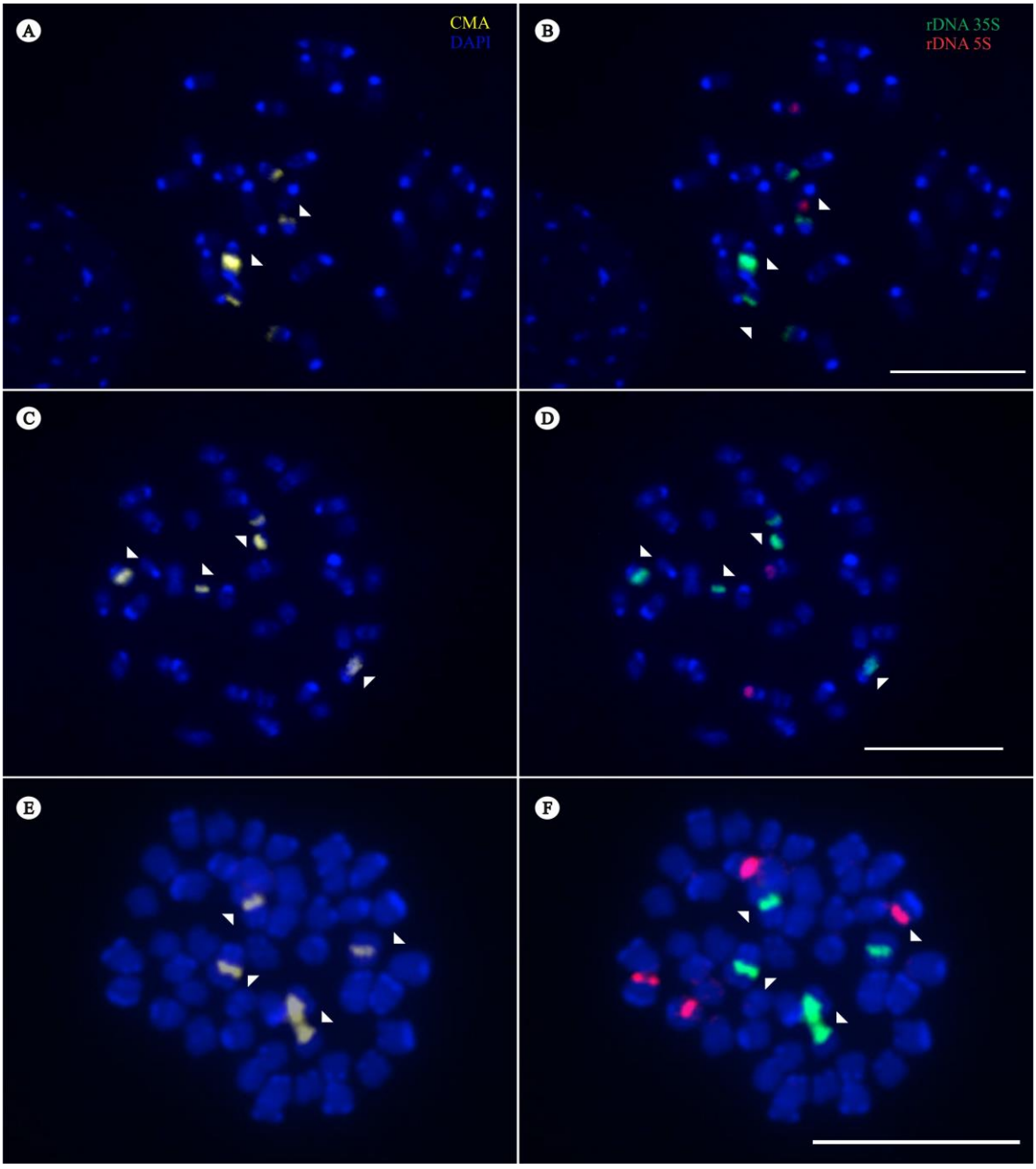
In the pentaploid *C. dactylon*, the relative length of the shortest chromosome pair corresponds to 13.91% of the total length of the haploid set while the longest corresponds to 7.98 %. We observed hemizygous state of chromosome pairs 2’ and 4’ in relation to 35S and 5S rDNA sites, respectively. The interstitial-terminal signals of 5S rDNA sites were located on the short arm on chromosome 17, 18, 19 and 20 with 0.35 µm average size and 0.12 µm average distance from the centromere. On the pericentromeric position, 35S rDNA sites were observed on chromosome pair 2, with 0.67 µm average size (Figure 2 and 3; Table 2).

