RESEARCH ARTICLE

KARYOMORPHOLOGICAL ANALYSIS OF SOME EDIBLE AROIDS OF UPPER BRAHMAPUTRA VALLEY OF ASSAM

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SUMMARY Aroids (Araceae) locally known as '*Kachu*' are among the most favoured edible plants throughout Assam. Karyomorphology of 16 collections belonging to 10 species of edible aroids of Upper Brahmaputra Valley of Assam has been studied. The species included here are, *Alocasia macrorrhizos* (L.) G. Don (2n = 42), *A. odora* (Roxburgh) K. Koch (2n = 28), *Amorphophallus bulbifer* (Roxb.) Blume (2n = 26), *Colocasia antiquorum* Schott (2n = 56), *C. esculenta* (L.) Schott (2n = 28, 42, 56), *Cyrtosperma merkusii* (Hassk.) (2n = 26), *Lasia spinosa* (L.) Thwaites (2n = 26, 28), *Steudnera assamica* Hook. f. (2n = 28), *Typhonium trilobatum* (L.) Schott (2n = 26) and *Xanthosoma sagittifolium* (L.) Schott (2n = 26). The lowest and highest diploid chromosome numbers in the taxa studied here are, 2n = 26 and 56 respectively and the intervening numbers being 2n = 28 and 42. Intraspecific variations in chromosome number and evolutionary significance of karyotypes are discussed. Satellite markers were not observed in the accessions.

Keywords: Edible aroids, karyomorphology, aneuploidy, polyploidy, Assam.

INTRODUCTION

Aroids (Araceae) colloquially known as *Kachu*, are one of the most important and favoured edible plants of not only the people of Upper Brahmaputra Valley of Assam but the entire Northeast India. This family has 8 subfamilies, 119 genera and 6450 species distributed mostly in tropical and subtropical regions (Mabberley 2017).

Chromosome data are known to be of taxonomic value and found to be essential in studies focusing on diversification (Stebbins 1971). Karyomorphological features also play an important role in determining the taxonomic status of the species as they help in the study of plant systematics and evolution (Clark & Wall 1996). When different taxa showed the same chromosome number and karyotypic features, then it is very problematic to distinguish between them by conventional cytological analysis (Sultana et al. 2011). Hence, there is a need to generate more cytological and morphological information which will be helpful in examining relationships within the species as well as in the genera (Sheffer & Croat 1983).

Various works have been done on cytological and karyomorphological features of Aroids both in edible and as well as in wild varieties by many workers from India and aboard viz.. Alam & Deen (2002), Bhattacharyya (1957), Begum et al. (2009), Chen et al. (2007), Coastes et al. (1988), Cotias-de-Oliveira et al. (1999). Cusimano et al. (2012), D'Emerico et al.(1993), Das et al. (2015), Delay (1951), Fu-Hua et al. (2001), Ghimire et al. (2012), Hore & Tanti (2014), Ito (1942), Kawahara (1978), Kurakubo (1940), Kuruvilla et al.(1981), Lakshmanan et al. (2015), Lukhtanov et al. (2011), Mookerjea (1955), Okada & Hambali (1989), Parvin et al.(2008), Petersen (1989), Pfitzer (1957), Rao (1947), Sharma & Das (1954), Sharma & Datta (1961), Sreekumari & Mathew (1991a, b), Yang et al. (2003), Yen & Wheeler (1968).

Different species of edible aroids were collected from the study area and identified based on their morphological features. Due to their phenotypic plasticity (Sultana et al. 2011) it is very difficult to reach authentic identification as they show morphological variations in different environmental conditions. The present study deals with chromosome number and morphology of 10 species of edible aroids viz., Alocasia macrorrhizos (L.) G. Don, A. odora (Roxburgh) K. Koch, Amorphophallus bulbifer (Roxb.) Blume, Colocasia antiquorum Schott, C. esculenta (L.) Schott, Cyrtosperma merkusii (Hassk.), Lasia spinosa (L.) Thwaites, Steudnera assamica Hook. f., Typhonium trilobatum (L.) Schott and Xanthosoma sagittifolium (L.) Schott.

