RESEARCH ARTICLE

Karyotype analysis of *Vanilla borneensis* Rolfe- a critically endangered orchid of Assam, India

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Abstract:

Vanilla borneensis Rolfe is a monopodial climbing orchid growing naturally in the state of Assam, India. It has been reported as a rare, endemic and threatened terrestrial orchid. In the present study, karyotypic analysis of the *V. borneensis* was carried out to characterize the chromosome content. Somatic chromosome number in *V. borneensis* was found with 2n = 28, out of which 13 were median, 11 metacentric and 4 sub-metacentric chromosomes. This report can be considered as the first cytological report of the *V. borneensis* which is endemic to Assam in India and can be of valuable information for further taxonomic identification as well as genetic characterization of the group.

Keywords: Cytology • chromosome contents • karyotype • *Vanilla borneensis*

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Introduction

Assam the gateway of the North-eastern India is considered as the reservoir of different varieties of plant and animal resources. In this region orchid comprises as the important vegetation showing different types of growth forms and habit (Bhagabati et al., 2006; Chowdhery, 2009). Vanilla borneensis Rolfe (synonym= V. pilifera Holtt) is considered to be a rare, endemic and threatened orchid of Assam, India (Anonymous, 2015; Deka et al., 2017). V. borneensis is a hot and warm environment loving plant that can be found in two conditions, viz. terrestrial and as epiphytic climbers. Miller first described this genus taking the Spanish word 'vanilla' in reference to its long, slender, pod like fruit. In Assamese local language it is called as 'harjora lota' and is generally used as an ornamental plant (Baruah et al., 2017). The species was reported in the foothill of Mikir Hills in Karbi Anglong district of Assam (Northeast India) along with Malays, whereas it was first reported in Thailand (Borthakur and Hajra, 1976). Chowdhury (2005) first reported this beautiful orchid species from Nambor Reserve Forest of Golaghat District and recently it has also been reported from Karbi Anglong district (Deka et al., 2017). A report by Barik et al. (2018) states that, V. borneensis Rolfe is a critically endangered orchid which needs to be conserved as soon as possible. Present population stock of V. borneensis is depleting day by day due to habitat destruction. Soto Arenas (2003) opined that 35 species of the genus Vanilla have aromatic compound. Among them, the mostly cultivated one is V. planifolia Andrews (Purseglove et al., 1981) and is the second most expensive spice traded in the world market (Anonymous, 2000) and 95% of the world commercial Vanilla originates from it.

The morphological study of chromosome i.e., karyomorphology has gained a new sphere of importance in understanding the systematic relationships, phylogeny and evolution of related plant groups, in addition to the study of karyotype variation within and between species (Mathew and Mathew, 1982; Aswathanarayana, 2003). Although in the present days, advance techniques has diverted the attention of classical cytological works but to determine the systematic position of a taxon at higher taxonomic level, it still serves as an efficient tool (Raven, 1975). Therefore, karyomorphological study serves as the initial step for deeper understanding of structure, organization and functioning of the genetic system (Lisha et al., 1994; Sarma and Tanti, 2014, 2015). In the present study we conducted a detail karyomorphological study of V. borneensis as adequate data of karyomorphology was not available for this important endangered orchid species.

Materials and methods

Plant material

The stem of V. borneensis is fleshy and leaves are deep green in color, stem branched, channelled, internodes 7-10 cm long, rooting arises from node; Leaves thick,

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oblong, 10-14 cm; apex acute; base obtuse; petioles 1-1.5 cm. Inflorescences arise from node, 5-6 cm long, with 6-12 fragment flowers arising during march to May; bracts 0.5-1 cm. long; pedicels 5-6 cm long; Sepal 3, free, oblong-lanceolate, greenish, shiny, 3-3.4 cm long; Lateral petals similar to sepals; labellum whitish pink with dark purple veins inside, 3 cm long with appendage hairs at apex down to the mouth close to 8-10 scales of brush, hairs 0.5 cm long; lower inside in front column dark red; column 1-1.8 cm long, most part connate to labellum; operculum green; pollinia 4; ovary green; fruit sickle shaped, up to 15cm long; fruiting during July-August.

For the present study fresh plant material of V. borneensis Rolfe was collected from Karbi Anglong district (Lahorijan Reserve Forest) (Fig. 1A). Cuttings were maintained well in greenhouse condition in polybags containing soil sand and organic cow manure mixture at Department of Botany, Gauhati University (Fig. 1B). Proper maintenance and frequent observations were done for the collection of roots for cytological studies. The collected specimens were processed into mounted herbarium sheets following standard herbarium techniques and submitted to ASSAM herbarium and GUBH herbarium (Jain et al., 1977).