Figure 1 Mitotic metaphases of *Cynodon* accessions with FISH and CMA/DAPI staining. 35S rDNA sites (green), 5S rDNA sites (red), CMA^{+} bands (yellow) and $DAPI^{+}$ bands (blue). (A-B) *Cynodon incompletus* (PI 29175301 PL 15; 2n = 2x = 18). (C-D) *Cynodon nlemfuensis* (ERX-7; 2n =2x = 18). (E-F) Diploid *Cynodon dactylon* (PI 291169-04; 2n = 2x = 18). (G-H) *Cynodon transvaalensis* (PI 289923-09; 2n = 4x = 36). 35S rDNA sites co-located along CMA bands (arrow). Scale Bar = 10μm.



Fonte: Do Autor (2018).

Figure 2 Mitotic metaphases of *Cynodon* accessions with FISH and CMA/DAPI staining. 35S rDNA sites (green), 5S rDNA sites (red), CMA^{+} bands (yellow) and $DAPI^{+}$ bands (blue). (A-B) Triploid *Cynodon dactylon* (PI 502336-23; 2n =3x = 27). (C-D) Tetraploid *Cynodon dactylon* (PI 291175 PL 12; 2n =4x = 36). (E-F) Pentaploid *Cynodon dactylon* (PI 289750-10; 2n =5x = 45). 35S rDNA sites co-located along CMA bands (arrow). Scale Bar = 10µm.



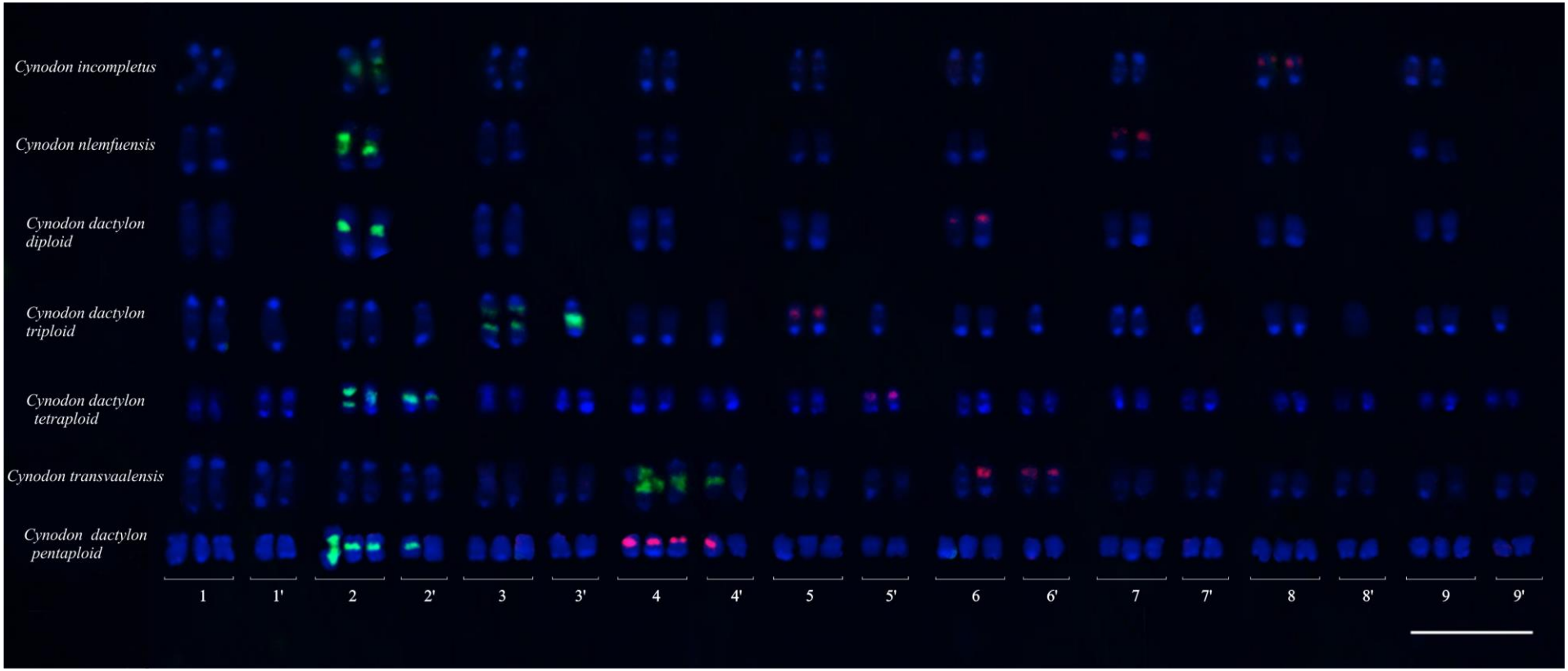
Fonte: Do Autor (2018).

