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Short Communication

Physical localization and probable transcriptional activity of 18S–5.8S–26S rRNA gene loci in some Asiatic Cymbidiums (Orchidaceae) from north-east India

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ABSTRACT

Fluorescence *in situ* hybridization based physical localization of 45S ribosomal DNA in eight horticulturally important species of *Cymbidium* (Orchidaceae) from north-east India (South-East Asia) has been carried for the first time. Observations revealed only one pair of chromosomes had NOR loci. Three, out of eight Cymbidiums showed decondensed, dispersed, extended form of hybridization signals of rDNA as dots of fluorescence (transcriptionally active), where as the rest of the Cymbidiums revealed condensed (non-active) forms, hence demonstrated the heteromorphism in size, intensities and their appurtenance which may be under epigenetic control. Except for the ribosomal genes, no other active genes have been reported to reside within the nucleoli. Such observations provide useful chromosome landmarks and provide valuable evidence about the genome evolution, speciation and ploidy both at molecular and chromosomal levels which is more or less highly ambiguous in family Orchidaceae.

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1. Introduction

India is endowed with rich treasure of phyto-diversity of agricultural, horticultural, and medicinal plants besides several unique flora that are endemic to the region. The northeastern region of India is regarded as one of the mega biodiversity hotspots (Myers et al., 2000) for a number of plant species including various orchids (Sharma et al., 2010a). The family Orchidaceae is considered as threatened and most of the genera are endangered in their natural habitat. *Cymbidium*, or boat orchid, is a genus comprising of 52 evergreen species, of which about 20 species are reported from India and found mostly in Arunachal Pradesh, Sikkim and Meghalaya provinces. It belongs to subtribe Cyrtopodiinae, tribe Cymbidieae and family Orchidaceae (Dressler, 1993). Chromosome variations in orchids are rampant and as a whole, this is quite intriguing since many of the genera exhibit higher ploidy levels with variable base numbers (Ehrendorfer, 1980; Goldblatt, 1980). Raven (1975) opined that it is premature to suggest a distinct base number for Orchidaceae. Cytogenetical studies in orchids are by and large lacking and still fewer reports are available for Indian orchids (Sharma and Chetterji, 1966; Singh, 1984; Sharma et al., 2010a). Most of the *Cymbidium* plants are epiphytic and hence, root tip mitosis and karyotype analysis is relatively difficult in Cymbidiums. Recently, Sharma et al. (2010a) reported details of karyotypes in three species of Asiatic *Cymbidium* viz. *C. eburneum*, *C. hookerianum* and *C. mastersii*. However, unequivocal differentiations between species are hampered by almost identical chromosome numbers (2n = 40) and only few differences with regard to chromosome morphology, presence of low heteromorphism with no clear indications for distinct satellite chromosomes (Sharma et al., 2010a). The basic chromosome number of several genera belonging to this family is still unclear leading to difficulties in determining accurate ploidy level and to understand the pattern of speciation and evolution vis-à-vis chromosomes in the family Orchidaceae (Sharma et al., 2010a).

Recent studies in Orchidaceae have utilized DNA sequence data to resolve phylogenetic relationships and infrageneric classification (Cox et al., 1997; Pridgeon et al., 1997; Ryan et al., 2000; Van den Berg et al., 2002, 2005). A similar approach has been employed to resolve phylogenetic and classification ambiguities in the genus *Cymbidium* using repeat unit length variation and internal transcribed spacer (ITS) sequences of nuclear ribosomal DNA coupled with plastid *matK* gene which basically revealed low level of variation and possible South-East Asian origin of the genus *Cymbidium* (Van den Berg et al., 2005). Genes coding for ribosomal RNA occur universally in all organisms. In eukaryotes, they consist of tandemly repeated rDNA units composed of transcribed regions, coding for

Abbreviations: FISH, Fluorescence *in situ* hybridization; ITS, Internal transcribed spacer; rDNA, Ribosomal DNA; SPAR, Single primer amplification reaction.

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18S, 5.8S and 26S rRNA, and non-transcribed regions. Nuclear ribosomal DNA units have been studied in species, populations and even individuals (or inbred lines) as in wheat (Flavell et al., 1986), maize (McMullen et al., 1986), *Vicia* (Raina and Ogihara, 1995), *Arachis* (Singh et al., 2002), *Nymphaea* (Dkhar et al., 2010) and many others. Repetitive sequence families are major components of plant genomes (Heslop-Harrison, 2000). These genes are organized into two multigene families in eukaryotes. One of these families contains the major rDNA (45S) that codes for 18S, 5.8S and 28S rRNAs, and the smaller, that codes for the minor rDNA (5S). The nucleolar organizing regions (NORs) that contain the 45S rDNA can be easily identified in chromosomes by using certain fluorochrome based *in situ* hybridization techniques (Leitch and Heslop-Harrison, 1992). This technique permits more exact chromosome identification and mapping, demonstrates the relatedness of individual species, and elucidates their phylogenetic relationships with valuable chromosomal landmarks (Heslop-Harrison, 2000). Few reports are available on physical localization of ribosomal DNA in orchids (Demerico et al., 2001; Clements, 2003; Cheng et al., 2004; Tsai et al., 2004; Cabral et al., 2006; Begum et al., 2009). Only a single report by Nagl (1977) showed the localization of amplified DNA in nuclei of *Cymbidium* by *in situ* hybridization and revealed the fact that the nuclear DNA of the orchid genus *Cymbidium* is unique among monocots indicating the location of the highly amplified AT-rich DNA fraction.

