Molecular Biology Reports (2020) 47:887–896
https://doi.org/10.1007/s11033-019-05179-7

ORIGINAL ARTICLE

GISH-based comparative genomic analysis in *Urochloa* P. Beauv.

Caio T. R. Corrêa[1] · Nathalia G. Z. Bonetti[1] · Sanzio C. L. Barrios[2] · Cacilda B. do Valle[2] · Giovana A. Torres[1] · Vânia H. Techio[1]

Received: 10 September 2019 / Accepted: 31 October 2019 / Published online: 16 November 2019

Abstract

The genus *Urochloa* P. Beauv. [syn. *Brachiaria* (Trin.) Griseb.] comprises species of great economic relevance as forages. The genomic constitution for the allotetraploid species *Urochloa brizantha* (cv. Marandu) and *Urochloa decumbens* (cv. Basilisk) and the diploid *Urochloa ruziziensis* was previously proposed as BBB^1B^1, $B^1B^1B^2B^2$ and B^2B^2, respectively. Evidence indicates *U. ruziziensis* as the ancestral donor of genome B^2 in *U. decumbens* allotetraploidy, but the origin of the genomes B and B^1 is still unknown. There are diploid genotypes of *U. brizantha* and *U. decumbens* that may be potential ancestors of the tetraploids. The aim of this study was to determine the genomic constitution and relationships between genotypes of *U. brizantha* (2x and 4x), *U. decumbens* (2x and 4x) and *U. ruziziensis* (2x) via genomic in situ hybridization (GISH). Additionally, chromosome number and genome size were verified for the diploid genotypes. The diploids *U. brizantha* and *U. decumbens* presented 2n = 2x = 18 chromosomes and DNA content of 1.79 and 1.44 pg, respectively. The GISH analysis revealed high homology between the diploids *U. brizantha* and *U. decumbens*, which suggests relatively short divergence time. The GISH using genomic probes from the diploid accessions on the tetraploid accessions' chromosomes presented similar patterns, highlighting the genome B^1 present in both of the tetraploids. Based on GISH results, the genomic constitution was proposed for the diploid genotypes of *U. brizantha* (B^1B^1) and *U. decumbens* ($B^{1\prime}B^{1\prime}$) and both were pointed as donors of genome B^1 (or $B^{1\prime}$), present in the allotetraploid genotypes.

Keywords *Brachiaria* · Genomic composition · Polyploidy · Cytogenomic analysis

Abbreviations

GISH	Genomic in situ hybridization
RAPD	Random amplification of polymorphic DNA
CTAB	Cetyltrimethylammonium bromide
gDNA	Genomic DNA
SSC	Saline sodium citrate
TNT	Tris-NaCl-Tween-20
DAPI	4′,6-Diamidino-2-phenylindole
H3K4me2	Dimethylation of the lysine residue at 4th position on the N-terminal tail of histone 3
H3K9me2	Dimethylation of the lysine residue at 9th position on the N-terminal tail of histone 3
FISH	Fluorescent in situ hybridization
rDNA	Ribosomal DNA

Introduction

The genus *Urochloa* P. Beauv. [syn. *Brachiaria* (Trin.) Griseb.] comprises approximately 135 species distributed in tropical and subtropical regions, mainly in East Africa, which is considered as their center of origin [1]. *Urochloa* species are the most commonly used forages in Brazil, representing 85% of the cultivated pastures (99 Mha). The species of greatest economic importance are *Urochloa brizantha* (Hoschst. ex A. Rich) R.D. Webster, *Urochloa decumbens* (Stapf) R.D. Webster, *Urochloa humidicola* (Rendle) Morrone & Zuloaga and *Urochloa ruziziensis* (R. Germ. & C.M. Evrard) Morrone & Zuloaga [2].

Due to the extensive use of *Urochloa* species in livestock nutrition and their wide adaptation and distribution, new

This study is part of C.T.R. Correa's thesis and the abstract can be found in the repository: http://repositorio.ufla.br/jspui/handle/1/33642.

✉ Vânia H. Techio
vhtechio@ufla.br

[1] Department of Biology/DBI – Plant Cytogenetics Laboratory, Federal University of Lavras (UFLA), P.O. Box 3037, Lavras, Minas Gerais, Brazil

[2] Embrapa Gado de Corte – Campo Grande, Campo Grande, Mato Grosso do Sul, Brazil

cultivars have been pursued through genetic breeding to overcome actual cultivar limitations [3]. Part of the current breeding programs are focused in the production of interspecific hybrids with the species *U. brizantha*, *U. decumbens* and *U. ruziziensis* [2]. These three species are known to form an agamic complex, in which *U. brizantha* and *U. decumbens* are predominantly apomictic tetraploids and *U. ruziziensis* is a sexual diploid [4].

The phylogenetic relationships between the agamic complex species demonstrate some controversy. Morphological analysis have grouped *U. brizantha*, *U. decumbens* and *U. ruziziensis*, indicating high similarity between the three species and closer proximity between the first two [5, 6]. Molecular phylogenies and dendrograms corroborate with the grouping of these three species, but they differ regarding the interrelations within the group. Studies based on RAPD markers [7, 8] and chloroplastid genes [9] agree with the morphological data on the closer proximity between *U. brizantha* and *U. decumbens*. According to Pessoa-Filho et al. [9], *U. ruziziensis* lineage would have diverged from the other two 5.67 mya, followed by a more recent divergence between the other two, approximately 1.6 mya. In contrast, Triviño et al. [10], in a study of genetic diversity and population structure based on microsatellites, verified a closer proximity between *U. decumbens* and *U. ruziziensis*, in relation to *U. brizantha*. It is worth noting that the majority of these studies used mostly polyploid genotypes and cultivars, with the exception of *U. ruziziensis*, which is exclusively diploid and a single diploid genotype of *U. decumbens* used in Triviño et al. [10] analysis.

Similarly, cytogenetic studies tend to assess predominantly polyploid species, considering its prevalence in the genus (specially tetraploids) and in cultivated pastures [3]. Chromosome numbers in *Urochloa* range from 2n = 14 to 2n = 90, and the most frequently observed basic chromosome number is x = 9. Regarding the agamic complex species, cytotypes (intraspecific variation on chromosome number) were reported for *U. brizantha* (2n = 18, 36, 45 and 54) and *U. decumbens* (2n = 18 and 36) [11, 12].