Table 2 Summary of results obtained through cytogenetic analyses conducted in species of *Cynodon*

Characteristic evaluated	*Cynodon incompletus*	*Cynodon nlemfuensis*	*Cynodon dactylon*	*Cynodon dactylon*	*Cynodon dactylon*	*Cynodon transvaalensis*	*Cynodon dactylon*
Chromosome number	2n=2x=18	2n=2x=18	2n=2x=18	2n=3x=27	2n=4x=36	2n=4x=36	2n=5x=45
Nuclear DNA content	1.17 ± 0.036 pg*	1.08 ± 0.094 pg*	1.22±0.067pg	2.07± 0.019pg	2.303± 0.111pg	1.95 ± 0.086 pg*	2.55 ± 0.098 pg*
Karyotypic formula	9m	9m	9m	9m	9m	9m	9m
Average relative length of the shortest and longest chromosome pairs	8.24 and 15.28%	7.73 and 14.85%	7.92 and 14.21%	7.58 and 16.25%	7.98 and 13.91%	7.70 and 16.81%	7.98 and 13.91%
Total length of haploid set	26.02±0.42	27.49±0.34	25.40±0.36	28.28±0.64	18.97±0.40	20.21±0.69	17.59±0.30
Number of 35S rDNA sites	2	2	2	3	4	3	4
Chromosome pairs with 35S rDNA sites and location	2 (Pericentromeric region)	2 (Pericentromeric region)	2 (Pericentromeric region)	3 (Pericentromeric region)	2 (Pericentromeric region)	3 (Pericentromeric region)	4 (Pericentromeric region)
Average size of 35S rDNA site	1.03µm	1.17µm	0.80µm	0.85µm	0.94µm	0.72µm	0.67µm
Variation amplitude size of 35S rDNA sites	0.69 to 1.46 µm	0.71 to 1.71 µm	0.33 to 1.36 µm	0.50 to1.33µm	0.52 to 1.80 µm	0.21 to1.18 µm	0.31 to 1.80 µm
Number of 5S sites	2	2	2	2	2	3	4
Chromosome pairs with 5S rDNA site and location	8 (Interstitial-proximal on short arm)	7 (Interstitial-proximal on short arm)	6 (Interstitial-proximal on short arm)	5 (Interstitial-terminal on short arm)	5 (Proximal on short arm)	6 (Interstitial-proximal on short arm)	4 (Interstitial-terminal on short arm)
Average size of 5S rDNA sites on chromosome pairs	0.54µm	0.46µm	0.33µm	0.46µm	0.35µm	0.39µm	0.50µm
Average distance of 5S rDNA sites from the centromere	0.24µm	0.28µm	0.38µm	0.50µm	0.12µm	0.23µm	0.54µm
Number of CMA^+ bands	2	2	2	3	4	3	4
Percentage of $DAPI^+$ bands	55.53%	33.9%	28.24%	38.34%	30.9%	34.62%	35.67%
MCA[1]	7.52±1.46	10.57±1.25	9.49±1.51	9.08±1.57	9.22±0.64	8.45±1.55	6.71±1.10
CVCL[2]	21.81±3.50	21.81±4.39	19.77±3.05	24.14±3.12	23.57±2.93	25.44±2.49	18.42±2.97
Presence of chromosomal heteromorphism	Difference in the total length of homologous chromosomes at pair 8	Difference in the total length of homologous chromosomes at pair 9		Hemizygous state of 5S rDNA site in the chromosomes sets 5 and 5’	Hemizygous state of 5S rDNA site in the chromosome pair 5 and 5’	Hemizygous state of 35 and 5S rDNA sites in the chromosome pair 4’ and 6’.	Hemizygous state of 35S and 5S rDNA sites in the chromosome pair 2’ and 4’. Difference in the total length of 35S rDNA site in the chromosomes sets 2.