MATERIALS AND METHODS

The materials for cytological analysis were collected from different places of Upper

Brahmaputra Valley of Assam (Jorhat, Golgahat, Sivasagar, Charaideo, Dibrugarh and Tinsukia) and were cultivated in home gardens. Karvomorphological investigations were carried out using the root tips of the accessions. For the study of somatic chromosomes, methods used by Sharma & Sharma (1980), Sreekumari & Mathew (1991a), Yang et al. (2003) and Zhang et al. (2010) were followed. The root tips were collected from the plants between 5:00 and 8:30 A.M. and washed them thoroughly with running water and then pretreated in paradichlorobenzene at room temperature for two and a half h or an ice water mixture solution for 24 h at 4° C. The root tips were fixed in freshly prepared Carnoy's solution (60% ethanol, 30% chloroform and 10% glacial acetic acid) for 24 h. After fixation, the root tips were washed with 70% alcohol and finally stored in 70% alcohol. Root tips were also directly fixed in Carnoy's solution without pretreatment in para-dichlorobenzene which had an advantage as they produce a clear picture in photographs of metaphase chromosomes instead of hazy appearance in pretreated materials. Moreover, the chromosome condensation was better and centromeres were easily recognized (Zhang et al. 2010). Therefore, root tips without pretreatment of paradichlorobenzene were used during the investigation.

The hydrolysis of the cells of root tips was done with a mixture of 1N HCl for 30 min at room temperature (25° C) and washed it with distilled water for four times. The materials were then stained in 1.5% aceto-orcein for 20 min. The deeply stained meristematic parts of the root tips were excised out and placed in a drop of 45 % acetic acid on a clean slide and squashed. For uniform dissociation of cells, gentle pressure with the thumb finger was applied on the coverslip and finally sealed with paraffin wax. The temporary preparations were observed under a compound microscope (Olympus CH20i). Well spread metaphase plates were selected for karyomorphological analysis of chromosomes. The measurements of the chromosome lengths were taken with the help of stage and ocular measurements (Govindarajan & Subramanian 1983, 1985). The mean length was calculated for each chromosome pair. Measurements of chromosome arms were based on three metaphase plates. In the present investigation the total complement length (TCL), arm ratio, gradient index, chromosome volume, TF%, relative length of chromosomes, asymmetry index, mitotic index and phase index were calculated following established standard procedure (Arano 1963, Arano & Saito 1980, Battaglia 1957a 1957b, Bora 1998, Deogade & Nasare 2016, Khosla & Sobti 1985, Levan et al. 1964, Li & Chen 1985, Pritchard 1967).

The chromosomes were classified according to the classifications followed by Tanaka (1971, 1977, 1980). Karyotype formulae were derived on measurements of metaphase chromosomes from photomicrographs. On the basis of length, chromosomes were categorized into Type A (>4.00 µm), Type B (3.00-4.00 µm), Type C $(2.00-2.99 \,\mu\text{m})$, Type D $(1.00-1.99 \,\mu\text{m})$ and Type $E (< 0.99 \,\mu\text{m})$. Based on the centromeric position, the chromosomes were classified into median (M), metacentric (m), sub-metacentric (sm), subtelocentric (st) and telocentric (t). Karyotype analysis and symbols for the description of chromosomes were done by following the system proposed by Levan et al. (1964). Karyotype symmetry was determined by the method proposed by Stebbins (1971). Idiograms of each accession was prepared by arranging the haploid set of chromosomes in order of decreasing length (Battaglia 1955, Stebbins 1971).

OBSERVATIONS

The chromosomes at mitotic metaphase of 10 species were examined (Figs 1–16). The karyomorphological characteristics of the accessions are summarized in Tables 1 and 2. Idiograms are given in Figs 17–32. Four different diploid chromosome numbers of 26, 28, 42 and 56 have been observed and in two species intraspecific variation in chromosome number has been noticed.