From the perusal of literature it is amply clear that besides chromosome number reports, the information about the 45S rDNA locus particularly in the genus *Cymbidium* is lacking and various Asiatic *Cymbidium* species did not attract the attention of scientists for such observations as yet. Therefore, the present investigations were carried out for physical localization of 45S ribosomal DNA in eight species of *Cymbidium* from India (South-East Asia).

2. Materials and methods

2.1. Plant materials and chromosome preparation

Eight species belonging to the genus *Cymbidium* were collected mainly from Arunachal Pradesh, Meghalaya and Sikkim provinces of northeastern region of India. Plant samples of *C. cyperifolium* Wall ex Lindl., and *C. tracyanum* L. Castle., were obtained from Dr. U. C. Pradhan, Chairman, Orchid Specialist Group, Government of India whereas *C. hookerianum* Rchb.f., *C. iridioides* D. Don, and *C. tigrinum* Parish ex Hook. f. were obtained from the Orchid Research Centre, Government of Arunachal Pradesh, Tipi, Arunachal Pradesh. The plant samples of other species were obtained from authentic nurseries viz. Green Light nursery, Upper Shillong, Meghalaya and International nursery, Kalimpong, West Bengal. The plants were grown in the greenhouse of the Plant Biotechnology Laboratory, Department of Botany as well as Department of Biotechnology and Bioinformatics of North-Eastern Hill University, Shillong. For each species, a minimum of five individuals and more than one population were studied. Actively growing root tips from the potted plants were fixed in Carnoy's fluid followed by pre-treatment with saturated solution of ρ-dichlorobenzene for 3 h at room temperature. Root tips were appropriately hydrolyzed with a mixture of 1 N HCl:45% acetic acid (2:1) for 10 min at room temperature and squashed after staining in 1% acetocarmine.

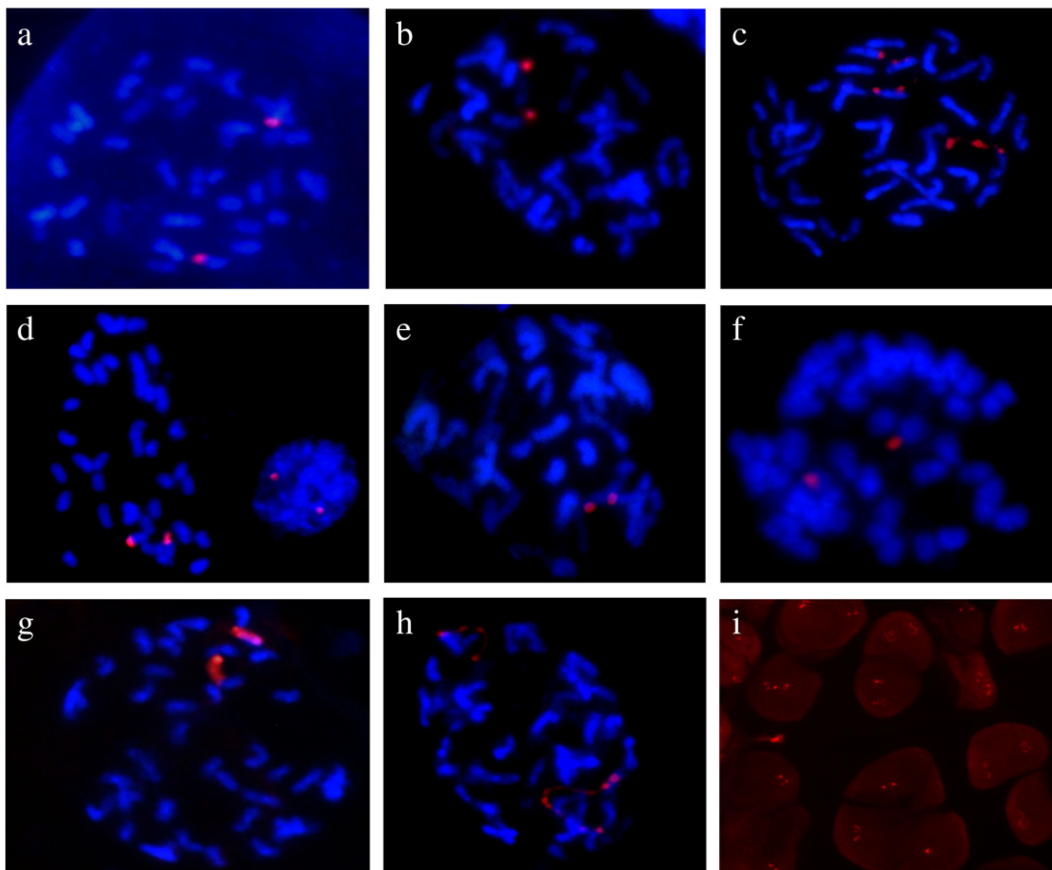

Fig. 1. Fluorescent *in situ* hybridization of 45S rDNA to root-tips cells showing two major sites in eight *Cymbidium* species (a) *C. elegans* (b) *C. cyperifolium* (c) *C. aloifolium* (d) *C. iridioides* (e) *C. hookerianum* (f) *C. mastersii* (g) *C. tracyanum* and (h) *C. tigrinum* (i) hybridization pattern at interphase of *C. mastersii.* (c, g and h) show the decondensed dispersed extended rDNA signals as dots of fluorescence (transcriptionally active) at pro-metaphases. (a, b, d, e, and f) show the condensed hybridization signals (non-active) at metaphase.

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