* Chiavegatto et al (2016). [1]Mean centromeric asymmetry; [2] Coefficient of variation of chromosome length. Fonte: Do Autor (2018).

Figure 3 Karyogram of *Cynodon* species/ accessions. 35S rDNA sites (green), 5S rDNA sites (red), CMA^{+} bands (yellow) and $DAPI^{+}$ bands (blue). *Cynodon incompletus* (PI 29175301 PL 15; 2n = 2x = 18). *Cynodon nlemfuensis* (ERX-7; 2n = 2x = 18). Diploid *Cynodon dactylon* (PI 291169-04; 2n = 2x = 18). Triploid *Cynodon dactylon* (PI 502336-23; 2n = 3x = 27). Tetraploid *Cynodon dactylon* (PI 291175 PL 12; 2n = 4x = 36). *Cynodon transvaalensis* (PI 289923-09; 2n = 4x = 36). Pentaploid *Cynodon dactylon* (PI 289750-10; 2n = 5x = 45). Scale Bar = 10µm.



Fonte: Do Autor (2018).

For *Cynodon incompletus*, the length of the shortest and longest chromosome pairs are respectively 8.24 and 15.28% of the total length of the haploid set. Interstitial-terminal signals of 5S rDNA were observed on chromosome pairs 8 on short arm, with 0.54 µm average size and 0.24 µm average distance from the centromere. On chromosome pair 2, 35 rDNA sites were located on the pericentromeric region, with 1.03 µm average size. The chromosome pair 9 presented heteromorphism in relation to the total length of the chromosome (Figure 1 and 3; Table 2).

In *Cynodon nlemfuensis*, the length of the largest and smallest chromosome pairs corresponded to 14.85 and 7.73%, respectively, of the TLHL. Interstitial-terminal signal, 5S rDNA, were found on chromosome pair 7 on short arm with 0.46 µm average size and 0.28 µm average distance from the centromere. On chromosome pair 2, were observed 35S rDNA sites on the pericentromeric position, with 1.17µm average size. Heteromorphism in relation to the total length of the chromosome was found on the chromosome pair 9 (Figure 1 and 3; Table 2).

Cynodon transvaalensis had the largest and smallest chromosome pairs, which corresponded to 16.81 and 7.70 %, respectively, of the total length of the haploid set. We observed hemizygous state of chromosome pairs 4' and 6 in relation to 35S and 5S rDNA sites, respectively. Interstitial-terminal signals of 5S rDNA were evidenced on chromosome pairs 6 and 6' on short arm, with 0.39 µm average size and 0.23 µm average distance from the centromere. On chromosome pair 4 and 4', 35 rDNA sites were located on the pericentromeric region, with 0.72 µm average size. (Figure 1 and 3; Table 2).

For all accessions studied, 35S rDNA sites were co-located along with CMA bands (regions rich in CG) and displays the extended chromatin fiber while 5S rDNA sites were co-located along with $DAPI^+$ bands (regions rich in AT), except in *C. nlemfuensis* and tetraploid *C. dactylon* (Figure 1, 2 and 3).

Our results suggest that *C. nlemfuensis* was the species with the most asymmetric and derived karyotype. Moreover, pentaploid *C. dactylon* was the access with the most symmetric karyotype.

3.2 Mapping of heterochromatin pattern

Heterochromatin bands were revealed by CMA/DAPI staining after fluorescent *in situ* hybridization. DAPI-banding pattern was observed with intraspecific in *C. dactylon* cytotypes and interspecific variations (Table 2). Regarding the variations, *C. incompletus* presented the highest amount of DAPI heterochromatin, with 55.53% of the total length of the haploid set. These bands were distributed in all chromosome pairs on the long arm (terminal region), short arm (terminal region), and pericentromeric region (Figure 1 and 3).