In Amorphophallus bulbifer, Cyrtosperma merkusii, L. spinosa (A1), T. trilobatum. and X. sagittifolium the diploid number was found to be 26. In Alocasia odora (A9), Colocasia esculenta (A5-7), L. spinosa (A2), and S. assamica the diploid number was found to be 28. In Alocasia macrorrhizos (A8) and Colocasia esculenta (A12) the diploid number was found to be 42 while in Colocasia antiquorum (A15) and C. esculenta (A16) the highest number of chromosomes 2n = 56 has been observed. The total complement length of 539.7 µm is highest in accession A12 of C. esculenta and lowest in L. spinosa (A1) with 78.65 µm. The chromosome length varies from 24.6 to $6.00 \ \mu m$ in C. esculenta (A12) and L. spinosa (A1), respectively. The mitotic index was highest in A7 of C. esculenta with 61.65% and lowest of 45.27% in A5 of C. esculenta. The phase index is highest in A5 of C. esculenta with 220.91% and lowest of 162.20% in A7 of C. esculenta whereas the karyotypic asymmetry index is highest in A16 of C. esculenta with 64.43% and lowest in A12 of C. esculenta with 52.97%. The size gradient within the genome i.e., gradient index is lowest (14.49%) in accession A11 of S. assamica and highest (49.02%) in A1 of L. spinosa.

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— 1.5μm

Figs 1–16: Somatic chromosomes of edible aroids. 1. *L. spinosa* (A1). 2. *L. spinosa* (A2). 3. *Cyrtosperma merkusii* (A3). 4. *T. trilobatum* (A4). 5. *Colocasia esculenta* (A5). 6. *C. esculenta* (A6). 7. *C. esculenta* (A7). 8. *Alocasia macrorrhizos* (A8). 9. *A. aodora* (A9). 10. *C. esculenta* (A10). 11. *Steudnera assamica* (A11). 12. *C. esculenta* (A12). 13. *Amorphophallus bulbifer* (A13). 14. *Xanthosoma sagittifolium* (A14). 15. *C. antiquorum* (A15). 16. *C. esculenta* (A16).

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Figs. 17–32: Idiograms. 17. L. spinosa (A1). 18. L. spinosa (A2). 19. Cyrtosperma merkusii (A3). 20. T. trilobatum (A4). 21. Colocasia esculenta (A5). 22. C. esculenta (A6). 23. C. esculenta (A7). 24. Alocasia macrorrhizos (A8). 25. A. odora (A9). 26. C. esculenta (A10). 27. Steudnera assamica ((A11). 28. C. esculenta (A12). 29. Amorphophallus bulbifer (A13). 30. Xanthosoma sagittifolium (A14). 31.C. antiquorum (A15). 32. C. esculenta (A16).

SI.	a .	· ·	Place of		chromosome length (um)		TCL TE a		Asymmetry	Mitotic	Phase	Gradient	
No.	Species	Local name	collection	Accessions	2n	LA	SA	(µm)	TF %	index (%)	(%)	(%)	(%)
1	Alocasia macrorrhizos	Maan Kachu	C, D, G, J, S , T	A8	42	18.0	4.5	466.5	42.12	57.92	55.05	181.66	24.17
2	A. odora	Dahi/Shillong Kachu	C, D, G, J, S , T	A9	28	13.5	3.0	231.0	37.66	62.34	58.75	170.21	22.22
3	Amorphophallus bulbifer	Ol Kachu	C, D, G, J, S , T	A13	26	12.0	3.0	205.5	39.42	60.58	53.47	187.02	33.33
4	Colocasia antiquorum	Mukhia Kachu	C, D, G, J, S , T	A15	56	9.0	3.0	316.0	38.86	61.14	58.10	172.10	33.33
5	C. esculenta	Ahinia Kochu	C, D, G, J, S , T	A7	28	15.0	6.0	234.0	35.9	64.10	61.65	162.20	30.00
6	<i>C esculenta</i> (with Light Brown Petiole)	Teli Kachu	C, D, G, J, S , T	A10	28	10.0	3.0	181.1	38.16	61.84	61.08	163.71	25.00
7	C. esculenta (with Light Green Petiole)	Pani Kachu	C, D, G, J, S , T	A5	28	10.5	3.0	194.2	39.44	60.56	45.27	220.91	28.57
8	C. esculenta (with Purple Petiole)	Nil Kochu	C, D, G, J, S , T	A6	28	12.0	2.5	209.6	40.55	59.45	54.73	182.73	23.53
9	C. esculenta (with Green Petiole)	Kachu	C, D, G, J, S , T	A12	42	18.9	5.7	539.7	47.03	52.97	50.43	198.29	30.16
10	C. esculenta (with Black Petiole)	Kolia/Kola Kachu	C, D, G, J, S , T	A16	56	8.7	3.0	317.1	35.57	64.43	51.99	192.33	34.48
11	Cyrtosperma merkusii	Aiti/Nol Kachu	C, S	A3	26	13.5	3.0	216.0	37.5	62.5	53.09	188.36	37.50
12	Lasia spinosa	Chengmora	C, D, G, J, S , T	A1	26	4.0	2.0	78.65	43.65	56.45	47.74	209.46	49.02
13	L. spinosa	Chengmora	C, D, G, J, S , T	A2	28	6.0	2.5	103.4	37.72	62.86	50.53	197.9	48.54
14	Steudnera assamica	Edalia Kachu	C, D, G, J, S , T	A11	28	14.25	3.0	246.0	40.24	59.76	49.84	200.63	21.05
15	Typhonium trilobatum	Chamakachu	C, D , S	A4	26	13.5	3.0	182.36	35.8	64.20	52.61	190.08	22.22
16	Xanthosoma sagittifolium	Bao Kachu	C, D, G, J, S , T	A14	26	12.0	4.5	207.6	35.93	60.58	49.96	200.17	25.00

