Chromosome localisation of nucleolar organizer region in *Rhodophiala bagnoldii* (Herb.) Traub (Asparagales: Amaryllidaceae) determined by silver nitrate staining

Localización cromosómica de la región organizadora nucleolar en *Rhodophiala bagnoldii* (Herb.) Traub (Asparagales: Amaryllidaceae) determinada por tinción con nitrato de plata

Pedro Jara-Seguel[1], Claudio Palma-Rojas[2],Javier Contreras[2] & Elisabeth von Brand[3]

[1]Escuela de Ciencias Ambientales, Facultad de Recursos Naturales, Universidad Católica de Temuco, Casilla 15-D, Temuco-Chile.
[2]Departamento de Biología, Facultad de Ciencias, Universidad de La Serena, Casilla 599, La Serena-Chile.
[3]Departamento de Biología Marina, Facultad de Ciencias del Mar, Universidad Católica del Norte, Casilla 117, Coquimbo, Chile.
pjara@uct.cl

RESUMEN

Estudios cariotípicos en *Rhodophiala bagnoldii* (Herb.) Traub (2n = 18) han descrito constricciones secundarias subteloméricas localizadas en el brazo largo del par cromosómico 7. En este trabajo, una señal Ag-NOR positiva fue observada en la constricción secundaria de ambos cromosomas homólogos. Nucléolos fusionados y no fusionados fueron también observados en núcleos interfásicos de células meristemáticas.

The study of nucleolar organizer regions (Ag-NORs) provides information on chromosomal distribution of active versus silent rRNA genes due to the presence of acidic/argyrophilic domains in the RNA polymerase I transcription machinery (Schwarzacher *et al*. 1978, Medina *et al*. 1983, Cermeño *et. al*. 1984, Hubbell 1985, Carvalho *et al*. 2010), which is stainable by silver nitrate (Goodpasture & Bloom 1975). The Ag-NOR technique has been complemented in the last years with the use of fluorescent *in situ* hybridization (FISH) using specific probes to indentify the physical localisation of ribosomal gene clusters (5S, 18S/25S) (Schwarzacher & Heslop-Harrison 2000). These methods, combined with the study of the number of nucleoli in interphase nuclei (Fernández-Gómez *et al*. 1969), have been a valuable tool to evaluate rDNA functionality in plant cells (Carvalho *et al*. 2010). However, the number of Chilean angiosperms for which the chromosome localisation of rDNA has been studied are little (ca. 19 species), and only two Amaryllidaceae species have been examined [e. g., *Placea amoena* Phil. and *Rhodophiala* aff. *advena* (Ker-Gawl.) Traub] (Baeza & Schrader 2004, Baeza *et al*. 2006). Other two foreign species of the family have also been studied using Ag-NOR technique (e. g., *Amaryllis belladona* L. and *Hippeastrum parodii* Hunz. et Cocc.) (Naranjo & Poggio 1988).

Rhodophiala is a South American genus represented by ca. 29 species. Karyotype studies in three *Rhodophiala* species from Chile (*R.* aff. *advena*, *R. bagnoldii* and *R. phycelloides*; 2n = 18) coincide in that the secondary constrictions located in chromosome pair 7 may carry NOR (Palma-Rojas 2000). This description has been corroborated in *R*. aff. *advena* using FISH method (Baeza *et al*. 2006). However, nothing has been described for other *Rhodophiala* species whose karyotypes have been previously reported.

In this work the Ag-NOR technique was performed to locate active rDNA in chromosomes of *R. bagnoldii*. This species is endemic to Chile and inhabits from 23º to 30ºS, being an important component of the flora of the Atacama dessert.

Plants and fruits of *R. bagnoldii* were collected in the Región de Coquimbo, Provincia de Elqui, Lagunillas locality, altitude 110 m (30º08`S - 71º21`W), 28-X-2008, C. Palma s.n. (ULS). Root of germinated seeds from ten mother plants were pre-treated with colchicine 0.05% (w/v) at 4ºC for 3 hours, fixed in ethanol-glacial acetic acid (3:1 v/v) at 4ºC for 24 hours and stored in ethanol 70% (v/v) at 4ºC until require. A part of fixed roots were stained with the Feulgen reaction. The remaining roots were washed with distilled water and treated with a solution of pectinase-cellulase (Fluka 2:1 w/w) at 7.5% (w/v) in solution 0.2 M of citrate buffer pH 4.2 at 37ºC for 30 min. Chromosome preparations were made by squashing the root tips in 50% glacial acetic acid. The procedure used to obtain Ag-NOR bands was based on a modification of the techniques described by Hizume *et al*. (1980), Sánchez-Rufas

et al. (1982) and Mehra & Brekrus (1985). In addtition, the count of nucleoli in interphase nuclei was made following the method described by Fernández-Gómez *et al*. (1969).

The results of silver nitrate staining performed in mitotic metaphases of *R. bagnoldii* showed a positive Ag-NOR signal in subtelomeric location on the long arm of both homologous of the chromosome pair 7 (five metaphases). This observation corroborates the presence of the NOR in the secondary constrictions previously described by Palma-Rojas (2000) for the species (Fig. 1a, c). A similar situation has been observed in *R.* aff. *advena* using FISH method (Baeza *et al*. 2006). On the other hand, the presence of two nucleoli in interphase nuclei of *R. bagnoldii* shows independent activity of both NORs observed in chromosome pair 7 (Fig. 1b). However, nucleolar fusion was also observed with the presence of one large nucleoli in some meristematic cells. This nucleolar dynamics among fused and unfused nucleoli has been broadly described in plants and animals having different ploidy levels (Gosh 1976, Jordan *et al*. 1982). In this context, recent works have discussed this nucleolar dynamics and have revealed additional functions of the nucleoli in plants, where virtually all eukaryotic RNA polymerase-transcribed RNAs are processed and where other important cellular processes are regulated (Kim 2009, Carvalho *et al*. 2010).

