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Cytotaxonomic studies of three ornamental aroids

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ABSTRACT

Cytotaxanomical analysis carried out on three ornamental aroids (*Anchomanes difformis, Anchomanes hookeri* and *Arum maculatum*) proved that cytological studies is none negligible tool in phylogeny and scientific classificationns of plants. Aceto-orcein stain squash technique was used in this study. *Anchomanes difformis* and *Anchomanes. hookeri* showed more relatedness in chromosome number and chromosome morphology, sharing the same chromosome number 2n=13, while *Arum maculatum* has 2n=8. The following karyotypes formular were revealed: 2n=8=3M+3SM+2ST in *Arum maculatum*; 2n=13=5M+1SM+7ST in *A. difformis*; and 2n=13=3M+6SM+4 *A. hookeri*. Other karyotype parameters investigated like CI%, AsI and degree of asymmetry of the genomes supported current taxonomic ranking.

***Keywords*:** aceto-orcein; *Arum maculatum*; *Anchomanes hookeri*; *Anchomanes difformis*; Karyotype; asymmetry

1. INTRODUCTION

Araceae is a group of monocotyledon flowering plants that bear a type of inflorescence called spadix. The spadix is usually enclosed in spathe or leaf-like bracts. The group belongs to the order *Alismatales*. Members are colloquially called aroid. They are herbaceous and often rhizomatous or tuberous.

Arum maculatum is a common woodland plant species, commonly known as arum, Jack in the pulpit, lords and ladies etc. it bears spadix enclosed by pale green spathe. They are monoecious and the female flowers form rings at the base while rings of male flower are at the top of the spadix. They bear poisonous red berries that remains long after the leaves and the flower have withered away. They bear starch storing roots. Bromfield (1856) identified two types based on the leave spot. The spotted and unspotted.

Anchomanes difformis (Engl) is a species of flowering plants in the family Araceae. It is herbaceous plant with pricky petiole, having huge divided leaf and spathe and the solitary leaf arise from a horizontal tuber. They normally grow as wild but sometimes are cultivated as ornamentals. They are native to West Africa (Eneojo *et al*, 2011). Afolayan, *et al* (2012) reported high potential for industrial applications especially in the food, textile and pharmaceutical industries (Eneojo *et al*, 2011). It is locally called "*Oje"* by the Igbos in East of Nigeria.

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Anchomanes hookeri (Schott) is a large herbaceous plant with stout pricky stem, which grow to 3 ft. or more in height with obovota leaves and palmate veination (Brown, 1902). The stem and the adaxial surface of the leaves have spines, the roots barely grow deep into the ground but can grow horizontally up to 13 cm. It takes a long time to completely unfold its leaves after breaking the soil. Its tubers are flattened, roughly spherical and weighing as much as 5-15 kg.

Karyotype and related studies have proved to be relevant tool in determining karyo-evolutionary trends within taxa (Srivastava and Srivastava, 2000; Hamidah et al, 2009). Recently, karyotype studies have contributed enormously to the systematic along with biochemistry, molecular, morphological and anatomical studies.

2. MATERIALS AND METHODS

2. 1. Supply of Root Tips

For karyotype studies and for the study of chromosome numbers, the plants of the three species were grown in saw-dust culture because the root tips needed for the studies are very delicate and if sown in soil can break in the process of uprooting and the particles do not adhere tightly and so can allow for the easy uprooting of the plants without breaking the delicate root tips

2. 2. Chromosome Investigation

Chromosome studies involve a number of steps such as determining the best harvest time for root tips, pretreatment, fixation, hydrolysis, squashing, staining and chromosome analysis. These steps are necessary so that on squashing and staining, chromosome would appear as distinct shortened, single bodies scattered within a given cell thus permitting easy counting and measurements. Thus metaphase plate is always preferred and was used.

2. 3. Harvest Time for Root Tips

The time of collection of root tips is of prime importance because it is desirable to arrest enough metaphase plates. This involved collection of root tips at half hourly interval from 9 a.m. to 3 p.m.

2. 4. Pretreatment and Fixation

Pretreatment is aimed at clearing the viscosity of the cytoplasm in the root tips and also a means of shortening the chromosome and inhibiting the formation of spindles, resulting in ‘thick’, distinct and disorganized bodies of chromosome. Two chemicals normally used are 0.003 M 8-hydrooxyquinoline and 0.1 % colchicines. The procedure applied involved cutting root tips about 4 cm long and a slit made at the cut end so as to enhance the absorption of the chemical by the root tips.

The plant materials were immediately immersed in the pretreatment solution and kept at room temperature of about 20 °C for two hours before storing in the refrigerator at a temperature of 4 °C for 2-3 hours. This low temperature treatment aided the shortening of the chromosome. The result presented here were obtained with 8-hydrooxyquinoline. Carnoy’s solution I, a mixture of absolute alcohol and glacial acetic acid in the ratio 3: 1 was the fixative used. For fixation, the pretreated roots were put in specimen bottles containing the solution and left in the refrigerator at 4 °C for at least 24 hours.