Meiotic studies on tetraploids cultivars of *U. brizantha* (cv. Marandu) e *U. decumbens* (cv. Basilisk) and hybrids revealed several abnormalities typical from allopolyploids, e.g., asynchronic chromosome segregation, intragenomic pairing and presence of multivalents and micronucleus [13–17]. More recently, the allotetraploidy was confirmed via genomic in situ hybridization (GISH) and a proposal for genomic composition was presented for *U. brizantha* (BBB^1B^1), *U. decumbens* ($B^1B^1B^2B^2$) and *U. ruziziensis* (B^2B^2) [18]. The authors have also demonstrated that the three genomes (B, B^1 and B^2) are homoeologous and that *U. decumbens* and *U. ruziziensis* share one genome, which highlights the greater proximity between the two species, as proposed in previous studies [10, 11, 19, 20]. This scenario points to *U. ruziziensis* as the ancestral donor of genome B^2, whereas the origin of the genomes B and B^1 is unknown and eligible candidates would be diploid genotypes of *U. brizantha* and *U. decumbens*.

Additional GISH analysis including diploid genotypes may contribute to validate the genomic constitution proposed by Paula et al. [18], as well as to assist in the investigation of the ancestral genomes involved in the polyploidization process and the genomic relationships between the diploid and tetraploid genotypes. Thus, the aims of this study were to determine the genomic constitution and the chromosome homology/homoeology relationship between genotypes of *U. ruziziensis* (2x), *U. brizantha* (2x and 4x) and *U. decumbens* (2x and 4x), via GISH.

Materials and methods

Plant material

The experiment was conducted with the diploid accessions of *U. brizantha* [B105 (2n = 2x = 18)] and *U. decumbens* [D04 (2n = 2x = 18)], from Embrapa Beef Cattle, municipality of Campo Grande, Mato Grosso do Sul State, Brazil and the commercial cultivars *U. ruziziensis* [cultivar Kennedy (2n = 2x = 18)], *U. brizantha* [cultivar Marandu (2n = 4x = 36)] and *U. decumbens* [cultivar Basilisk (2n = 4x = 36)].

Slide preparation

Root tips were collected and pretreated with cycloheximide (12.5 mg/l) for 2 h at room temperature. Subsequently, they were washed in distilled water and fixed in ethanol/acetic acid (3:1) solution. Cell wall digestion was performed with an enzyme solution consisting of cellulase Onozuka R10 (0.7%), cellulase Sigma-Aldrich (0.7%), pectolyase Sigma-Aldrich (1%) and cytohelicase Sigma-Aldrich (1%) for 90 min at 37 °C. Slides were prepared according to the flame-drying technique [21], with adaptations.

GISH

Genomic DNA (gDNA) from *U. brizantha* (B105), *U. decumbens* (D04) and *U. ruziziensis* was isolated using CTAB protocol [22]. The genomic probes were labeled by nick translation with digoxigenine-12-dUTP. The GISH was performed by hybridizing gDNA of the diploid genotypes reciprocally and on the tetraploid cultivars (Fig. 1).

Previously selected slides were denatured in 70% formamide at 85 °C for 1 min and 25 s, followed by dehydration in alcohol series (70, 90 and 100%) for 5 min each. The hybridization mixture containing formamide (50%), dextran

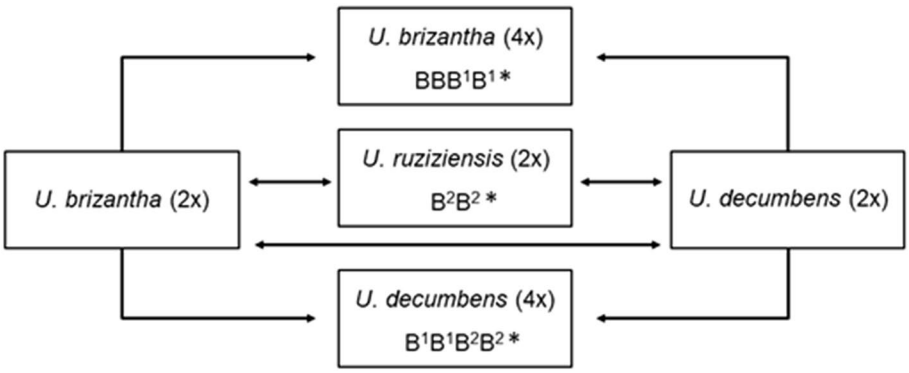

Fig. 1 Schematic of GISH probing in *Urochloa* species. Aesterisk—genomes determined by Paula et al. [18]

sulfate (10%), 2x saline sodium citrate (SSC) buffer (pH 7.0) and 50 to 100 ng of probe was denatured at 95 °C for 8 min and applied to the slides. Hybridization process took place in humid chamber at 37 °C for 24 to 48 h. No blocking DNA was used.

Probes were detected using anti-digoxigenin conjugated with rhodamine after washes in 2x SSC buffer at 42 °C (80.7% of estringency) and 1× tris-NaCl-Tween-20 (TNT). The chromosomes were stained with 4′,6-diamidino-2-phenylindole (DAPI)/Vectashied and images were captured by QImaging Retiga EXi CCD camera attached to a fluorescence microscope Olympus BX 60.

The genomic relationship analysis was based on the genomic composition proposed by Paula et al. [18]. Chromosome segments stained by the genomic probe (GISH+ signals) were measured in the Karyotype software 2.0 [23] to assess the hybridization proportion on the metaphases. Chromosomes were grouped according to extension of GISH+ signals, based on the chromosome regions (centromeric and pericentromeric, interstitial and terminal) described by Heslop-Harrison and Schwarzacher [24] with adaptations. Image processing was done in the Photoshop CC 2017 Software.

