Bangladesh J. Bot. **34**(1): 31-36, 2005 (June)

KARYOTYPE ANALYSIS OF SEVEN ORCHID SPECIES FROM BANGLADESH

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Key words: Karyotype, Orchid, Bangladesh.

Abstract

Seven orchid species growing in Bangladesh were karyomorphologically investigated. Orcein staining showed that interphase nuclei and prophase chromosomes of *Oberonia iridifolia, Peristylus constrictus, Luisia grovesii, Phaius tankervilliae* and *Sarcanthus appendiculatus* possessed facultative heterochromatin, whereas *Pholidota pallida* and *Rhyncostylis retusa* showed constitutive heterochromatin. In *L. grovesii, P. constrictus, S. appendiculatus* and *R. retusa* chromosomes are 2n = 38. In *O. iridifolia* and *P. tankervilliae* chromosome are 2n = 30 and 2n = 52, respectively. *P. pallida* possessed 2n = 40 chromosomes. The low number of submetacentric chromosomes indicated a tendency towards symmetric karyotype in all the species. *P. pallida* possessed all metacentric chromosomes, which revealed a primitive feature, but gradual decrease in chromosome length indicated an advanced feature.

Introduction

Bangladesh is rich in orchids, with 159 species and two varieties under 63 genera (Huda *et al.* 1999). These species are distributed mainly in the hilly areas of greater Sylhet, Chittagong and Mymensingh districts. Only a few of them were cytogenetically investigated (Zaman and Sultana 1983, 1984, Alam *et al.* 1993). In this investigation, comparative karyotype analysis of seven orchid species of Bangladesh were carried out to find out karyotypic diversification among these species.

Materials and Methods

Seven orchid species, mentioned hereafter were collected from different districts of Bangladesh. The plant materials have been maintained in the Botanical Garden, Department of Botany, University of Dhaka, Bangladesh.

The healthy roots of these species were collected at 9:00-9:30 a.m. during the rainy season (June-August). Root tips of *ca.* 0.5 cm were cut and treated with 0.002 M 8-hydroxyquinoline for 5 hrs at 18^{o} C followed by 15 min fixation in 45% acetic acid at 4^{o} C. Then the treated root tips were hydrolysed in a mixture of 1N HCl and 45% acetic-acid (2 : 1) at 60^{o} C for 13 sec. These were stained with 1% aceto-orcein for 5 hrs at room temperature and squashed.

Results and Discussion

Oberonia iridifolia (Roxb). Lindl.: Few small chromocenters in interphase nuclei (Fig. 1A), indicated simple chromocenter type of interphase nuclei. The mitotic prophase chromosomes stained uniformly throughout the length (Fig. 1B), indicating continuous type. Tanaka (1971) mentioned that simple chromocenter type of interphase nuclei generally show gradient type of prophase chromosomes. Earlier Alam *et al.* (1993) reported simple chromocenter type of interphase nuclei and gradient type of prophase chromosomes in this species. Therefore, the present findings did not correlate with the above mentioned reports. The reason may be the presence of facultative heterochromatins, which coil in the interphase nuclei but are uniformly distributed in prophase chromosomes.

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The chromosome number of this species was found to be 2n=30 (Fig.1C). The same number was reported for this species by Jorapur and Hedge (1980) and Alam *et al*. (1993). The individual chromosome length ranged from 1.30 to 2.90 µm. The chromosomes were more or less homomorphic. There was no gradual decrease in chromosome length (Table 1). The centromeric formula of this species was 24 m + 6 sm.