Fixation kills and stabilizes the root tips rapidly thus preserving them in a near natural condition for a long time.

2. 5. Hydrolysis

After fixation, the roots were hydrolyzed with 0.1 N HCL at 60 °C in a water bath. This helped to soften the root tissues and enhanced the absorption of stain. To obtain a good result, series of time were tried ranging from three to fifteen minutes.

The optimal results were obtained using 0.1 N HCl for 12 minutes for all the three species and 7 minutes, using 1 N HCl also for all the species. The reason for long period of hydrolysis was that roots were big and often with tough 'skin' covering.

2. 6. Squashing and Staining

Squashing is aimed at separating the cells from one another to enhance the study of chromosome. The aim of staining is to bring about contrast between the chromosomes which remained stained.

After hydrolysis, the root tips appeared milky in color and were washed four times with distilled water. A root tip was then placed on a clean slide on top of a filter paper. The milky tip was next cut with scapel and sliced into fine bits in two drops of stain-aceto orcein. Thick particles were removed.

After staining, the cover slip was placed in position and filter paper was folded over the slide just to remove excess stain. A gentle tapping with a match stick was given so as to further scatter the chromosomes two ends of the filter paper were again folded over the slide and then pressed with the right thumb. This helped to further spread the chromosomes, expel any trapped air as well as blot out the excess stain.

2. 7. Slide Preservation

For temporary preservations, the sides of the cover slip were sealed with a nail varnish which preserved the cell for up to one week. For permanent preparations, the slides were first smeared with egg albumen and allowed to dry. This served as an adhesive. Squashes were made on the slide and a cover slip put in place and pressed down as normal.

Inspection was then made at a lower power to ensure that the slide had a reasonable number of diving cells. A razor blade was used to sharply lift the cover slip from the slide. Both cover slip and slide were passed through grades of alcohol for 30 seconds beginning with 50-75-90 % - Absolute – Absolute alcohol / xylene [50 % : 50 %] and then finally xylene for 1 minute. After xylene a drop of Canada balsam was added to the squashing position on the slide and fresh cover slip was carefully lowered. On another slide, another drop of Canada balsam was added and the original cover slip was placed making sure that no air bubbles were entrapped. Inspections were then made and a good slide was allowed to dry for 24 hours.

2. 8. Karyotype Analysis

Chromosomes at metaphase were measured according to previous studies (Kalvande *et al*, 2012; Hamidah *et al*,2009; Hejazi, 2011; Gonzalez et al, 2013; Truta et al, 2013; Lavania and Srivastava , 1992; and Gunjan and Roy, 2010). Chromosome designation followed Levan *et al.* (1964), and the homology was assigned according to similarities in length, morphology, and centromere position, respectively, on the basis of CI (Centromeric Index) and r (arm ratio) values. Thus, the chromosomes are metacentric when they have a mean arm ratio r (r

≤1.7 and CI = 37.5 – 50.0) submetacentric (r=1.70-2.99, CI=25.0-37.5), subtelocentric (r=3.00-6.99 and CI=12.5-25.0), or telocentric (r≥7.00 and CI ≤ 12.5) (Truta *et al,* 2013). In karyotype, the chromosome pairs were grouped in descending order of their length. The karyotypes were characterized numerically estimating the followings: long arm (LA), short arm (SA), absolute length of each chromosome CL [LA+SA], arm ratio R[LA/SA], centromeric index (CI) [SA/CL]. To evaluate the karyotype asymmetry /symmetry, the following estimates were used:
The total form percent (TF %).

$$TF\% = \left(\frac{\sum SA}{\sum CL}\right)$$

Asymmetry Index (AsI%) (synonymous with Ask%)

$$AsI\% = \left(\frac{\sum LA}{\sum CL}\right) x100$$

The intrachromosomal asymmetry idex (A_1) and the interchromosomal asymmetry index (A_2) were calculated according to (Srivastava and Srivastava, 2000; Truta et al, 2013)

$$\mathrm{A1} = \left|1 - \left(\sum \frac{SA}{SA}\right)/n\right|$$

where SA stand for the mean of short arm, LA is the mean of the long arm, (n) is the number of homologues chromosomes. It measures the average position of the centromere in karyotype and ranges from 0 (completely symmetrical) to 1 (completely asymmetrical).

$$A2 = \left(\frac{SCL}{XCL}\right)$$

where S_{CL} = standard deviation of chromosome length, X_{CL} = mean chromosome length for each genotype. It is defined as a coefficient to evaluate heterogeneity of chromosome length.