Cynodon transvaalensis has 34.62% of the total length of the haploid set with DAPI heterochromatin bands, distributed in all chromosome pairs on long arm (terminal region). By contrast, on short arm, terminal region $DAPI^+$ bands were stained on chromosome pairs 1, 1', 2, 2', 4, and 4'. Interstitial-proximal $DAPI^+$ bands were also observed on chromosome pair 6 and 6', on short arm. On chromosome pairs 1, 1', 2, 2', 5, and 5' were found $DAPI^+$ bands on pericentromeric region (Figure 1 and 3).

In *Cynodon nlemfuensis* we observed 33.9% of the $DAPI^+$ bands of the TLHL. The DAPI heterochromatin pattern showed 9 bands on terminal region (long arm) of all chromosome pairs, two terminal regions (chromosome pairs 1 and 2) and one interstitial-proximal (chromosome pair 4) on short arm. In addition, we found one pericentromeric region $DAPI^+$ bands on chromosome 2 (Figure 1 and 3).

In relation to both intraspecific and interspecific levels, diploid *C. dactylon* had the least amount of $DAPI^+$ bands, with 28.24% of the total length of the haploid set. These bands were found in all chromosome pairs (terminal region on long arm). On chromosome pairs 1 and 3 (terminal region on short arm) and chromosome pair 4 (interstitial-proximal) were stained with DAPI. Pericentromeric region on chromosome pairs 3, 7, 8 e 9, were observed $DAPI^+$ bands (Figure 1, 3, and 4).

Tetraploid *C. dactylon* had 30.09% of the $DAPI^+$ bands of the TLHL. On long arm there were no $DAPI^+$ bands just on the chromosome pairs 9 and 9'. On short arm, we found no $DAPI^+$ bands. Pericentromeric region were no $DAPI^+$ bands on chromosome pairs 1, 1', 2, and 2' (Figure 2, 3, and 4).

Pentaploid *C. dactylon* has 35.67% of the total length of the haploid set with DAPI heterochromatin, distributed in all chromosome pairs on long arm (terminal region). The

short arm have terminal DAPI$^+$ bands on chromosome sets 1, 1', 3 and '3. Interstitial-terminal DAPI$^+$ bands were also found on chromosome sets 4 and 4', on short arm. The pericentromeric region was stained just on chromosome pair 1 (Figure 2, 3, and 4).

Pentaploid *C. dactylon* has 35.67% of the total length of the haploid set with DAPI heterochromatin, distributed in all chromosome pairs on long arm (terminal region). The short arm has terminal DAPI$^+$ bands on chromosome sets 1, 1', 3 and '3. Interstitial-terminal DAPI$^+$ bands were also found on chromosome sets 4 and 4', on short arm. The pericentromeric region was stained just on chromosome pair 1 (Figure 2, 3, and 4).

At the intraspecific level, triploid *C. dactylon* has the highest amount of DNA with 38.34% of DAPI heterochromatin of the total length of the haploid set. These bands were distributed in all chromosome pairs on long arm (terminal region), short arm on chromosome sets 1, 1', 2, 2', 3, 3', 6, 6', 7 and 7' (terminal region) and chromosome pair 5 (interstitial-terminal), and pericentromeric region on chromosome sets 1, 1', 2, 2', 7 and 7' (Figure 2, 3, and 4).

