Aquatic Botany 136 (2017) 52–55

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Short communication

Karyotype variations in seagrass (*Halodule wrightii* Ascherson—Cymodoceaceae)

Silmar Luiz da Silva [a], Karine Matos Magalhães [b,*], Reginaldo de Carvalho [a]

[a] *Graduate Program in Botany—PPGB and Cytogenetic Plant Laboratory of the Federal Rural University of Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP: 52171-900, Recife, Pernambuco, Brazil*

[b] *Aquatic Ecosystems Laboratory of the Federal Rural University of Pernambuco, Rua Dom Manoel de Medeiros, s/n, Dois Irmãos, CEP: 52171-900, Recife, Pernambuco, Brazil*

ARTICLE INFO

Article history:
Received 26 June 2015
Received in revised form 5 August 2016
Accepted 15 September 2016
Available online 16 September 2016

Keywords:
Cytomixis
Seagrass
B chromosomes
C-banding
Chromosome number

ABSTRACT

Karyotype variations in plants are common, but the results of cytological studies of some seagrasses remain unclear. The nature of the variation is not clearly understood, and the basic chromosomal number has still not been established for the majority of the species. Here, we describe karyotype variations in the seagrass *Halodule wrightii*, and we suggest potentially causative mechanisms involving cytomixis and B chromosomes. We prepared slides using the squashing technique followed by conventional Giemsa and C-banding, and silver nitrate and a CMA/DAPI staining. Based on intraspecific analysis, the diploid chromosome number of *H. wrightii* exhibited a variation from $2n = 24$ to $2n = 39$; $2n = 38$ was the most frequent. In general, we characterized the karyotype as an asymmetrical, semi-reticulated interphase nucleus with a chromosomally uniform condensation pattern. Cytomixis stands out as the primary event responsible for this variation in chromosome number. C-banding revealed a fully heterochromatic chromosome, which was described as a B chromosome. Staining with CMA and DAPI revealed preferential binds to GC-rich and AT-poor DNA. These chromosomes were located in proximal, interstitial and sub-terminal regions. Our fluorochrome differential staining results are the first for this genus. The chromosome number variation observed for *Halodule wrightii* is directly associated with the cytomixis process and B chromosomes.

1. Introduction

The occurrence of chromosome number variations in aquatic plants has been reported for quite some time (Talavera et al., 1993). This effect has been observed in the group of marine submersed vascular plants known as seagrasses. The main variations registered in the chromosome numbers of seagrasses consist of aneuploidy and polyploidy (Kuo, 2013; Talavera et al., 1993). In the *Halodule* genus, it is not clear which mechanism generates the variability in chromosome numbers because the initial idea of polyploidy (den Hartog et al., 1979) was refuted (Ito and Tanaka, 2010).

Cytogenetic studies of seagrass have largely focused on providing information about the taxonomic delimitation problem in several species (Semroud et al., 1992; Snoeijs and van der Ster, 1983), as also proposed for *Halodule* (Phillips and Meñez, 1988). However, cytogenetics has not been effective because of the difficulty in determining chromosome number in this genus (Kuo, 2013). The study of variations in chromosome numbers may also reveal possible karyotype evolution processes (Guerra, 2008).

Numerical variation is a rearrangement event (Guerra, 2008) that may also result from disploidy, the presence of B chromosomes (Bs) (Guerra, 2008) and, less frequently, cytomixis (Mursalimov et al., 2013). Chromosome banding techniques such as double staining with the fluorochromes chromomycin A_3 (CMA) and 4′,6-diamidino-2-fenilindol (DAPI) and C-banding are important for determining how these rearrangements occur (Barros e Silva and Guerra, 2010). Here, we report karyotype variations in *H. wrightii*, and we suggest potentially causative mechanisms involving variations in chromosome number.

2. Materials and methods

Roughly 150 tips of roots and rhizomes were collected from the *H. wrightii* population in the spring tide with the aid of snorkels and diving masks on the beach of Suape on the southern coast of Pernambuco, Brazil (8°21′24.91″S, 34°57′22.20″W) between August 2013 and September 2014. The *H. wrightii* population is located in an extensive discontinuous meadow in a mosaic pattern with

* Corresponding author.
E-mail address: magalhaeskm@gmail.com (K.M. Magalhães).

http://dx.doi.org/10.1016/j.aquabot.2016.09.005
0304-3770/

patches up to 10 m in distance. Since two *Halodule* species are found on the Brazilian coast, we identified all plant material to ensure that only *H. wrightii* were cytogenetically tested. We followed a specific dichotomous identification key based on morphological characters (leaf characteristics: apex and width); reproductive structures are rare, and variations between species have not yet been described (den Hartog and Kuo, 2006; Oliveira Filho et al., 1983). We pretreated the roots tips with 8-hydroxyquinoline 2 mM for 24 h and fixed them in Carnoy 3:1 (v/v).

2.1. Giemsa C-banding

We performed the Giemsa C-banding procedure as described by Guerra and Souza (2002). Modifications involved root tip digestion. The digestion was performed in an enzymatic solution of 2% cellulase (Serva, Carl-Benz-Str. 7, Frankfurt, Germany) and 20% pectinase (Sigma Aldrich, St. Louis, Missouri, USA) at 37 °C for 40 min. The roots tips were then squashed in a drop of 45% acetic acid, frozen in liquid nitrogen and then air dried.