Genome size estimation

Samples of 20–30 mg of young foliar tissue of *U. brizantha* and *U. decumbens* were macerated with leaves of *Pisum sativum* L. (internal standard-DNA 2C = 9.09 pg) in 1 mL of frozen $MgSO_4$ buffer to obtain a nuclear suspension [25]. The suspension was stained with 25 µL of propidium iodide (1 mg/mL) and a minimum of 10.000 nucleus were quantified in a Fascalibur (Becton Dickinson) cytometer. Histograms were obtained using the Cell Quest software and analysed on the WinMDI 2.9 software. Genome size was estimated in picograms (pg).

Results

The accessions B105 (*U. brizantha*) and D04 (*U. decumbens*) were both confirmed with 18 chromosomes and presented nuclear DNA content (2C) of 1.79 and 1.44 pg, respectively.

GISH+ signals varied in the extension of hybridization region (centromeric/pericentromeric and interstitial regions or fully hybridized chromosomes) on chromosomes within the same set and between different accessions (Figs. 2, 3). The genomic probe of *U. decumbens* 2x and *U. ruziziensis* hybridized 65.43% and 45.20%, respectively, on *U. brizantha* 2x chromosomes (Table 1; Fig. 2a, b).

In *U. decumbens* 2x, the proportion of hybridized genome was 100% with *U. brizantha* 2x probe (Table 1; Fig. 2c) and 60.89% with *U. ruziziensis* probe (Table 1; Fig. 2d). As for *U. ruziziensis*, the gDNA of *U. brizantha* 2x and *U. decumbens* 2x hybridized 70.20% and 51% (Table 1; Fig. 2e, f), respectively.

Regarding the tetraploid cultivars, *U. brizantha* 2x and *U. decumbens* 2x probes produced similar hybridization pattern in *U. brizantha* 4x (Fig. 3a, b) and proportion of hybridized genome of 41.36% and 51.56%, respectively. *U. decumbens* 4x hybridized 58.29% with *U. brizantha* 2x probe (Table 1; Fig. 3c) and 49.38% with *U. decumbens* 2x probe (Table 1; Fig. 3d).

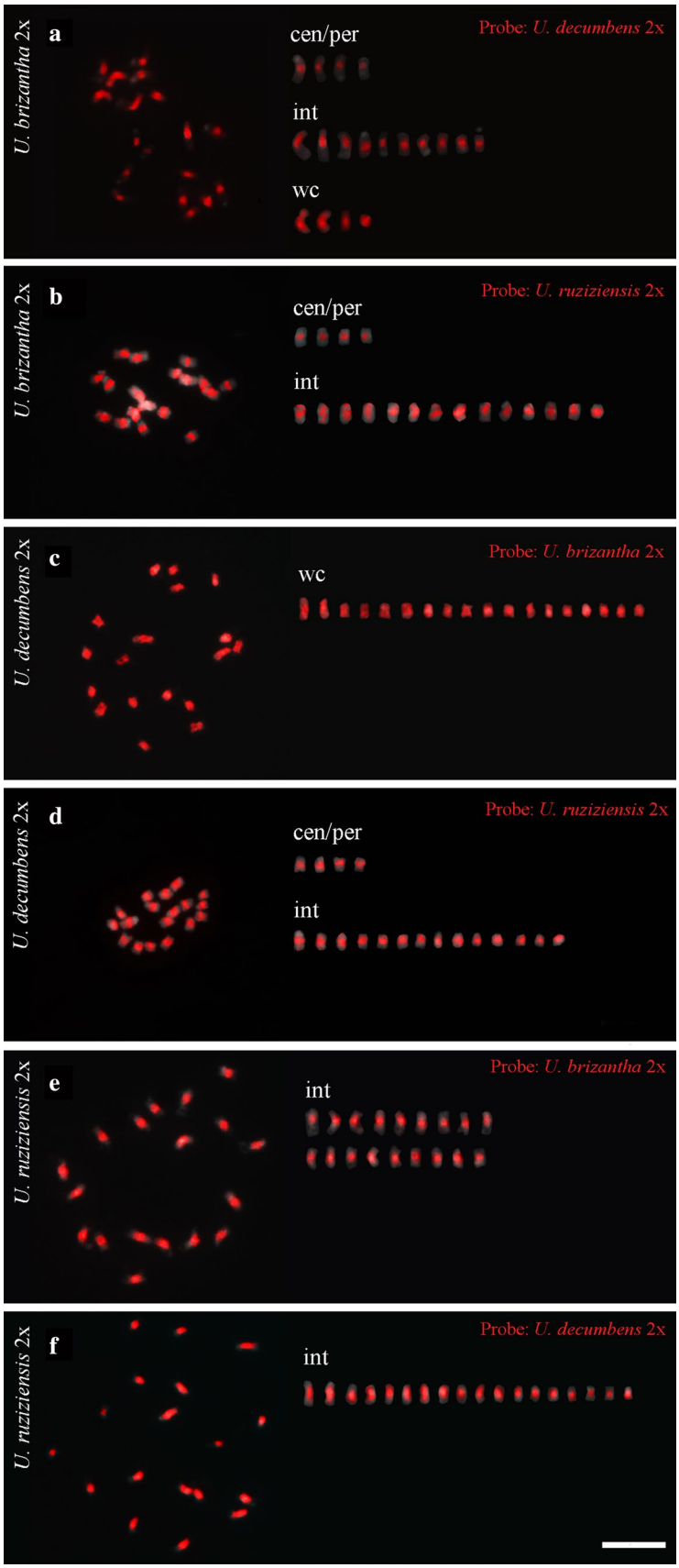

Fig. 2 Metaphases of *U. brizantha* (2n = 2x = 18) with probes of *U. decumbens* 2x (**a**) and *U. ruziziensis* 2x (**b**). Metaphases of *U. decumbens* (2n = 2x = 18) with probes of *U. brizantha* 2x (**c**) and *U. ruziziensis* 2x (**d**). Metaphases of *U. ruziziensis* (2n = 2x = 18) with probes of *U. brizantha* 2x (**e**) and *U. decumbens* 2x (**f**). Chromosomes are stained with DAPI (grey) and probe signals are indicated by red fluorescence. The GISH+ signals were classified as centromeric/pericentromeric (cen/per), interstitial (int) or whole chromosome (wc). The bar represents 10 µm. (Color figure online)