(A) (B) (C)

Fig. 1. (A) -*Vanilla borneensis* Rolfe in natural habitat; (B) Flowering stage of *V. borneensis*; (C) Cutting done for root growth

Collection of root tips

Young root tips of the experimental plant specimen were collected after 30 days of propagation. The collection time was preferred between 7am – 7.30am in the morning. Soon after collection the root tips were washed using distilled water and were placed in a clean tissue paper to soak out the excess surface water from the root tips. Then the root tips were pre- treated with 0.05% (w/v) colchicine for 50 min. Following which root fixation was done with 3:1 (v/v) ethanol/acetic acid for at least 6 h. After fixation, the root tips were thoroughly washed and preserved in 70% ethyl alcohol (Sarma et al., 2017).

Preparation for chromosome counts

The collected fixed root tips were hydrolysed in 1N HCl for 20 min at 30 - 40°C. After hydrolysis, the roots were stained with 1.5% (w/v) aceto-orcien for about 1.5 h. After staining, root tip tissue squashes were prepared in slides and were observed under compound microscope at a magnification of 1600x (16x*100x) using oil immersion. Well scattered metaphase plates were selected for karyo-morphological analysis of the chromosomes and perfectly stained chromosomes were photographed. The following parameters were considered for karyological studies:

(i) total chromosome length; (ii) arm ratio = length of long arm/ length of short arm; (iii) volume of chromosomes ($\pi r^2 h$); (iv) relative length of chromosome = (length of individual chromosome/total chromatin length of the diploid set) × 100 (Khosla and Sobti, 1985); (v) chromosome type based on length – chromosomes were categorised as: Type A (≥ 3.51 µm), Type B (3.01-3.50 µm), Type C 2.51-3.00 µm), Type D (2.01-2.50 µm), Type E (1.51-2.00 µm), Type F (≤ 1.50 µm); (vi) F% = length of short arm/total length of individual chromosome) ×100 (Levan et al., 1964); (vii) total form percent (TF%) = (total sum of short arm length/total sum of chromosome length) × 100 (Huziwara, 1962) ; (viii)

chromosome type based on centromeric position (Levan et al., 1964) .

Results

Chromosome characterization

The detailed report on the karyomorphological analysis of V. borneensis was described below. The chromosome number of V. borneensis was found to be 2n = 28 in the somatic cells. The chromosome length varied from 1.36 µm to 4.08 µm while their volumes ranged from 0.004 µm3 to 0.235 µm3. The relative length of the chromosomes varied from 2.32 µm to 6.98 µm. On the basis of the length, chromosomes were classified into Type A, Type B, Type C, Type D, Type E and Type F [Table 1; Fig. 2(a-b)]. The total genomic chromosome length was found to be 65.28 µm. The different types of chromosomes categorized on the basis of the length could be represented as: A4 + B4 +D10 + E4 + F6 = 28. In the present investigation no chromosome with secondary constriction was recorded.

Chromosome length composition and karyotype analysis

In the present investigation, karyotype analysis of V. borneensis revealed that the chromosomes are small in size with a diploid set of 2n = 28. The 28 somatic chromosomes comprised of 14 median chromosomes, 11 metacentric chromosomes and 3 sub-meta centric chromosomes. The karyotype formula for V. borneensis Rolfe. could be represented as: M14 + m10 + sm4 = 2n = 28. Here, chromosomes showed an abundance of median chromosomes in the karyotype.