Karyotypic characteristics were determined using the symmetry classes of Stebbins (SC) (Stebbins, 1971).

3. RESULTS

Table 1. The classification of karyotypes in relation to their degree of asymmetry according to stebbins (1971).

	Proportion of chromosomes with arm ratio>2			
Ratio (Largest/smallest)	1.00	0.99-0.51	0.50-0.01	0.00
<2(A)	1A	2A	3A	4A
2-4(B)	1B	2B	3B	4B
>4(C)	1C	2C	3C	4C

Table 2. Average of cytogenetic parameters of *Arum maculalum* utilized for idiogram construction (CL = chromosome lenght, LA = long arm lenght, SA = short arm lenght, AR = arm ratio (LA/SA), CI = Centromeric index, RL = relative length.

	TYPE	CL	LA	SA	AR	CI	RL
1	SM	2.10	1.40	0.70	2.00	33.33	4.69
2	SM	4.20	2.80	1.40	2.00	33.33	9.39
3	SM	4.55	3.15	1.40	2.25	30.77	10.17
4	M	5.60	2.80	2.80	1.00	50.00	12.51
5	M	5.60	2.80	2.80	1.00	50.00	12.51
6	ST	5.60	4.20	1.40	3.00	25.00	12.51
7	ST	7.35	5.60	1.75	3.20	23.81	16.42
8	M	9.45	5.60	3.85	1.45	40.74	21.11
T		44.45	28.35	16.10		286.98	99.99
M							

Table 3. Average values of cytogenetic parameter of *Anchomanes differmis* utilized for idiogram construction (CL = chromosome lenght, LA = long arm lenght, SA = short arm lenght, AR = arm ratio (LA/SA), CI = Centromeric index, RL = relative lenght).

	TYPE	CL	LA	SA	AR	CI	RL
1	ST	5.90	4.50	1.40	3.21	23.73	4.02
2	ST	9.10	7.70	1.40	5.50	15.39	6.20
3	ST	9.10	7.70	1.40	5.50	15.39	6.20
4	ST	9.80	8.40	1.40	6.00	14.29	6.68
5	SM	9.90	7.10	2.80	2.54	28.28	6.74
6	M	10.50	5.25	5.25	1.00	50.00	7.16
7	M	10.85	6.65	4.20	1.58	38.71	7.40
8	ST	12.60	9.80	2.80	3.50	22.22	8.59

9	M	12.60	7.00	5.60	1.25	44.44	8.59
10	ST	13.30	10.50	2.80	3.75	21.05	9.07
11	ST	14.00	11.20	2.80	4.00	20.00	9.54
12	M	14.35	8.40	5.95	1.41	41.46	9.78
13	M	14.70	7.35	7.35	1.00	50.00	10.02
T		146.70	101.55	45.15		384.96	99.99

Table 4. Average values of cytogenetic parameter of *Anchomanes hookeri* utilized for idiogram construction (CL = chromosome lenght, LA = long arm lenght, SA = short arm lenght, AR = arm ratio (LA/SA), CI = Centromeric index, RL = relative lenght).

	TYPE	CL	LA	SA	AR	CI	RL
1	ST	8.40	7.00	1.40	5.00	16.67	5.09
2	ST	8.40	6.30	2.10	3.00	25.00	5.09
3	ST	9.10	7.00	2.10	3.33	23.08	5.51
4	SM	11.55	8.05	3.50	2.30	30.30	6.99
5	M	11.90	7.05	4.90	1.44	41.18	7.20
6	M	11.90	5.95	5.95	1.00	50.00	7.20
7	SM	12.60	8.40	4.20	2.00	33.33	7.63
8	ST	12.60	9.80	2.80	3.50	22.22	7.63
9	SM	13.30	8.75	4.55	1.92	34.21	8.05
10	M	13.65	8.40	5.25	1.60	38.46	8.26
11	SM	15.40	10.50	4.90	2.14	31.82	9.32
12	SM	16.10	11.90	4.20	2.83	26.09	9.75
13	SM	20.30	14.70	5.60	2.63	27.59	12.29
T		165.20	113.20	51.40	32.7	399.95	100.01

Table 5. Karyotype characteristics of the three aroids.