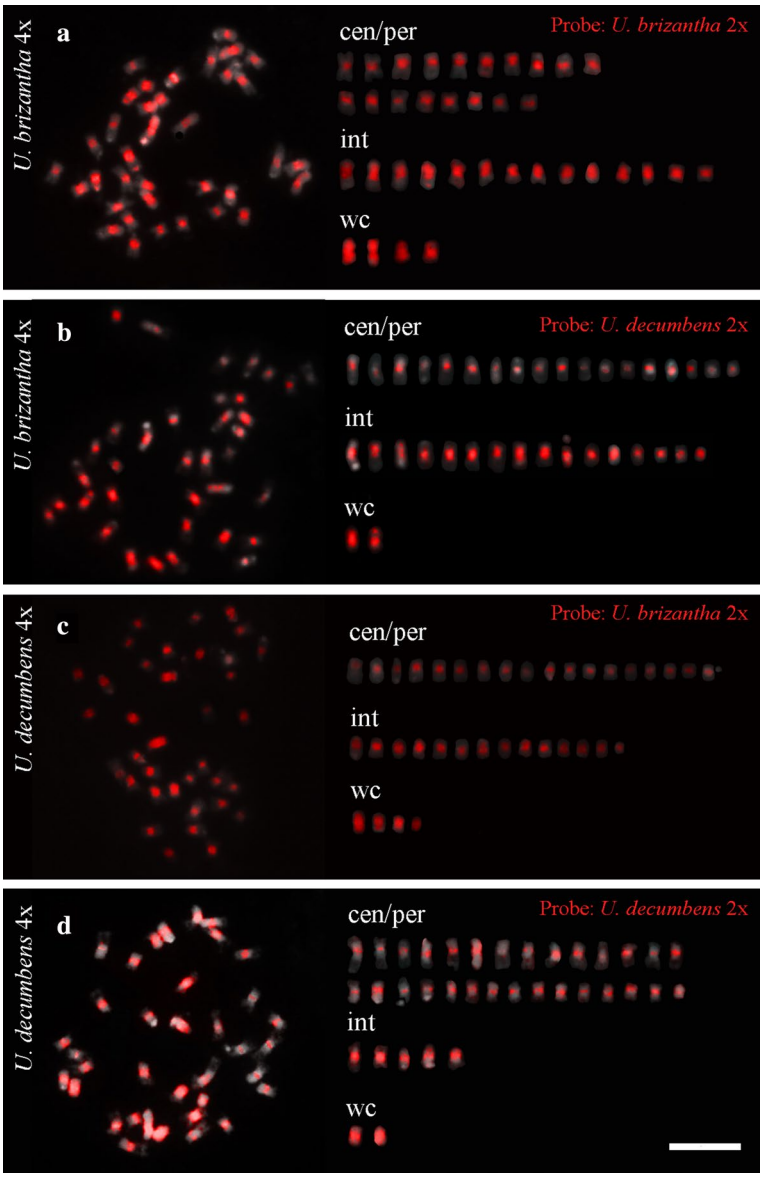

Fig. 3 Metaphases of *U. brizantha* (2n = 4x = 36) with probes of *U. brizantha* 2x (**a**) and *U. decumbens* 2x (**b**). Metaphases of *U. decumbens* (2n = 4x = 36) with probes of *U. brizantha* 2x (**c**) and *U. decumbens* 2x (**d**). Chromosomes are stained with DAPI (grey) and probe signals are indicated by red fluorescence. The GISH+ signals were classified as centromeric/pericentromeric (cen/per), interstitial (int) or whole chromosome (wc). The bar represents 10 µm. (Color figure online)

Table 1 Proportion (%) of hybridized *Urochloa* genomes, obtained via GISH

Metaphase	Probe		
	U. brizantha 2x	*U. decumbens* 2x	*U. ruziziensis* 2x
U. brizantha 2x	–	65.43 ± 4.63	45.20 ± 0.53
U. decumbens 2x	100	–	60.89 ± 1.45
U. ruziziensis 2x	50.93 ± 1.57	70.12 ± 4.43	–
U. brizantha 4x	41.36 ± 1.98	51.56 ± 2.15	–
U. decumbens 4x	58.54 ± 3.34	49.38 ± 1.28	–

Discussion

Chromosome number and genome size

The chromosome number (2n = 2x = 18) confirmed for *U. brizantha* (B105) and *U. decumbens* (D04) had previously been reported [26–29]. The C value obtained for the diploid genotypes (0.89 and 0.72 pg respectively) is proportionally consistent with the values reported for the tetraploid cultivars Marandu (*U. brizantha*) and Basilisk (*U. decumbens*), respectively 1.43 and 1.66 pg [30]; 1.75 and 1.89 pg [31].

Urochloa brizantha and *U. decumbens* genomes are considered 'small' according to the classification proposed by Leitch et al. [32] and the difference in the DNA content between the diploid accessions (0.17 pg) may be associated

with the proportion of repetitive DNA on the genomes. Bennetzen et al. [33] indicated that differences in the repetitive DNA content, specifically regarding the activity of various transposable elements, are the main reason for variations in genome size between related species.

Ishigaki et al. [30] analyzed the C value of five cultivars of four *Urochloa* species and observed that the genome size was dependent on the ploidy level, with a tendency of larger C values as the ploidy increases. This can also be inferred for *U. decumbens* when comparing the C values obtained in the present study with values previously reported for the tetraploid cultivars [30, 31].

Genomic relationship between diploid *Urochloa* genotypes

The proximity/distance relationship between the different genomes was inferred based primarily on the number and extension of GISH+ signals and also on the proportion of hybridized genome. All chromosomes observed presented GISH+ signals at least up to the centromeric/pericentromeric regions, regardless the probe used. Such results can also be seen in Paula et al. [18] study and shows the homology of the centromeric repeats among the different *Urochloa* species. The conservation of centromeric repeats has been demonstrated in other genera in Poaceae, such as *Secale*, *Hordeum*, *Festuca*, *Semiarundinaria*, *Arundo* e *Zea* [34].