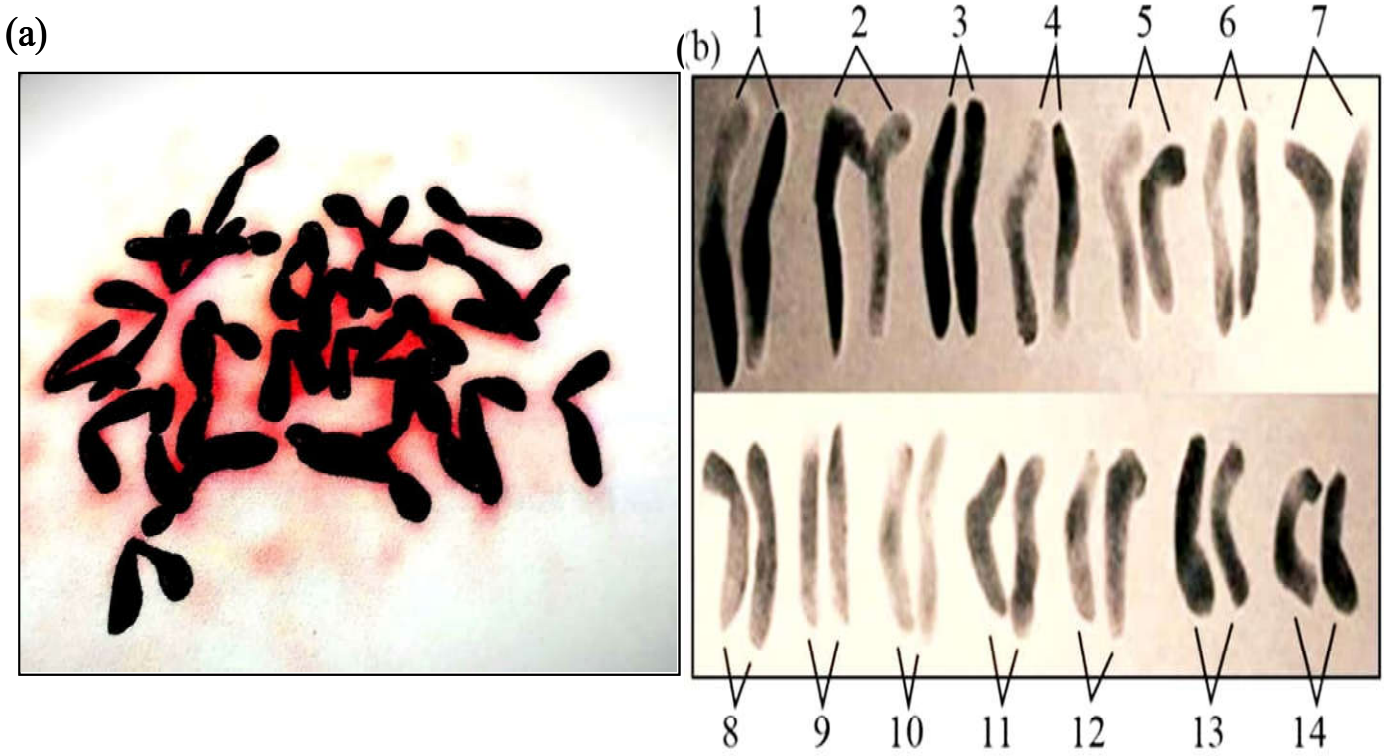

Fig. 2. (a) Microphotograph of the chromosome of *V. borneensis* (1600× magnification); (b) karyotype of *V. borneensis*.

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Table 1: Karyomorphological characteristics of *V. borneensis*

Type	No.	Length					Chromosome				
		long arm (l) (µm)	short arm (s) (µm)	total length (l + s) (µm)	relative length (µm)	Arm ratio (l/s)	Radius (r) µm	Vol. ($\pi r^2 h$) μm^3	Centromeric index (F %)	Position of centromere	Nomenclature of chromosome
F	1	0.68	0.68	1.36	2.32	1	0.034	0.004	50	M	Median
F	2	0.68	0.68	1.36	2.32	1	0.034	0.004	50	M	Median
F	3	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
F	4	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
F	5	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
F	6	0.68	0.68	1.36	2.97	1	0.017	0.0012	50	M	Median
E	7	1.02	0.68	1.7	3.71	1.5	0.017	0.0015	40	m	Metacentric
E	8	1.02	0.68	1.7	3.71	1.5	0.017	0.0015	40	m	Metacentric
E	9	1.19	0.68	1.87	4.08	1.75	0.051	0.0152	36.36	Sm	Sub metacentric
E	10	1.19	0.68	1.87	4.08	1.75	0.051	0.0152	36.36	Sm	Sub metacentric
D	11	1.36	0.68	2.04	4.46	2	0.051	0.0166	33.33	Sm	Sub metacentric
D	12	1.36	0.68	2.04	4.46	2	0.051	0.0166	33.33	Sm	Sub metacentric
D	13	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	14	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	15	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	16	1.02	1.02	2.04	4.46	1	0.034	0.0074	50	M	Median
D	17	1.36	0.85	2.21	4.83	1.6	0.051	0.018	38.46	m	Metacentric
D	18	1.36	0.85	2.21	4.83	1.6	0.051	0.018	38.46	m	Metacentric
D	19	1.36	1.02	2.38	5.2	1.33	0.051	0.0194	42.85	m	Metacentric
D	20	1.36	1.02	2.38	5.2	1.33	0.051	0.0194	42.85	m	Metacentric
B	21	1.7	1.36	3.06	5.23	1.25	0.102	0.099	44.85	m	Metacentric
B	22	1.7	1.36	3.06	5.23	1.25	0.102	0.099	44.44	m	Metacentric
B	23	1.7	1.36	3.06	5.23	1.25	0.102	0.099	44.44	m	Metacentric
B	24	1.7	1.36	3.06	5.23	1.25	0.102	0.099	44.44	m	Metacentric
A	25	2.04	2.04	4.08	6.98	1	0.136	0.235	50	M	Median
A	26	2.04	2.04	4.08	6.98	1	0.136	0.235	50	M	Median
A	27	2.04	2.04	4.08	6.98	1	0.136	0.235	50	M	Median
A	28	2.04	2.04	4.08	6.98	1	0.136	0.235	50	M	Median