SPECIES	KARYOTYPE	CI %	R	AsI	TF	A1	A2	CLASSES
A. maculatom	2n=8=3M+3SM+2ST	35.87	4.50	63.35	35.98	0.923	0.367	3C
A. difformis	2n=13=5M+1SM+7ST	29.61	2.49	69.22	30.78	0.966	0.219	3B
A. hookeri	2n=13=3M+6SM+4ST	30.77	2.42	68.52	31.11	0.965	0.249	3B

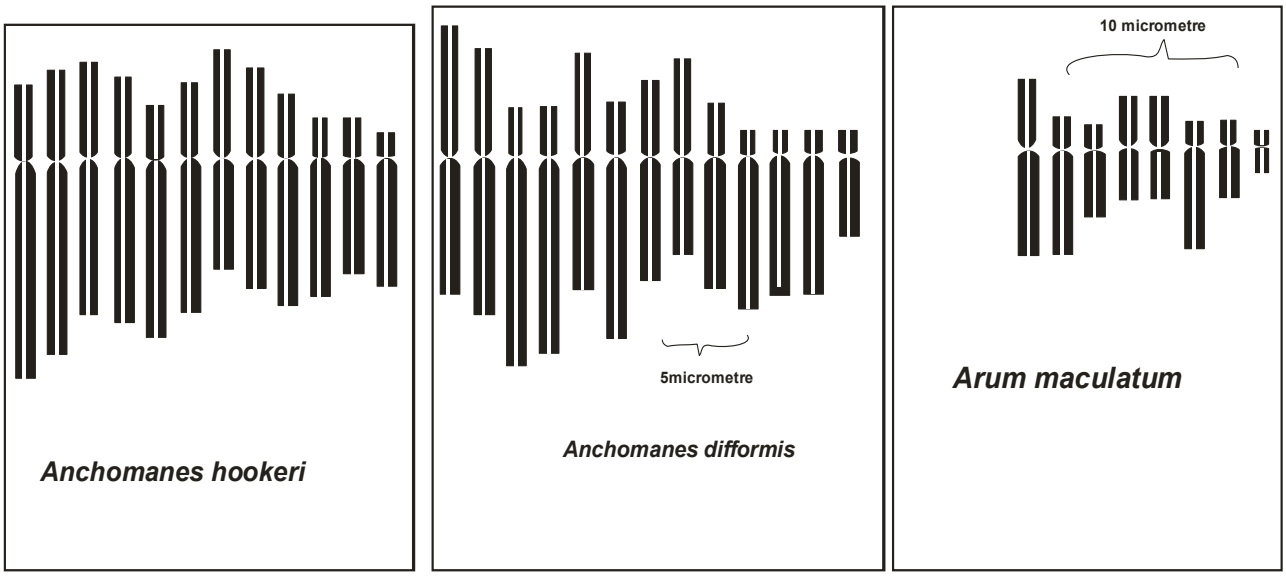


Fig. 1. Ideogram.

4. DISCUSSIONS AND CONCLUSIONS

Arum maculatum is morphologically distinct from the other two *Anchomanes* in this study. The true reason of investigating the *Arum* along the *Anchomanes* is to prove a concomitant distinction in their karyograms. The first indicator is the chromosomal numbers. In *Arum maculatum* 2n=8 while in both Anchomanes, 2n=13. The TF, total form percent that is used to show what percentage of the total chromosome constitutes the short arm indicated 35.98 % for *Arum maculatum*, 30.78 % for *A. difformis* and 31.11 % for *A. hookeri*. A lucid picture is made to manifest in these data. The dissociation between the *Arum* and the *Anchomanes*; and then the relatedness of *A. difformis* to *A. hookeri* is put in the picture. The slight difference shown between the *Anchomanes* also qualifies them to be proclaimed different species.

The AsI, the asymmetric index, a measure of the genomes long arm indicated a visible line of demarcation that is associated with the taxonomic ranking. The following AsI % 63.35 %, 69.22 % and 68.52 % for *Arum maculatum*, *A. difformis* and *A. hookeri* respectively. The difference between *Arum* and *Anchomanes* are significant.

The karyotype (see Table 5) indicated metacentric, submetacentric and subtelocentric chromosomes in the three plants. *A. differmis* has more than half of its diploid chromosome to be subtelocentric (7ST) and *A. hookeri* has 4ST chromosomes while *Arum maculatum* has only 2ST chromosomes.

Truta *et al* (2013) A1 measures the length of short and long arms of each pair of homologues and the average position of the centromere in the karyotype and ranges from 0 (completely symmetrical) to 1 (completely asymmetrical) (Paknia and Karimzadeh, 2010). A1 analyses in this study showed 0.923 for *Arum maculatum*, 0.966 for *A. differmis* and 0.965 for *A. hookeri* (Peruzzi et al, 2009).

The idiogram (see Fig. 1) tries to picture the karyomorph. It shows the chromosome number; the relationship between each short arm to the long arm of genomes and the degree of specialization, which is also the degree of asymmetry. According to Stebbins (1950), more primitive plants show high symmetrical chromosome structures. It then means that *Arum maculatum* is primitive to the *Anchomanes* and that *A. differmis* is specialized than *A. hookeri* as indicated by their chromosomal symmetry.

The karyomorph differences indicates structural changes in their chromosomes that may results from inversions, deletions, translocations etc.

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