C, Charaideo; D, Dibrugarh;G, Golaghat; J, Jorhat; S, Sivasagar; T, Tinsukia.

Sl. No.	Species	Accn No.	Karyotypic formula	Chromosome formula
1	Alocasia macrorrhizos	A8	$M_8 + m_{18} + sm_{14} + st_2$	$A_{42} + B_2$
2	A. odora	A9	$M_6 + m_{10} + sm_8 + st_4 \\$	$A_{26} + B_2$
3	Amorphophallus bulbifer	A13	$M_8 + m_6 + sm_{12}$	$A_{26} + B_2$
4	Colocasia antiquorum	A15	$M_8 + m_{12} + sm_{16} + st_{20}$	$A_{54} + B_2$
5	C. esculenta (with Black Petiole)	A16	$M_2 + m_{12} + sm_{14} + st_{30}$	$A_{54} + B_2$
6	C. esculenta (with Green Petiole)	A12	$M_{10} + m_{32}$	A ₄₂
7	C. esculenta (with Light Brown Petiole)	A10	$M_2 + m_{16} + sm_8 + st_2$	$A_{20} + B_8$
8	C. esculenta (with Light Green Petiole)	A5	$M_6 + m_{11} + sm_{10} + st_2 \\$	$A_{26} + B_2 + C_2$
9	C. esculenta (with Purple Petiole)	A6	$M_2 + m_{16} + sm_{10}$	$A_{22} + B_4 + C_2$
10	C. esculenta	A7	$M_2 + m_{10} + sm_{16}$	A ₂₈
11	Cyrtosperma merkusii	A3	$M_6 + m_8 + sm_8 + st_4 \\$	$A_{24} + B_2$
12	Lasia spinosa Type I (Non-pinnatifid)	A1	$M_7 + m_{13} + sm_6$	$A_6 + B_8 + C_{12}$
13	L. spinosa Type II (Pinnatifid)	A2	$M_0 + m_{12} + sm_{16}$	$A_9 + B_{13} + C_6$
14	Steudnera assamica	A11	$M_4 + m_{12} + sm_6 + st_2$	$A_{26} + B_2$
15	Typhonium trilobatum	A4	$M_2 + sm_{16} + m_4 + st_4$	$A_{22} + B_4$
16	Xanthosoma sagittifolium	A14	$M_8 + m_6 + sm_{12}$	$A_{24} + B_2$

TABLE 2: Karyotype analysis in 16 accessions of edible aroids.

M, Median; m, metacentric; sm, submetacentric; st, subtelocentric.