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Discussion

The importance of karyotype analysis in distinguishing plants species is well known. The role of alteration of chromosome morphology in speciation and in determining inter-relationships between species, varieties and even strains has been reviewed earlier by (Sharma and Varma, 1959). The ecological and morphological data only is not sufficient in differentiating the species of a genus and in determining their phylogenetic relationships. In such case, a clear cytological and karyomorphological data can be of effective tool for more accurate understanding and justification (Hanelt and Mettin, 1966; Dewey, 1984).

In the present investigation, the somatic chromosome number of V. borneensis was reported for the first time with 2n = 28. There is no earlier report regarding the chromosome number of this species from the state of Assam, India. These 28 somatic chromosomes comprised of 14 median chromosomes, 10 metacentric chromosomes and 4 sub-metacentric chromosomes. The chromosomes were more or less homomorphic in length, showing no gradual decrease in chromosome length. The proportions of metacentric and median chromosomes were high in the karyotypes of this species. Therefore, a tendency of symmetric karyotypes exists in this species, indicating a primitive character.

Nair (2013) reported the somatic chromosome number of V. borneensis collected from the Andaman and Nicobar Island with 2n = 40 and other Vanilla species viz. V. tahitensis showed 2n=32 as most frequent chromosome number which is the one reported for V. planifolia also by many of the earlier workers. The same report also highlights the interspecific hybrids of V. planifolia × V. tahitensis revealing a variation in chromosome number ranging from 2n = 28 to 2n = 32 while the other wild Vanilla species viz. V. aphylla and leafless Vanilla sp. from Godavari showed 2n=72 and 2n=36 respectively. The variation in the intra specific chromosome number is due to the phenomenon of natural polyploidy which is very much common in the Vanilla species as reported by many workers. Bory et al., (2008) reported the first broad cytogenetic study of V. planifolia, where most of the accessions of V. planifolia were suggested to be diploid with 2C- value of 5 pg and the rare natural triploids and autotetraploids have recently formed in Reunion Island.

In the cultivated species of V. planifolia has been reported with highly variable intra – individual somatic chromosome number ranging from 2n = 13 and 2n = 32. Nair and Ravindran (1994) reported with similar observation where the root tip cells represented variable chromosome number ranging from 2n = 20 and 2n = 32, with 2n = 28 the most common. However, 2n = 32 is traditionally reported as the chromosome number for V. planifolia (Hoffmann, 1929; Heim, 1954; Martin, 1963; Das et al., 2017). In this finding, the proportions of maximum metacentric and median chromosomes is high in the karyotypes of V. borneensis showing tendency towards symmetrical karyotype which is an indication of primitive character.

Conclusion

Chromosome morphology is one of the most suitable features when it comes to calculating the plant's chromosome number; the size, structure and form of the chromosome are diverse. Different classification groups have different karyotypes, irrespective of their families, subfamilies genera and species (Shi et al., 2009; Hore and Tanti, 2014; Tanti et al., 2009). The karyological data represented here is the first ever report of the species with somatic chromosome number of 2n= 28. Our findings can play a important role with many perspectives in further phylogenetic and taxonomic studies as well as for the improvement of its population stock. This report adds to a very few number of scientific reports available for the species. Moreover, the potentiality of the cultivated Vanilla species gives us a major goal for finding many more information from this wild stock available in North East India for the betterment of mankind (Tanti et al., 2012; Toijam et al., 2012). In conclusion, our data support the idea that V. borneensis is the more primitive because it possesses mostly median and metacentric chromosomes.

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