3.3 Chromosome evolution and character mapping

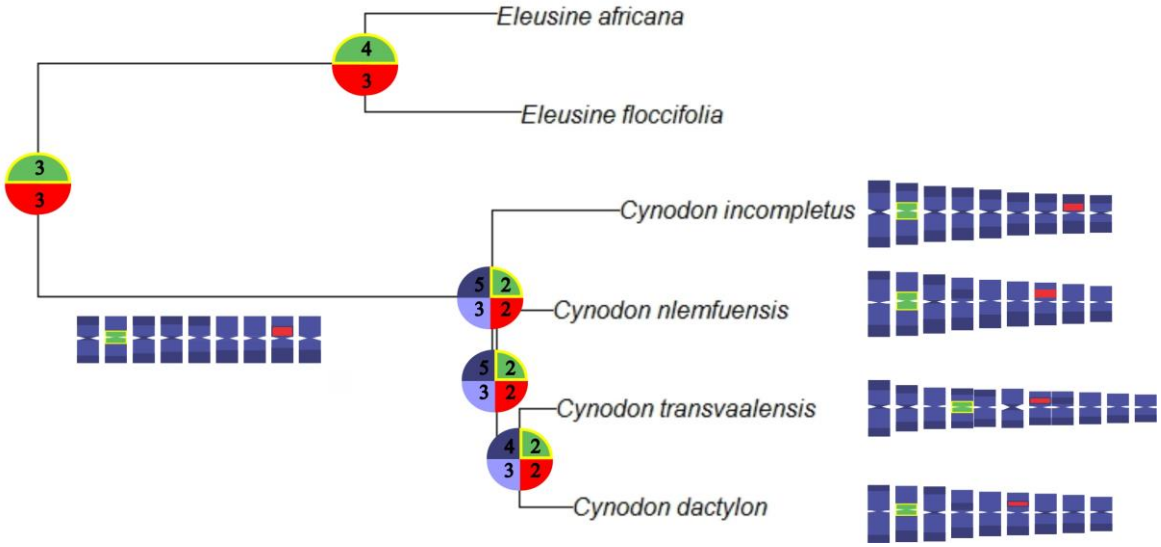
The chromosome evolution of *C. dactylon* cytotypes occurred via polyploidization, hybridization and genomic reorganization. Tetraploid *Cynodon dactylon* was originated from hybridization/polyploidization of diploid *C. dactylon*. Triploide *Cynodon dactylon* originated from the union of reduced gametes of diploid and tetraploid cytotypes. Pentaploid *Cynodon dactylon* were originated from union of reduced gametes of diploid and unreduced gametes of tetraploid cytotypes.

In our results, most recent common ancestor of *Cynodon* had two 35S and 5S rDNA sites, two CMA bands and nine, five and three DAPI bands on long arm, short arm, and pericentromeric region, respectively (Figure 5).

Figure 4 Hypothesis of the karyotype evolution in *Cynodon dactylon* cytotypes. 35S rDNA sites (green), 5S rDNA sites (red), CMA^+ bands (yellow) and $DAPI^+$ bands (blue).

Fonte: Do Autor (2018).

Figure 5 The most recent common ancestral karyotype of *Cynodon incompletus* (2*n* = 2*x* = 18), *Cynodon nlemfuensis* (2*n* = 2*x* = 18), *Cynodon transvaalensis* (2*n* = 4*x* = 36) and *Cynodon dactylon* (2*n* = 2*x* = 18). 35S rDNA sites (green), 5S rDNA sites (red), CMA^+ bands (yellow), $DAPI^+$ bands on short arm (dark blue), and $DAPI^+$ bands on centromeric region (light blue).



Fonte: Do Autor (2018).

4 DISCUSSION

4.1 Karyotype variation in polyploidy event

Classical and molecular cytogenetic techniques, such as CMA/DAPI staining and FISH, 35S and 5S rDNA sites, provided useful markers for chromosome identification and valuable data to enhance knowledge about genome organization and chromosome evolution in *Cynodon*. This information coupled with phylogenetic analyzes allowed the construction of hypotheses about speciation processes and chromosome evolution in the genus. In this study, we reconstructed the most recent common ancestor karyotype for *C. dactylon*, *C. incompletus, C. nlemfuensis*, *C. transvaalensis* using chromosome character mapping. In addition, we investigated the karyotype variation during polyploidy event in *C. dactylon* cytotypes. Most of the variants were characterized by the absence or presence of signals of the heterochromatin bands and ribosomal DNA due to duplications or deletions. Our result turned out to provide a powerful insight in the chromosome/genome characterization in *Cynodon,* as the mapping of rDNA loci was described for the first time. By using this approach, we reinforced the potential of using molecular karyotype data as a mean of explaining the genetic diversity present in this genus.