The available data for *Rhodophiala* suggest that the subtelomeric localisation of the NOR in the long arm of the chromosome 7 is a conservative character within the karyotype of the genus and may be a robust marker to evaluate cytoevolutionary patterns (Palma-Rojas 2000, Baeza *et al*. 2006). Nevertheless, all these data should be interpreted with caution due to that only two species of the genus have been studied using specific techniques to study localisation and/or expression of ribosomal cistrons. In this sense, FISH method and/or silver nitrate staining are valuable tools to study NOR localisation and/or expression in plants such as those discussed by Carvalho *et al*. (2010).

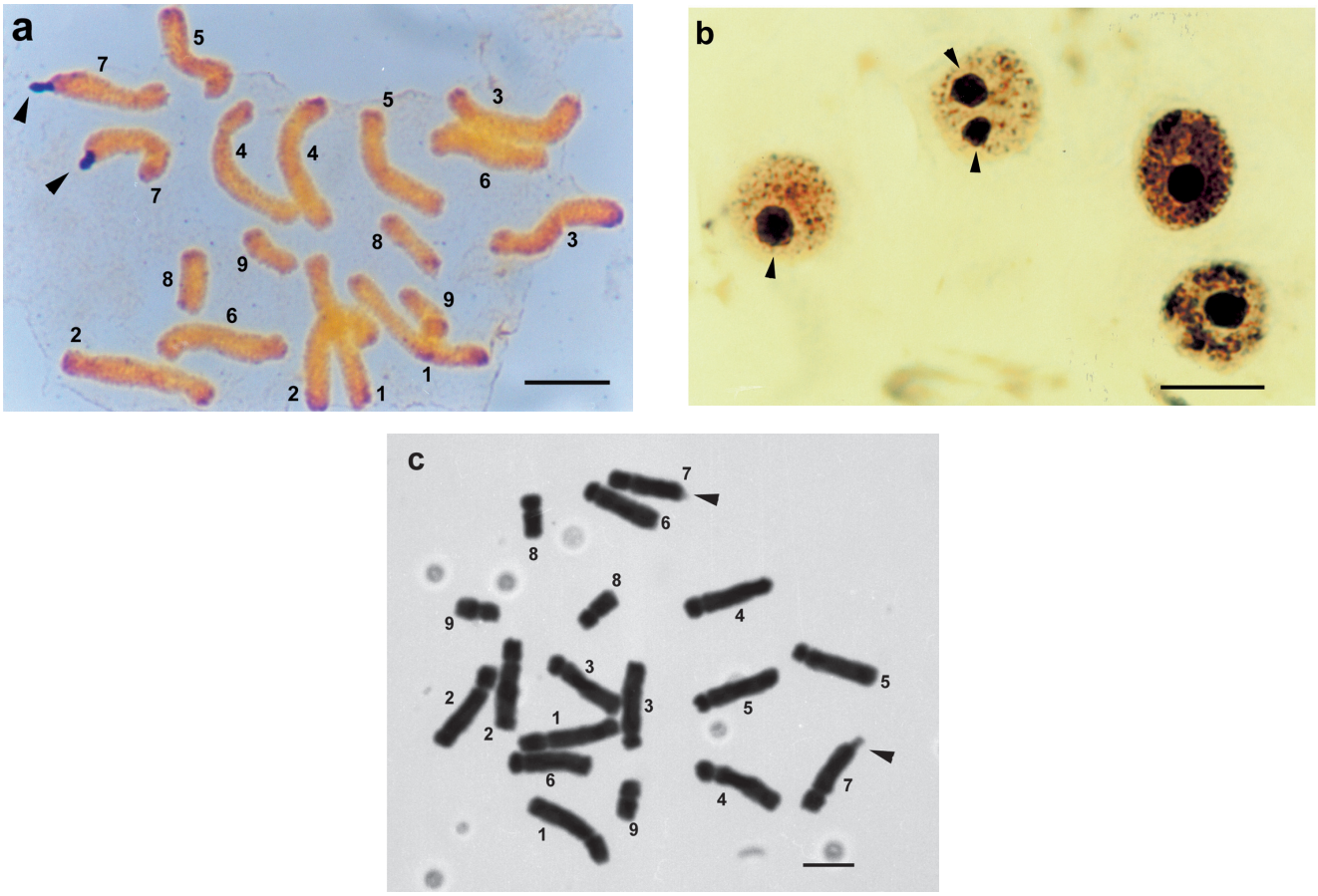

Figure 1. *Rhodophiala bagnoldii* (2n = 18). (a) Metaphase with Ag-NOR signals in subtelomeric localisation of the long arm of chromosome pair 7, (b) interphase nuclei with one and two nucleoli, and (c) Feulgen stained metaphase showing secondary constrictions in pair 7 (head arrows indicate Ag-NOR, nucleoli, and secondary constrictions, respectively). Bar = 10 µm.

Figura 1. *Rhodophiala bagnoldii* (2n = 18). (a) Metafase con señal Ag-NOR en localización subtelomérica en el brazo largo del par 7, (b) núcleos interfásicos con uno y dos nucléolos y (c) metafase teñida con Feulgen que muestra constricciones secundarias en el par 7 (puntas de flecha indican Ag-NOR, nucléolos y constricciones secundarias, respectivamente). Barra = 10 µm.

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