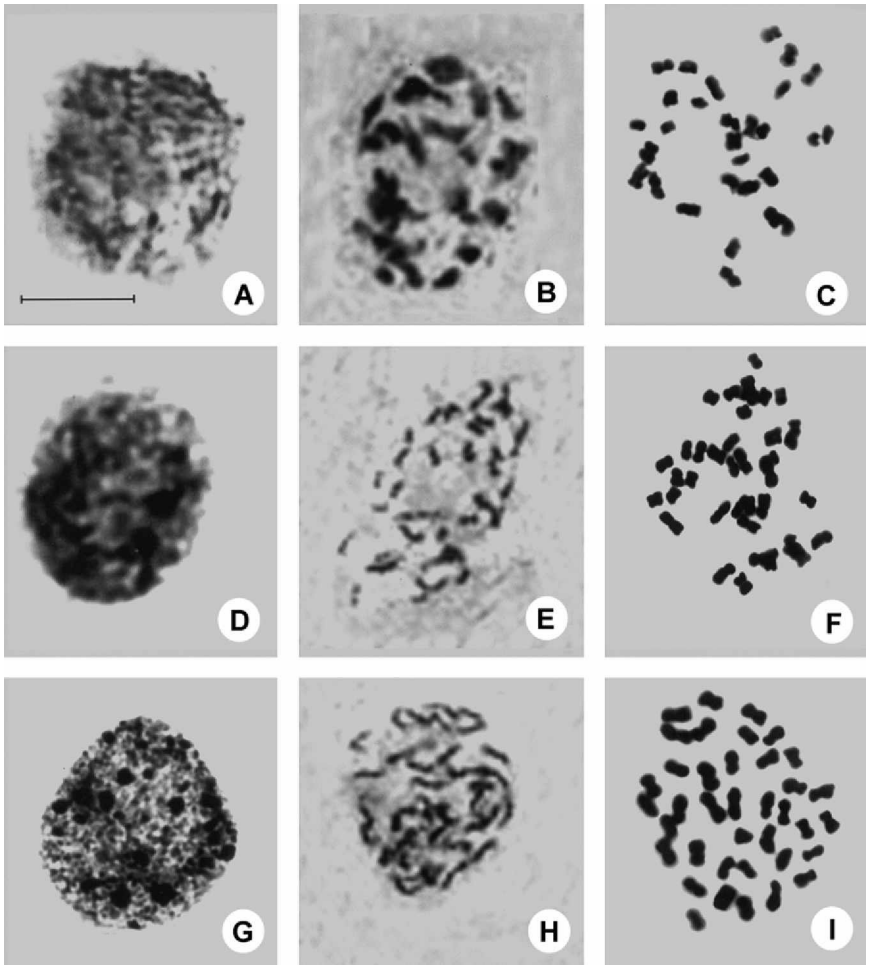

Fig. 1. A-C. *Oberonia iridifolia*. D-F. *Peristylus constrictus.* G-I. *Luisia grovesii.* A, D and G. Interphase nuclei. B, E and H. Propahase chromosomes. C, F and I. Metaphase chromosomes. Bar = 10 µm.

Peristylus constrictus (Lindl.) Lindl.: The interphase nuclei of this species also possessed few small chromocenters (Fig.1D). The mitotic prophase chromosomes stained uniformly (Fig. 1E). Alam *et al.* (1993) reported the same type of interphase nuclei and prophase chromosomes in this species.

The chromosome number of this species was found to be 2n=38 (Fig.1F) which was also reported earlier by Alam *et al*. (1993). The individual chromosome length ranged from 1.60 to 3.20 µm. The chromosomes were more or less homomorphic in length and there was no gradual decrease in chromosome length (Table 1). The centromeric formula was 28 m + 10 sm.

Luisia grovesii Hook. f.: Many darkly stained heterochromatic blocks were found in the interphase nuclei (Fig. 1G). According to Tanaka (1971), it revealed complex chromocenter type of its interphse nuclei. The prophase chromosomes had gradient type of staining (Fig. 1H). Alam *et al.* (1993) reported similar findings in interphase nuclei and prophase chromosomes of this species. Tanaka (1971) mentioned that in case of complex chromocenter type of interphase nuclei, prophase chromosomes generally show interstitial type of staining. The reason of this disagreement could be due to the presence of some facultative heterochromatins that preferentially coil in the interphase nuclei and gradually uncoil during the course of cell division.

The chromosome number of this species was found to be 2n=38 (Fig. 1I). Alam *et al*. (1993) reported the same 2n number for this species. The individual chromosome length ranged from 2.10 to 4.10 µm. The gradual decrease in chromosome length was insignificant. The centromeric formula of this species was 34 m + 4 sm, showing a good number of metacentric chromosome in the karyotype (Table 1).

Table 1. Comparative karyotype analysis of seven orchid species.