For the majority of *Cynodon* species studied, there are reported of polyploids cytotypes with diploid (2n = 2x = 18) and tetraploid (2n = 4x = 36) forms (HARLAN et al., 1970; ASSEFA et al., 1999; CHIAVEGATTO et al., 2016). However, for *C. dactylon* that presents from diploid (2n = 2x = 18) to hexaploid (2n = 6x = 54) (DHALIWAL; GUPTA 2011), but the tetraploid (2n = 4x = 36) is the prevalent cytological form (WU, 2011).

Nuclear ribosomal DNA (rDNA) encoding 5S and 5.8S-18S-26S rRNA. Both 5 S and 35S rDNA are organized in tandem arrays. However, each 35S rDNA unit contains 18S, 5.8S and 26S rRNA genes, the internal transcribed spacers (ITSs), and an intergenic spacer (IGS) (GARCIA et al., 2012; GARCIA; KOVARÍK, 2013). C-G nucleotides are present in high ratio in composition of 35S rDNA sites and are stained by chromomycin A (CMA) (CABRAL et al., 2006). Therefore, the co-localization between rDNA 35S sites and CMA bands, as observed in *Cynodon*, is highly reported in the literature (GUERRA, 2000; NANI et al., 2016; COSTA et al., 2017) as well as for the grass family, such as in

Brachiaria (NANI et al., 2016), *Setaria* (NANI et al., 2015) and *Lolium* (ROCHA et al., 2015).

In the diploid species studied, the number of rDNA 35S and 5S are conserved. However, the position for rDNA 5S sites ranged from chromosomal pairs 6 to 8, while the position for rDNA 35S sites were always in the chromosome pair 2.

For polyploid species/accessions, the gain of 35S rDNA loci appear to have occurred more frequently than 5S rDNA loci. In other words, the gain of 35S rDNA loci followed the ploidy level, except in *C. transvaalensis* and pentaploid *C. dactylon*. Their position were observed more often on chromosome pairs 2 and 2', except in *C. transvaalensis* and in the triploid *C. dactylon*. Number of the 5S rDNA loci were observed in hemizygous only in *C. transvaalensis* and in the pentaploid *C. dactylon*. For the other polyploid accessions, the number of the 5S rDNA loci maintained the two sites observed in diploid species/ accessions, but their positions ranged from chromosomal pairs 4 to 8.

In the study conducted by Zhi-Yun et al. (2013) in two tetraploid *Cynodon* (Bermudagrass) using FISH technique, three 35S rDNA and 5S rDNA sites were observed in genotype C121. While, in C299, 35S and 5S rDNA probe exhibited four hybridization signals, for each sites. The 35S rDNA sites were located at the centromeric regions for both accessions. Meiotic studies in tetraploid *C. dactylon* showed one bivalent connected to the nucleolus and rarely two or three (SILVA et al., unpublished data). However, Brilman et al. (1982) mentioned the presence of the nucleolus organizer region (NOR) in the chromosomal pair 4, different from that observed in this study in mitotic metaphases. In the diploid cytotype of *C. dactylon* presented by Dhaliwal et al. (2011) a nucleolar bivalent was observed, coinciding with the number of rDNA sites observed in this study for the diploid cytotype of *C. dactylon*.

Ribosomal DNA positions are highly polymorphic since these genes are mobile and are able to have high potential intragenomic mobility. In general, the action of tranposable elements has been associated to the intraspecific and interspecific variations in the number and localization of rDNA sites (SCHUBERT; WOBUS, 1985; SCHUBERT, 2007; LYSÁK; SCHUBERT, 2013). In this case, comparative studies using specific genes, such as 5S and 35S rDNA sites, allow to identify chromosome rearrangements (inversions, deletions, or translocations) and trace karyotype evolution (SCHUBERT; LYSÁK, 2011).

On the other hand, in some groups the rDNA sites are highly conserved, so these regions are often used in cytotaxonomic studies (GUERRA; 2012). For this species/accessions studied, the 35S rDNA sites on chromosome pairs 2 is a characteristic plesiomorphic. Probably, 35S rDNA sites in *C. transvaalensis* and triploid *C. dactylon* along the evolution of the group, were carried with the transposon elements for chromosome pair 4 and 4' in *C. transvaalensis* and chromosome sets 3 and 3' in triploid *C. dactlylon*.