2.2. Silver nitrate staining

Silver nitrate staining was performed as described by Guerra and Souza (2002). Fifty μl of silver solution was applied to the slides, and they were then covered with nylon screen and stored in a wet chamber at 60 °C for 15 min. Next, we washed the slides to remove excess stains, and they were air dried and mounted with Entellan Merk.

2.3. CMA/DAPI staining

We adopted the method described by Guerra and Souza (2002) for the fluorochrome staining. The roots were digested for 40 min in a cellulase (2%) and pectinase (20%) solution at 37 °C and then crushed in 45% acetic acid. The slides were aged for two days at room temperature and then stained with CMA at 0.5 mg/mL for 60 min in dark room; they were then washed with distilled water and air dried. Next, they were stained with DAPI 2 μg/mL for 30 min, washed with distilled water and air dried. Finally, the slides were mounted in McIlvaine-glycerol buffer 1:1 (v/v).

2.4. Morphometry and karyotype asymmetry

For the karyotype characterization, we analyzed the morphometry and karyotype asymmetry. We measured the chromosomes using MicroMeasure (version 3.3) (Reeves and Tear, 2000). We calculated the ratio values between the long and short arm (r) and the centromeric index (IC) to determine the position of the centromere (Guerra, 1988). Karyotype asymmetry was described using the intrachromosomal (A1) and interchromosomal (A2) indices (Romero Zarco, 1986) and the total form percent (TF%) (Huziwara, 1962). For the analysis, we used at least five metaphases with similar condensation patterns.

3. Results and discussion

Our results revealed that *H. wrightii* has high intrapopulation variation in its chromosome number. The most frequently observed chromosome number was $2n = 38$ (present in 45% of the cells that we analyzed), but the number varied from $2n = 24$ to $2n = 39$ (Fig. 1C–E).

den Hartog et al. (1979) first described that the chromosome number for *H. wrightii* as being $2n = 44$, the highest number reported to date for the species. These authors concluded that *Halodule* species with this number of chromosomes are tetraploid. However, the hypothesis that a high chromosome number indicates that a species is tetraploid has been refuted in studies of *H. pinifolia* (Miki) den Hartog, in which Ito and Tanaka (2010), using molecular analysis, inferred that polyploidy does not occur in the *Halodule* genus based on the number of copies of the *phy*B gene. Others cytogenetic studies of the genus *Halodule* recovered different chromosome numbers, as reported by Kuo (2013); see Table 1. According to the chromosome number available for *Halodule* species, it is not possible to establish a relationship between ploidy level and different data from the species *H. pinifolia* and *H. wrightii*. Therefore, one can assume that there is another mechanism (other than polyploidy) that promotes numerical variation in these species.

Table 1
Chromosome numbers of *Halodule* species previously described in the literature.

Species	2n	Site	Reference
Halodule hybrid	44	Okinawa, Japan	Ito and Tanaka (2010)
Halodule pinifolia	44	Okinawa, Japan; Tamil Nadu coast, India	Ito and Tanaka (2010); Vanitha et al. (2016)
	32	Cairns, Queensland	Kuo (2013)
Halodule sp.	44	Ishigaki, Japan	Kuo (2013)[a]
Halodule tridentata	32	Denham, W Australia	Kuo (2013)
Halodule uninervis	32	Denham, W Australia	Kuo (2013)
	44	Tamil Nadu coast, India	Vanitha et al. (2016)
Halodule wrightii	44	Curaçao, Netherlands Antilles; Tamil Nadu coast, India	den Hartog et al. (1979); Vanitha et al. (2016)
	24, 38, 39	Cabo de Santo Agostinho, Brazil	Present study

[a] cited by Kuo (2013).

When we analyzed different stages of cell division for *Halodule wrightii*, we noted the occurrence structures similar to DNA bridges were observed between neighboring cells observed in different individuals, which were associated with the cytomixis phenomenon, especially during prophase in meristematic tissue (Fig. 1A, B). These findings led us to believe that the number variation in *H. wrightii* may be coordinated by this phenomenon; during the cytomixis process there may be transfer of complete chromosomes (Mursalimov et al., 2015a), which can result in aneuploid cells, polyploid cells and cells without nuclei (Mursalimov et al., 2013).

Cytomixis frequency increases with the ploidy level increasing, although considered a genetic variability promoter mechanism though its evolutionary implications are still not to fully understand (Sidorchuk et al., 2015). Reis et al. (2016) concluded that by enabling DNA losses on a large scale, this process can help genome adjustments and accelerate the adaptation/stability of new polyploids and neopolyploids. Although the polyploidy of *H. wrightii* may be contestable, it seems reasonable to consider that the karyotype is suffering from intense rearrangement.

The species exhibited a semi-reticulated interphase nucleus with the presence of some chromocenters; morphometric parameters of the chromosomes of *H. wrightii* indicated that the smallest and largest chromosomes were 1.56 and 10.18 μm in length, respectively. The average chromosome length was 4.33 μm, and the karyotype formula was 11m + 27sm (Supplementary Table 1). One of the largest submetacentric chromosome pairs presented a nucleolar organizing region on its short arm. This amplification was confirmed by staining with silver nitrate, which revealed up to two formed nucleoluses (Supplementary Fig. 1B). The asymmetry indices A_1, A_2 and TF% in *H. wrightii* were 0.97, 0.52 and 38.24%, respectively (Supplementary Table 2), which indicates that *H. wrightii* possesses an asymmetric karyotype. Vanitha et al. (2016), presented different chromosomal number data that indicated the