The predominance of GISH+ pericentromeric signals has also been reported for *Brassica* chromosomes [35]. Such pattern was associated to the high proportion repetitive DNA families in centromeric and pericentromeric regions, whereas distal regions are probably richer in genes and do not hybridize as distinctively with the genomic probe. This is supported by an analysis with epigenetic signals in *U. ruziziensis* and tetraploid cultivars of *U. brizantha* and *U. decumbens* [36], which indentified the chromosomes terminal and interstitial-terminal regions as eucromatic, via immunolocalization of H3K4me2. Complementarily, H3K9me2 signals were displayed in tipically heterochromatic domains, including centromeric and pericentromeric regions. It is also worth considering that GISH+ signals preferentially located in centromeric/pericentromeric regions is seen even in species with small genomes, which have a relatively low proportion of repetitive DNA families, such as rice [37] and *Brachypodium distachyon* [38].

Telomeric regions, with the exception of fully marked chromosomes, did not present GISH+ signals, although they are composed of repetitive DNA and considered highly conserved in plants [39, 40]. Majka et al. [41], in a comparative GISH analysis, also observed absence of terminal signals in different species of Poaceae. The authors attributed this result to the complex telomeres composition of the species in question, which may contain, in addition to the basic telomeric sequence (T/A) 1-4 G1-8, other families of DNA organized in tandem, as previously reported for the wheat [42]. Another possible related factor is the late condensation in the terminal regions of *Urochloa* chromosomes, reported by Nani et al. [43], and also observed in this study. This phenomenon, associated to the DNA denaturation inherent to the FISH/GISH technique, causes certain degradation in the chromosome terminations, making it difficult to obtain signals in this region.

The full hybridization of *U. brizantha* 2x gDNA on all *U. decumbens* 2x chromosomes indicates that both species have a high degree of homology between their genomes. However, the reciprocal GISH revealed only 65.43% homology, suggesting that *U. brizantha* 2x has a greater diversity of repetitive sequences. Since the majority of the DNA in plants (and eukaryotes in general) is composed of blocks of repetitive sequences and that in Poaceae these sequences can represent up to 85% of the genome [44, 45], the large-scale differentiation of the genome (which can be observed at the chromosome level) between distinct taxa necessarily involves variation in the frequency of the various classes of repeats [46, 47]. In this context, it is possible that *U. brizantha* 2x possesses most of the repeats that are present in large scale in *U. decumbens* 2x, but have a greater variety that is not represented in the latter's genome.

The similarity between *U. brizantha* and *U. decumbens* have already been mentioned for the tetraploid cultivars in two taxonomic reviews [6, 48]. Both studies highlighted that the two species are frequently difficult to differentiate morphologically and there are even reports of *U. brizantha* identified as *U. decumbens* [6] and vice versa [49]. Ambiel et al. [7] questioned the identification of the cultivar Basilisk as *U. decumbens*, since the dendrogram based on RAPD markers positioned this species among *U. brizantha* accessions. However, Triviño et al. [10], in a study of genetic diversity and population structure using microsatellites of diploid and polyploid accessions, found that the cultivar Basilisk is in fact closer to the other tetraploid accessions of *U. decumbens*.

The similar hybridization pattern in *U. ruziziensis* chromosomes with *U. brizantha* 2x and *U. decumbens* 2x probes confirms that the genomes present in the latter two species are homoeologous to the genome B^2 of *U. ruziziensis*, as already mentioned by Paula et al. [18]. The proportion of hybridized genome was higher in *U. ruziziensis* and *U. decumbens* 2x than in *U. ruziziensis* and *U. brizantha* 2x, which indicates that the first two share more repetitive DNA sequences. Such relationship is corroborated by Triviño et al. [10] study, which concluded that *U. decumbens* 4x and *U. ruziziensis* are closer to each other than to *U. brizantha* 4x and pointed that one *U. decumbens* subgenome would be related to *U. ruziziensis* and other to *U. brizantha*. This had already been proposed for tetraploid genotypes, based

in meiotic analysis [13, 14] and reiterated in Paula et al. [18] cytogenomic study, which demonstrated that the three species are strictly related and that *U. decumbens* occupies an intermediate position in terms of genomic relationship.

Genomic constitution and relationship between diploid and tetraploid *Urochloa* genotypes

The higher affinity of *U. decumbens* 2x with *U. brizantha* 4x rather than with *U. decumbens* 4x was also observed by Triviño et al. [10], who analyzed the genetic diversity between *Urochloa* accessions and verified that diploid and polyploid accessions of *U. decumbens* formed two distinct subclusters, with some tetraploids being even closer to *U. brizantha*. According to the authors, the genetic distance between diploid and tetraploid accesses of the same species is not surprising, since the difference of ploidy level and apomictic reproduction represent reproductive barriers.

These results are plausible when considering the allopolyploid origin of the tetraploid *Urochloa* cultivars, as indicated by studies based in meiotic behavior [13, 14], rDNA mapping [43, 50, 51] and GISH [18]. An allopolyploid is not necessarily the sum of its parental genotypes, since the genome undergoes a series of evolutionary processes post polyploidization, such as genome reorganization and downsizing, gene expression alterations, gene fragmentation, gene conversion and sub- and neofunctionalization of duplicated genes [52]. Many of these processes have been associated to the extensive and rapid changes in polyploid genomes towards diploidization and stability [35], as described for maize [53] and *Arabidopsis thaliana* [54]. In this aspect, it is reasonable that the tetraploid genomes in *Urochloa* present variations when compared to the diploids, due to post-polyploidization genomic adjustments, as proposed by Nani et al. [43].

Moreover, the identification of *Urochloa* species is based on floral morphology and does not consider ploidy level or mode of reproduction (apomictic or sexual). The evolution of morphological traits does not necessarily reflects evolution of molecular characters [55]. Thus, the genetic proximity between two species may be masked by morphological differences, and vice versa. In this sense, it is inferred that *U. decumbens* 2x share a higher proportion of homologous repetitive regions with *U. brizantha* 4x than with *U. decumbens* 4x and that the morphology-based classification is not able to capture this genetic proximity, allocating them in distinct taxa.