The DAPI bands^{+} did not present distribution patterns and were different in amounts and sizes in all species/accessions. According to Guerra (2000), polymorphism for the number and size of heterochromatin bands is frequent in plants and transposable elements together with polyploidization are responsible for genome evolution (VICIENT; CASACUBERTA, 2017). These events result in changes of chromosome morphology by addition, inversions, deletions, or translocations of heterochromatin segment to chromosomes and consequently in variations in karyotype asymmetry in a group (HIZUME; SHIBATA, 2016). These rearrangements can explain the heteromorphisms on pairs 8 and 9, observed in the diploid species *C. incompletus* e *C. nlemfuemsis.*

Chiavegatto et al. (2016) studied the karyotypic asymmetry, via classical cytogenetics, of eight *Cynodon* species/accessions. These authors found only metacentric chromosomes (m) and showed karyotypic formula 9m, except for *C. incompletus* that presented only submetacentric chromosomes (sm) and karyotypic formula 9sm. In addition, *C. incompletus* showed the highest asymmetric karyotype, while *C. nlemfuensis* had the most symmetrical karyotype as compared to the polyploids accessions. In our results *C. nlemfuensis* had the most asymmetrical karyotype and pentaploid *C. dactylon* had the the most symmetrical karyotype. In both studies, triploid and tetraploid species/accessions showed the closest karyotypes. These differences may be related to the condensation degree of the metaphases used to obtain the karyological data. Moreover, in this study we used molecular cytogenetic techniques that allows greater refinement of the analyzes and facilitates the discrimination of chromosome pairs.

4.2 Intraspecific karyotype variation in *Cynodon dactylon*

Polyploid species have high adaptive capacity an edge over their diploid parents, because duplicated gene copies could allow the masking of deleterious recessive mutations or these genes can assume new or slightly varied functions (neofunctionalization or subfunctionalization) (MADLUNG, 2013). *Cynodon dactylon* presented five cytotypes, with a wide variation in levels ploidy, from diploid to hexaploid (DHALIWAL; GUPTA, 2011). For this reason it is the most adapted species of the genus and widely distributed throughout the tropical and sub-tropical regions in the world. Our results showed that *C. dactylon* cytotypes have a high karyotype variation, agreeing with the results of Wu (2011). The latter was based on molecular markers and showed that *C. dactylon* also has a high genetic variability.

Variation in the number and position of heterochromatin bands and ribosomal rDNA sites in *C. dactylon* cytotypes indicate that genomic reorganization has occurred during polyploidy events in the group. Likewise, that other polyploid species, after polyploidization events, occur chromosome rearrangements in order to remove complexity chromosome pairing and segregation interactions on the meiosis (COMAI, 2005) aiming at diploidization in meiotic behavior. A recent study (SILVA et al., unpublished data) with two tetraploid accessions of *C. dactylon* var. *dactylon* (PI 224141-29 and PI 29117102 PL 18) showed the formation of multivalents, but predominance of bivalents. These results indicate the search for a diploid-like meiosis behavior as suggest in our study based on comparative karyotype analyses.

Based on the distribution pattern variations (number and position) of ribosomal DNA and heterochromatin bands, including hemizygous status in some chromosomes, our results, indicate the presence of different genomes within *C. dactylon* cytotypes. These proposal is corroborated by meiotic analysis (SILVA et al. unpublished data) and molecular marks (GUO et al., 2015; 2016), suggesting that genomes are homoeologous and that polyploidy cytotypes are segmental allopolyploids.

5 CONCLUSION

Genus *Cynodon* have a high frequency of karyotype variation.

The number and position of 35 rDNA sites and number of the 5S rDNA sites are conserved in diploid species.

Cynodon dactylon is a segmental allopolyploid species.

The most recent common ancestor of *Cynodon* had two 35S and 5S rDNA sites, two CMA bands and nine (long arm), five (short arm) and three (pericentromeric region) DAPI heterochromatin.

During the events of polyploidization of the species there were losses and gains of heterochromatic regions which were responsive by the karyotype variation in *Cynodon*.

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