Species	2n	Range of chromosomal length (µm)	Total length of 2n chromosome complement (µm)	Centromeric formulae
Oberonia iridifolia	30	1.30-2.90	62.10	24m + 6sm
Peristylus constrictus	38	1.60-3.20	36.50	28m + 10sm
Luisia grovesii	38	2.10-4.10	114.60	34m + 4sm
Pholidota pallida	40	0.90-3.80	63.40	40m
Phaius tankervilliae	52	0.70-2.60	85.10	44m + 8sm
Sarcanthus appendiculatus	38	2.0-4.70	121.40	32m + 6sm
Rhyncostylis retusa	38	1.80-3.40	102.00	32m + 6sm

Pholidota pallida Lindl.: Many heterochromatic blocks were found in the interphase nuclei (Fig. 2A). Some of these were large. The possible reasons for the appearance of larger blocks were that the neighbouring heterochromatic regions fused together forming these blocks (Fig. 2A). It indicated that this species possessed complex chromocenter type of interphase nuclei as per Tanaka's classification (1971). The prophase chromosomes stained much more darkly at the centromeric regions indicating proximal type of chromosomes (Fig. 2B). Alam *et al*. (1993) reported small sized heterochromatic blocks in the interphase nuclei. In respect of heterochromatin size in the interphase nuclei, the present findings differs from Alam *et al's.* observation (1993). However, proximal type of prophase chromosomes confirmed the presence of abundant heterochromatin at the centromeres of this species.

The chromosome number of this species was found to be 2n=40 (Fig. 2C), the same number was also reported before for this species (Mehra and Sehgal 1980, Alam *et al.* 1993). The individual chromosome length ranged from 0.90 to 3.80 µm, indicating a gradual decrease in chromosome length (Table 1). All the 40 chromosomes were metacentric, showing a complete symmetric karyotype. The centromeric formula was 40 m.

Phaius tankervilliae Bl.: Large heteropycnotic blocks were observed in the interphase nuclei (Fig. 2D). This feature indicated the complex chromocenter type of interphase nuclei. The mitotic prophase chromosomes were of gradient type (Fig. 2E). Alam *et al.* (1993) also reported similar findings in this species.

The chromosome number of this species was found to be 2n=52 (Fig. 2F), as it was reported earlier for this species (Alam *et al*. 1993). Variation in chromosome number was also found in this species *viz.* 2n=38 (Li *et al*. 1992), 2n=48 (Teoh 1980), 2n=50 (Tanaka 1965). Variation in 2n