Both *U. brizantha* 2x and *U. decumbens* 2x probes showed high chromosome homology with *U. brizantha* 4x and *U. decumbens* 4x, suggesting that the diploid genomes are closely related to one subgenome from the tetraploids. Given the previously mentioned proximity between the diploid genomes with each other, it is reasonable to assume that both diploid probes are evidencing the same genome on the tetraploids. Considering the genomic constitution proposed by Paula et al. [18] for the tetraploid cultivars of *U. brizantha* (BBB^1B^1) and *U. decumbens* ($B^1B^1B^2B^2$), the GISH results from the present study indicate that the chromosomes fully marked and with GISH+ signals up to the interstitial region belong to the genome B^1B^1. In this context, any of the diploid genotypes *U. brizantha* (B105) and *U. decumbens* (D04) may have donated the genome B^1B^1 in these species allopolyploidization events, although it was not possible to distinguish the exact parents. Thus, in order to differentiate them (considering the *U. brizantha* 2x genomic differences in their reciprocal GISH), we suggest the genomic constitution B^1B^1 for and $B^{1\prime}B^{1\prime}$ for *U. decumbens* 2x. The genomic composition proposed for the tetraploids is complemented by the comparative GISH analysis of *U. brizantha* 2x and *U. decumbens* 2x with *U. ruziziensis*, which evidenced the genomic affinity and homoeology between B^1 and $B^{1\prime}$ with B^2.

Considering this scenario, it is possible that the chromosomes and their respective genomes from the diploid genotypes have undergone structural modifications/rearrangements throughout evolution. This is evidenced by the variation on the genome size between the two. However, more studies, including karyotypic and repetitive DNA analyzes associated to molecular phylogenies, are required to further investigate the divergence time between the diploid and tetraploid genotypes and confirm the origin of *Urochloa* allotetraploid genomes. Pessoa-Filho et al. [9] observed high genetic similarity and relatively divergence between the tetraploid cultivars of *U. brizantha* and *U. decumbens* (~ 1.6 million years) and indicated that probably a single polyploidization event occurred to establish these lineages, although no diploid accessions were included their analysis.

Integrated analysis of *Urochloa* genomic relationships

The recent recognition of genomes B, B^1 and B^2 for *Urochloa* has elucidated some questions about the allopolyploidy present in the agamic complex formed by *U. brizantha*, *U. decumbens* and *U. ruziziensis*. Paula et al. [18] indicated that *U. ruziziensis* carries the genome B^2 and it can be assumed that this species may have been the ancestral parent of *U. decumbens* 4x. The same had already been suggested by Basappa et al. [11], based on morphological characters and chromosome number. The present study brought new elements that point to the origin of the genomes B^1 or $B^{1\prime}$ involving the diploid accesses of *U. brizantha* 4x and *U. decumbens* 4x. However, the ancestry of the B genome, present in the tetraploid *U. brizantha* (genome BBB^1B^1), remains unknown. Future genomic studies using GISH

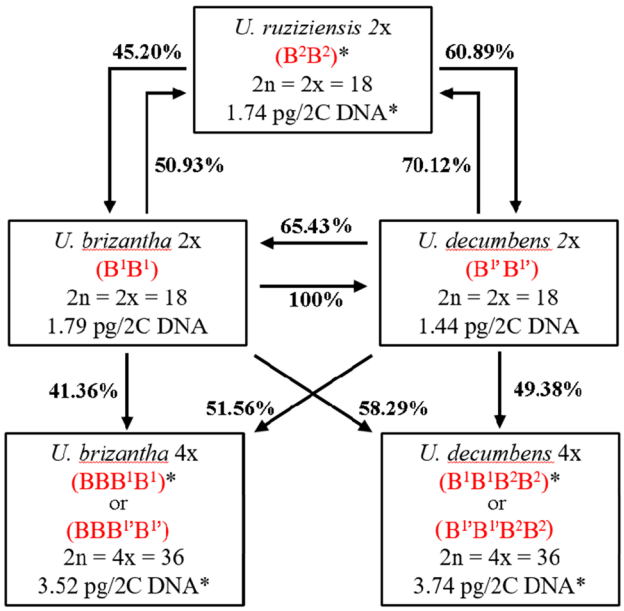

Fig. 4 Interrelations between *Urochloa* species. The arrows represent the hybridization percentage between genomes

should investigate the origin of genome B, considering other diploid *Urochloa* accessions.

Substantial evidence, such as (i) genomic differences between diploid and tetraploid genotypes of *U. brizantha* and *U. decumbens*; (ii) confirmation of the segmental allotetraploidy for *U. brizantha* and *U. decumbens*; (iii) meiotic behavior observed for the tetraploid species and interspecific hybrids; (iv) restricted gene flow between diploids and tetraploids and the different modes of reproduction (sexual diploids and apomictic tetraploids), indicates that *U. brizantha* 2x and 4x, as well as *U. decumbens* 2x and 4x, may be considered distinct taxa and demonstrate the necessity of a taxonomic review for the group.

A summary of the interrelations between the diploid and tetraploid species/genotypes of *Urochloa* is presented in Fig. 4.

Conclusion

The diploid accessions of *U. brizantha* and *U. decumbens* presented chromosome number and genome size as expected from the tetraploid accessions.

Genomic constitution for the diploid accessions of *U. brizantha* and *U. decumbens* is B^1B^1 and $B^{1\prime}B^{1\prime}$, respectively, with a higher diversity of sequences in *U. brizantha* genome.

Urochloa brizantha 2x and *U. decumbens* 2x are potential ancestors of allotetraploids that bear genome B^1 in their composition.

Acknowledgements The authors thank the support of the Foundation for Research Support of the State of Minas Gerais (FAPEMIG), the Coordination for the Improvement of Higher Education Personnel (CAPES), the National Council for Scientific and Technological Development (CNPq) for financial support for the development of this study. All authors read and approved the final manuscript.