61 3 7		Present study		Previous reports			
SI. No.	Species and Accn No.	2n	x	2n	Authors		
1	Alocasia macrorrhizos (A8)	42	14	28	Das (2018)		
2	A. odora (A9)	28	14	56	Sultana et al. (2011)		
3	Amorphophallus bulbifer (A13)	26	13	26	Chandler (1943)		
				26	Hotta (1971)		
				39	Marchant (1971b)		
				39	Ramachandran (1977)		
				39	Chauhan & Brandhan (1985)		
				26	Kuruvilla et al. (1989)		
				39	Shete et al. (2015)		
4	Colocasia antiquorum (A15)	56	14	28	Kurakubo (1940)		
				28	Ito (1942)		
				28	Sharma & Das (1954)		
				28, 42	Fedorov (1969)		
5	C. esculenta (A7)	28	14	42	Nakajima (1936)		
				42	Janaki Ammal (1945)		
				36	Rao (1947)		
				42	Mookerjea (1955)		
				42	Rattenbury (1957)		
					(Conte		

TABLE 3: Chromosome numbers of edible aroids from Upper Brahmaputra Valley of Assam.

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TABLE 3:	(Concluded)
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<i>a</i> 1 3 7		Present study		Previous reports		
SI. No.	Species and Accn No. —	2n	х	2n	Authors	
				42 28, 42 14, 28, 36, 42, 48	Pfitzer (1957) Mace & Godwin (2002) Dastidar (2009)	
				28	Wahidi & Nursyahra (2018)	
6	C. esculenta (A16)	56	14	-	-	
7	C. esculenta (A10)	28	14	-	-	
8	C. esculenta (A12)	42	14	28	Alam & Deen (2002)	
9	C. esculenta (A5)	28	14	28	Alam & Deen (2002)	
10	C. esculenta (A6)	28	14	28	Alam & Deen (2002)	
11	Cyrtosperma merkusii (A3)	26	13	-	-	
12	Lasia spinosa (A1)	26	13	26	Sultana et al. (2006)	
				24	Hore & Tanti (2014)	
13	L. spinosa (A2)	28	14	28	Sultana et al. (2006)	
				26	Hore & Tanti (2014)	
14	Steudnera assamica (A11)	28	14	-	-	
15	Typhonium trilobatum (A4)	26	13	18	Banerji (1947)	
				26	Simmonds (1954)	
				18	Sharma & Mukhopadhyay (1965)	
				18	Subramanian & Munian (1988)	
16	Xanthosoma sagittifolium (A14)	26	13	26	Janaki Ammal (1945)	

DISCUSSION

Chromosomes are the carriers of genetic material in the nucleus and any changes in chromosome structure provide the basis of speciation (Landry & Aubin-Horth 2014, Muratovicc et al. 2010). The karyotype is the highest level of the structural and functional organization of chromosomes (Weiss-Schneeweiss & Schneeweiss, 2013). Karyotypic properties are conferred by evolutionary changes (Leitch et al. 2013) that result from natural selection (Petrov 2001) and may substantially influence plant performance (Cronk 2004). For many years, karyotypes have received much attention as an important cytological trait in modern-day research. Cytological data are widely used to explain the relationships of the species, whether they are intraspecific or interspecific and are used to validate the identification of a specimen (Hong & Zhang 1990, Raven 1975, Stebbins 1971). Cytological studies, especially the karyotypic data are very useful in plant systematics. Work done by Barua (1995), Khosla & Sobti (1985), Lombello & Fomi-Martins (2002), Madhusoodan & Nazeer (1983), Mathew & Mathew (1982), Muramoto et al. (2008) Raven & Kyhos (1965) and Subramanyam & Chowdary (1992) are very important in taxonomic delimitation of the plant species.

The present investigation reveals 2n = 28 in *A*. *odora* which is contrary to the previous reports of 2n = 56 by Sultana et al. (2011) suggesting the occurrence of diploid and tetraploid conditions in one and the same species. Similarly, contrary to earlier report of 2n = 28 by Das (2018), in the present study, *Alocasia macrorrhizos* shows 2n = 42 indicating a triploid state on basic number of 14.

Amorphophallus bulbifer studied here revealed 2n = 26 as reported by Kuruvilla et al. (1989) on basic number 13. However, previous workers reported different chromosome numbers for *A. bulbifer*. Whereas Chandler (1943) and Hotta (1971) recorded 2n = 36, Chauhan & Brandhan (1983), Merchant (1971), Ramachandran (1977) and Shete et al. (2015) recorded 2n = 39 for this species.