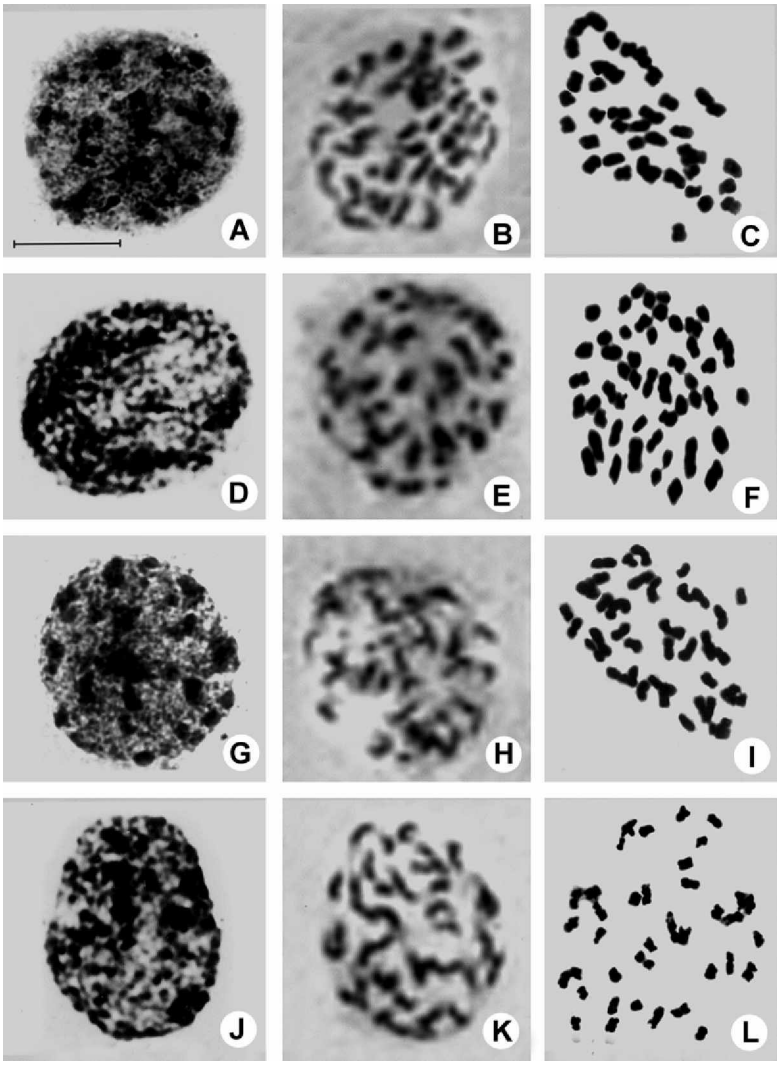

Fig. 2. A-C. *Pholidota pallida.* D-F. *Phaius tankervilliae.* G-I. *Sarcanthus appendiculatus.* J-L. *Rhyncostylis retusa.* A, D, G and J. Interphase nuclei. B, E, H and K. Prophase chromosomes. C, F, L and I. Metaphase chromosomes. Bar = 10 µm.

chromosome number indicated the probable presence of different cytotypes of this species. The individual chromosome length ranged from 0.70 to 2.60 µm (Table 1). The centromeric formula of this species is 44 m + 8 sm. The proportion of sub-metacentric chromosome is much less and hence showed a tendency to symmetric karyotype.

Sarcanthus appendiculatus Hook. f.: A number of big heterochromatic blocks were found in the interphase nuclei (Fig. 2G), which revealed a complex chromocenter type of interphase nuclei. The prophase chromosomes were of gradient type (Fig. 2H). But Alam *et al*. (1993) reported complex chromocenter type of interphase nuclei and proximal type of prophase chromosomes in this species. The present findings do not correlate to previous report, and this may be due to the presence of some facultative heterochromatins that preferentially coil in the interphase nuclei, and become uncoil in prophase.

The chromosome number of this species was found to be 2n=38 (Fig. 2I). The same chromosome number was reported for this species earlier (Kamemoto *et al*. 1964, Chatterjii 1990, Alam *et al*. 1993). The individual chromosome length ranged from 2.0 to 4.70 μm. The centromeric formula of this species was 32 m + 6 sm, showing less proportion of sub-metacentric chromosome.

Rhyncostylis retusa (L.) Bl.: Big heterochromatic blocks were present in interphase nuclei revealing complex chromocenter type (Fig. 2J). The prophase chromosomes stained at different interstitial regions (Fig. 2K). This nature of interphase nuclei and prophase chromosomes fully agree with previous reports (Tanaka 1971, Alam *et al.* 1993).

The chromosome number of this species was found to be 2n=38 (Fig. 2L). The same chromosome number was reported earlier for this species (Tara and Kamemoto 1970, Kulkarni and Jorapur 1979, Mehra and Kashyap 1981, Singh 1981, Vij *et al.* 1981, Alam *et al*. 1993). The individual chromosome length ranged from 1.80 to 3.40 μm. The chromosomes were more or less homomorphic in length, showing no gradual decrease in chromosome length (Table 1). The centromeric formula of this species is 32 m + 6 sm showing the abundance of metacentric chromosome in its karyotype.

The present study showed variations in karyomorphology of the seven species**.** Five species (*O. iridifolia, P. constrictus, L. grovesii, P. tankervilliae,* and *S. appendiculatus*) possessed facultative heterochromatin which coiled in interphase nuclei but became uncoil in prophase chromosomes. In contrast, *P. pallida* and *R. retusa* had constitutive heterochromatin throughout the cell cycle.

The proportion of metacentric chromosomes was high in the karyotype of these species (Table 1). Therefore, a tendency of symmetric karyotypes exists in these species, indicating a primitive character. Homomorphicity regarding chromosome length was found in six species except in *P. pallida.*

Since *P. pallida* had a fully symmetric karyotype, it may be regarded as the most primitive among the seven species. On the other hand, the sharp heteromorphicity in chromosome length was also observed only in this species and thus this species could also be considered as the most advanced among the seven species. The reason for this contradiction is unknown. Further investigation with differential banding and *in situ* hybridization is necessary to elucidate the cause for karyotypic diversity of these orchids.

Acknowledgement

The first author is grateful to the University Grand Commission for providing her UGC Fellowship (UGC/scholarship/2.21/Ph.D./04/5719).

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(Manuscript received on 2 May, 2005; revised on 5 June, 2005)