Author contributions CTRC—carried oed out flow cytometry analysis, helped prepared slides and image processing. SCLB and CBV—responsible for the breeding program of *Urochloa*; provided hybrid seeds of *Urochloa* and reviewed the manuscript. GAT—helped the GISH analysis and has been involved in drafting the manuscript. VHT—conceived the study, participated in its design and coordination and helped to draft the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Stevens PF (2001 onwards) Angiosperm Phylogeny Website. Version 14, July 2018 [and more or less continuously updated since]. http://www.mobot.org/MOBOT/research/APweb/. Accessed 2018
2. Jank L, Barrios SC, Valle CB et al (2014) The value of improved pastures to Brazilian beef production. Crop Pasture Sci 65:1132–1137
3. Valle CB, Jank L, Resende RM (2009) O melhoramento de forrageiras tropicais no Brasil. Rev Ceres 56:460–472
4. Valle CB, Savidan YH (1996) Genetics cytogenetics and reproductive biology of Brachiaria. In: Miles JW, Maass BL, Valle CB (eds) *Brachiaria* biology agronomy and improvement. Empresa Brasilera de Pesquisa Agropecuaria, Brasilia, pp 163–180
5. Assis GML, Euclydes RF, Cruz CD, Valle CB (2002) Genetic divergence in Brachiaria species. Crop Breed Appl Biotechnol 2:331–338. https://doi.org/10.12702/1984-7033.v02n03a02
6. Renvoize SA, Clayton WB, Kabuye CHS (1996) Morphology, taxonomy and natural distribution of Brachiaria (Trin.) Griseb. In: Kuumble V, Miles JW, Maass BL (eds) Brachiaria: biology, agronomy and improvement. Empresa Brasilera de Pesquisa Agropecuaria, Brasilia, pp 1–15
7. Ambiel AC, Guaberto LM, Vanderlei TM, Neto NBM (2008) Agrupamento de acessos e cultivares de três espécies de brachiaria por RAPD. Acta Sci Agron 30:457–464. https://doi.org/10.4025/actasciagron.v30i4.5298
8. Ambiel AC, Neto NBM, Guaberto LM, Vanderlei TM (2010) Brachiaria germplasm dissimilarity as shown by RAPD markers. Crop Breed Appl Biotechnol 10:55–64
9. Pessoa-Filho M, Martins AM, Ferreira ME (2017) Molecular dating of phylogenetic divergence between Urochloa species based on complete chloroplast genomes. BMC Genomics 18:516. https://doi.org/10.1186/s12864-017-3904-2
10. Triviño NJ, Perez JG, Recio ME et al (2017) Genetic diversity and population structure of *Brachiaria* species and breeding populations. Crop Sci 57:2633–2644. https://doi.org/10.2135/cropsci2017.01.0045
11. Basappa GP, Muniyamma M, Chinnappa CC (1987) An investigation of chromosome numbers in the genus *Brachiaria* (Poaceae: Paniceae) in relation to morphology and taxonomy. Can J Bot 65:2297–2309
12. Valle CB, Pagliarini MS (2009), Cytogenetics, and breeding of *Brachiaria*. In: Singh RJ (ed) Genetic resources, chromosome