Colocasia esculenta exhibits chromosomal instability. While studying 7 varieties of this species Parvin et al. (2008) reported 2n = 21 to 42 and Dastidar (2009) reported 2n=14, 28, 36, 42, and 48. However, in the present study, in 4 out of 6 accessions studied, the diploid number of 28 has been found as reported by Alam & Deen (2002) and Wahidi & Nursyahra (2018); the other two accessions showed 2n = 42 and 56. While the former is in the conformity with previous reports by Dastidar (2009), Mace & Godwin (2002) and Parvin et al. (2008), 2n = 56 reported here is a new record for this species. In C. antiquorum, present study revealed 2n = 56 for the first time deviating from the earlier reports of 2n = 14, 28and 42 (Ito 1942, Kurakubu 1940, Sharma 1956, Sharma & Das 1954). These variations in chromosome number of Colocasia species show that they are polymorphic, allogamous and protogynous species (Quero-Garcia et al. 2006).

In *L. spinosa* Hore & Tanti (2014) reported 2n = 24 and 26 and Sultana et al. (2006) reported 2n = 24 and 27. But our studies show 2n = 26 in the accession A1 and 28 in the accession A2 indicating intraspecific variation of chromosome number.

Typhonium trilobatum studied here revealed 2n = 26 as reported by Simmonds (1954) which differs from earlier reports of 2n = 18 by Banerji (1947) and Sharma & Mukhopadhyay (1965).

The total complement length (TCL) of all 16 accessions ranged from 78.65 µm to 539.70 µm. According to Stebbins (1950), a decrease in TCL is one of the factors indicating the trend for evolution. Thus, L. spinosa (A1) may be considered as the most advanced and Colocasia esculenta (A16) as primitive among all the accessions studied. Based on TF% T. trilobatum may be considered as most advanced and Colocasia esculenta (A12) as most primitive. Metacentric chromosomes are predominant in the karyotypes of all the accessions. According to Stebbins (1971) the abundance of metacentric chromosomes is characteristic of symmetrical karyotype which is primitive in nature. On the other hand, the species having asymmetrical karyotype is supposed to be more advanced than symmetrical ones (Stebbins 1950). Generally, karyotype shows a shift from symmetry to asymmetry in higher plants (Stebbins 1971). Primitive species mostly have a symmetrical type of karyotypes, whereas the advanced plant species show the asymmetrical type of karyotypes. If the karyotype asymmetry index is close to or equal to 50%, the karyotype symmetry is considerably higher, and the karyotype is considered as most primitive. The karyotype analysis of 16 accessions of edible aroids show

karyotype asymmetry index (As.K %) which ranges from 52.97% to 64.43%. Therefore, the karyotype symmetry is high. More cytological analysis in a larger number of edible aroids is important for establishing the basic number of chromosomes and for understanding trends in chromosome evolution within the species (Cotias-de-Oliveira et al. 1999).

In Colocasia esculenta with variable chromosome numbers, 2n = 26, 28, 42 and 56, are well supported by their colour of the petioles, while the difference in leaf morphology of L. spinosa are well supported by their chromosomal counts of 2n = 26 and 28. Hence, they may be called *C. esculenta* with light green petiole Type I, C. esculenta with purple petiole Type II, C. esculenta with light brown petiole Type III, C. esculenta with green petiole Type IV and C. esculenta with black petiole Type V while L. spinosa non-pinnatifid Type I and L. spinosa pinnatifid Type II, respectively. Further studies relating to chemotaxonomy, molecular taxonomy, palynology, etc. are needed in these taxa to decide the taxonomic status of these morphotypes.

Variation of chromosome numbers among the genera (Table 3), suggests that there is an increase in chromosome number in some phyletic lines to a higher level of ploidy in *C. esculenta* and *L. spinosa* while in some it has reduced which may be due to the spontaneous mutation occurring in the accessions (Mayo et al. 1997). According to Petersen (1989) the basic chromosome numbers of 14 or 12 may be the starting points for all the chromosome numbers known in Araceae due to aneuploid changes at the diploid as well as polyploid levels.

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