engineering, and crop improvement. CRC Press, Boca Raton, pp 103–143
13. Mendes-Bonato AB, Filho RGJ, Pagliarini MS et al (2002) Unusual cytological patterns of microsporogenesis in *Brachiaria decumbens*: abnormalities in spindle and defective cytokinesis causing precocious cellularization. Cell Biol Int 26:641–646. https://doi.org/10.1006/cbir.2002.0929
14. Mendes-Bonato AB, Pagliarini MS, Forli F et al (2002) Chromosome numbers and microsporogenesis in *Brachiaria brizantha* (Gramineae). Euphytica 125:419–425
15. Mendes-Bonato AB, Pagliarini MS, Silva N, Valle CB (2001) Meiotic instability in invader plants of signal grass *Brachiaria decumbens* Stapf (Gramineae). Acta Sci 23:619–625
16. Mendes-Bonato AB, Risso-Pascotto C, Pagliarini MS, Valle CB (2006) Cytogenetic evidence for genome elimination during microsporogenesis in interspecific hybrid between *Brachiaria ruziziensis* and *B. brizantha* (Poaceae). Genet Mol Biol 29:711–714
17. Mendes DV, Boldrini KR, Mendes-Bonato AB et al (2006) Cytological evidence of natural hybridization in *Brachiaria brizantha* Stapf (Gramineae). Bot J Linn Soc 150:441–446
18. Paula CMP, Souza Sobrinho F, Techio VH (2017) Genomic constitution and relationship in Urochloa (Poaceae) species and hybrids. Crop Sci 57:2605–2616. https://doi.org/10.2135/cropsci2017.05.0307
19. Lutts S, Ndikumana J, Louant BP (1991) Fertility of *Brachiaria ruziziensis* in interspecific crosses with *Brachiaria decumbens* and *Brachiaria brizantha*: meiotic behavior, pollen viability and seed set. Euphytica 57:267–274
20. Ndikumana J (1985) Etude de l'hybridation entre espèces apomitcques et sexuées dans le genre Brachiaria. Universite Catholique de Louvain, Belgium
21. Dong F, McGrath JM, Helgeson JP, Jiang J (2001) The genetic identity of alien chromosomes in potato breeding lines revealed by sequential GISH and FISH analyses using chromosome-specific cytogenetic DNA markers. Genome. https://doi.org/10.1139/gen-44-4-729
22. Doyle J, Doyle JL (1990) Isolation of plant DNA from fresh tissue. Focus (Madison) 12:39–40
23. Altinordu F, Pesuzzi L, Yu Y, He X (2016) A tool for the analysis of chromosomes: karyotype. Taxon 65(3):586–592
24. Heslop-Harrison JSP, Schwarzacher T (2011) Organisation of the plant genome in chromosomes. Plant J 66:18–33. https://doi.org/10.1111/j.1365-313X.2011.04544.x
25. Dolezel J (1997) Application of flow cytometry for the study of plant genomes. J Appl Genet 3:285–302
26. Nielen S, Almeida LM, Carneiro VTC, Araujo ACG (2009) Physical mapping of rDNA genes corroborates allopolyploid origin in apomictic *Brachiaria brizantha*. Sex Plant Reprod 23:45–51. https://doi.org/10.1007/s00497-009-0124-1
27. De Penteado MI, Santos ACM, Rodrigues IF et al (2000) Determinação de poliploidia e avaliação da quantidade de DNA total em diferentes espécies de gênero *Brachiaria*. Bol Pesqui Embrapa 11:1–32
28. Pinheiro AA (2000) Duplication of the chromosome number of diploid *Brachiaria brizantha* plants using colchicine. Plant Cell Rep 18:274–278
29. Ricci GCL, Souza-Kaneshima AM, Felismino MF et al (2011) Chromosome numbers and meiotic analysis in the pre-breeding of *Brachiaria decumbens* (Poaceae). Indian Acad Sci 90:289–294
30. Ishigaki G, Gondo T, Ebina M et al (2010) Estimation of genome size in *Brachiaria* species. Grassl Sci 56:240–242. https://doi.org/10.1111/j.1744-697X.2010.00200.x
31. Timbó AL, Pereira RC, Souza Sobrinho F, Davide LC (2014) Nuclear DNA content and chromosome numbereira in *Brachiaria* spp. genotypes. Rev Cienc Agron 45:62–67
32. Leitch IJ, Soltis DE, Soltis PS, Bennett MD (2005) Evolution of DNA amounts across land plants (Embryophyta). Ann Bot 95:207–217. https://doi.org/10.1093/aob/mci014
33. Bennetzen JL, Ma J, Devos KM (2005) Mechanisms of recent genome size variation in flowering plants. Ann Bot 95:127–132. https://doi.org/10.1093/aob/mci008
34. Belyayev A, Raskina O, Nevo E (2001) Evolutionary dynamics and chromosomal distribution of repetitive sequences on chromosomes of *Aegilops speltoides* revealed by genomic in situ hybridization. Heredity (Edinburgh) 86:738–742. https://doi.org/10.1046/j.1365-2540.2001.00891.x
35. Maluszynska J, Hasterok R (2005) Identification of individual chromosomes and parental genomes in *Brassica juncea* using GISH and FISH. Cytogenet Genome Res 109:310–314. https://doi.org/10.1159/000082414
36. Paula CM, Sobrinho FS, Techio VH (2016) Chromosomal distribution of H3K4me2, H3K9me2 and 5-methylcytosine: variations associated with polyploidy and hybridization in *Brachiaria* (Poaceae). Plant Cell Rep 35:1359–1369
37. Li C, Zhang D, Ge S et al (2001) Identification of genome constitution of *Oryza malampuzhaensis*, *O. minuta*, and *O. punctata* by multicolour genomic in situ hybridization. Theor Appl Genet 103:204–211
38. Jenkins G, Mur L, Bablak P et al (2004) Prospects for functional genomics in a new model grass. In: Leister D (ed) Plant functional genomics. CRC Press, Boca Raton
39. Roa F, Guerra M (2015) Non-random distribution of 5S rDNA sites and its association with 45S rDNA in plant chromosomes. Cytogenet Genome Res 146:243–249. https://doi.org/10.1159/000440930
40. Watson JM, Riha K (2010) Comparative biology of telomeres: where plants stand. FEBS Lett 584:3752–3759. https://doi.org/10.1016/j.febslet.2010.06.017
41. Majka J, Majka M, Kwiatek M, Wiśniewska H (2017) Similarities and differences in the nuclear genome organization within Pooideae species revealed by comparative genomic in situ hybridization (GISH). J Appl Genet 58:151–161. https://doi.org/10.1007/s13353-016-0369-y
42. Salina EA, Sergeeva EM, Adonina IG et al (2009) Isolation and sequence analysis of the wheat B genome subtelomeric DNA. BMC Genomics 10:414. https://doi.org/10.1186/1471-2164-10-414
43. Nani TF, Pereira DL, Souza Sobrinho F, Techio VH (2016) Physical map of repetitive DNA sites in *Brachiaria* spp.: intravarietal and interspecific polymorphisms. Crop Sci 56:1769–1783. https://doi.org/10.2135/cropsci2015.12.0760
44. Flavell RB, Gale MD, O'dell M et al (1993) Molecular organization of genes and repeats in the large cereal genomes and implications for the isolation of genes by chromosome walking. Chromosomes today. Springer, Dordrecht, pp 199–213
45. Schnable PS, Ware D, Fulton RS et al (2009) The B73 maize genome: complexity, diversity, and dynamics. Science 326:1112–1115. https://doi.org/10.1126/science.1178534
46. Biscotti MA, Olmo E, Heslop-Harrison JS (2015) Repetitive DNA in eukaryotic genomes. Chromosom Res 23:415–420. https://doi.org/10.1007/s10577-015-9499-z
47. López-Flores I, Garrido-Ramos MA (2012) The repetitive DNA content of eukaryotic genomes. Repetitive DNA 7:1–28. https://doi.org/10.1159/000337118
48. Morrone O, Zuloaga FO (1992) Revision de las especies sudamericanas nativas e introducidas de los generos *Brachiaria* y *Urochloa* (Poaceae: Panicoideae: Paniceae). Darwiniana 31:43–109
49. Maass BL (2004) Identifying and naming *Brachiaria* species. In: Miles JW, Valle CB (eds) Brachiaria: biology, agronomy and improvement. Empresa Brasilera de Pesquisa Agropecuaria, Brasilia, pp 9–12

50. Akiyama Y, Yamada-Akiyama H, Ebina M (2010) Morphological diversity of chromosomes bearing ribosomal DNA loci in *Brachiaria* species. Grassl Sci 56:217–223. https://doi.org/10.1111/j.1744-697X.2010.00197.x
51. Nielen S, Almeida LM, Carneiro VTC, Araujo ACG (2010) Physical mapping of rDNA genes corroborates allopolyploid origin in apomictic *Brachiaria brizantha*. Sex Plant Reprod 23:45–51. https://doi.org/10.1007/s00497-009-0124-1
52. Renny-Byfield S, Wendel JF (2014) Doubling down on genomes: polyploidy and crop plants. Am J Bot 101:1711–1725. https://doi.org/10.3732/ajb.1400119
53. Gaut B, Thierry d'Ennenquin M, Peek A, Sawkins N (2000) Maize as a model for the evolution of plant nuclear genomes. Proc Natl Acad Sci U S A 97:7008–7015
54. Ermolaeva M, Wu M, Eisen J, Salzberg S (2003) The age of the *Arabidopsis thaliana* genome duplication. Plant Mol Biol 51:859–866
55. Doyle JA, Endress PK (2000) Morphological phylogenetic analysis of basal angiosperms: comparison and combination with molecular data. Int J Plant Sci 161:121–153