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Karyotype Analysis on Two *Abrus* Adanson (Papilionaceae) Species in Nigeria

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Abstract: Cytological studies were undertaken on *Abrus precatorius* L. and *A. pulchellus* Wall. with a view towards confirming the chromosome number of each species and determining their karyotypes. Roots from germinating seeds of each species were collected and pretreated with 0.002M 8-hydroxyquinoline before fixing in 1: 3 glacial acetic acid: absolute alcohol. Hydrolyzed root tips were subsequently stained and squashed in a drop of FLP-Orcein. The chromosome number of *A. precatorius* is observed to be 2n = 22 while that of *A. pulchellus* is 2n = 44. Both species possess metacentric, submetacentric and acrocentric chromosomes. Chromosome length in *A. precatorius* was observed to vary from 0.40 to 1.00 μm while in *A. pulchellus,* the range was from 0.50 to 1.40 μm. The total length of haploid complement of *A. precatorius* is 7.30 μm. This is approximately double the total length of haploid complement of 15.98 μm observed in *A. pulchellus*. Karyological details of these species which confirm that *A. pulchellus* is a polyploidy, are reported for the first time in this study.

Key words: *Abrus,* acrocentric, chromosome, haploid, metacentric, Papilionaceae, polyploid

INTRODUCTION

Abrus Adanson is one of the small and lesser known genera of the Papilionaceae. Three species, *A. precatorius* L., *A. pulchellus* Wall. and *A. canescens* Welw. ex Bak. have been confirmed to occur in Nigeria (Ozoemenam *et al*., 2007). Though lesser known, these species posses overwhelming ethnomedical, ethnopharmacological and toxicological potentials (Anam, 2001; Acharya, 2004; Ohba, 2004; Moshi *et al*., 2005; Pillay *et al*., 2005).

Recent reports of the usefulness of chromosome numbers and morphology in cytogenetic and taxonomic studies include those of Mercado-Ruaro and Delgado-Salinas (2000), Maurat *et al*. (2005), Esra *et al*. (2006), Mustafa (2006), Pavlova (2008) and Yilmaz *et al*. (2009). In *Abrus,* chromosome numbers have been reported for *A. precatorius* (Borgen, 1980; Gill and Husaini, 1986; Yeh *et al*., 1986; Kumari and Bir, 1990). While Gill and Husaini (1986) gave a gametophytic count of 11 for *A. precatorius*, the other authors expectedly reported sporophytic count of 22 for the same species. For *A. pulchellus,* Gill and Husaini (1986) and Chatterjee *et al*. (1989) reported gametophytic counts of 12. However, Agbagwa and Okoli (2005) investigated several samples from Nigeria and discovered that *A. pulchellus* is actually a polyploid with 44 chromosomes. Apart from these chromosomes reports, general paucity of information on the cytogenetics of the genus exists possibly as a result of the small size of the chromosomes. These species even with their immense potentials in ethnomedicine are threatened with extinction due to gradual destruction of galleried forests, groves and shrines (Ozoemenam *et al*., 2007; Agbagwa and Obute, 2007) where the occur naturally. This study therefore, aims at describing the chromosome morphology of *A. pulchellus,* a polyploid and one of its possible diploid progenitors, *A. precatorius*, as a precursor for further genetic studies. Details of the karyotype of these species are reported for the first time.

MATERIALS AND METHODS

Seed samples of both species were collected between November 2000 and January 2001 from different localities in southern Nigeria. Seeds were germinated in buckets containing white sand within the University of Port Harcourt Botanical Garden, Port Hacourt Nigeria between 12th to 19th January 2001. For *A. precatorius* seeds, scarification with office pin was undertaken to facilitate germination. Root tips for the mitotic studies were obtained from germinating seeds between 11 and 12 h. The roots were washed and excised using a pair of forceps, pretreated with 0.002 M 8-hydroxyquinoline solution for 3 h and fixed in 1: 3 glacial acetic acid:absolute alcohol. Roots were hydrolyzed in 18% HCl aqueous for 3 min, stained and squashed in a drop of FLP-Orcein (i.e., a solution of Orcein and equal

portions of formic, lactic and propionic acids in water) under No.0 or No.1 cover glass on slides following Okoli (1992). The cells were flattened-out by pressing firmly with the thumb. Slides were examined under a Leitz Diaplan microscope and good chromosome plates photographed with Leica WILD MPS 52 microscope camera on Leitz Diaplan microscope. The cytological investigations were carried out from January 2010 to March 2001.

The karyograms were drawn from mitotic metaphase. Chromosome measurements were based on five metaphase plates for each species. Karyotype analysis followed the method of Levan *et al*. (1964).

RESULTS

***A. precatorius*:** The chromosome number of this species is 2n = 22 (Fig. 1). The karyotype (Fig. 3) consists of eleven chromosomes pairs with different morphological appearances viz: four metacentric pairs (1, 3, 6th and 7th), four submetacentric pairs (2, 4, 9th and 10th) and three acrocentric pairs (5, 8th and 11th). The length of chromosomes in this taxon varied from 0.40 to 1.00 μm. The longest arm is 0.60 μm and the shortest arm is 0.07 μm. Chromosome 5 had the highest L/S arm ratio. The total haploid length of *A. precatorius* is 7.30 μm. Details of the karyotype are shown in Table 1.

***A. pulchellus*:** The chromosome of *A. pulchellus* is 2n = 44 (Fig. 2). The karyotype (Fig. 4) consists of twenty-two chromosome pairs in three morphological categories: eight metacentric pairs (2, 3, 7, 9, 10, 16, 19th and 22 nd), eight submetacentric pairs (1, 4, 5, 6, 8, 11, 18th and 21 st), and six acrocentric pairs (12, 13, 14, 15, 17 and 20th). The length of chromosomes in *A. pulchellus* varied from 0.50 to 1.40 μm. The longest arm is 0.94 μm and the

Table 1: Karyotype details of metaphase chromosomes of *A. precatorius*

Chromosome pairs	Chromosome arms (μm)		Total length (μm)	Arm ratio (r =L/S)	Relative length (%)	Centromeric position
	Long arm (L)	Short arm (S)				
1	0.50	0.05	1.00	1.00	13.69	Metacentric
2	0.60	0.30	0.90	2.00	12.32	Submetacentric
3	0.40	0.30	0.70	1.33	9.58	Metacentric
4	0.47	0.23	0.70	2.04	9.58	Submetacentric
5	0.50	0.10	0.60	5.00	8.22	Acrocentric
6	0.25	0.25	0.50	1.00	6.85	Metacentric
7	0.40	0.30	0.70	1.33	9.58	Metacentric
8	0.58	0.12	0.70	4.83	9.58	Acrocentirc
9	0.33	0.17	0.50	1.98	6.85	Submetacentric
10	0.40	0.20	0.60	2.00	8.22	Submetacentric
11	0.33	0.07	0.40	4.71	5.48	Acrocentric

Total length of haploid complement of *A. precatorius* chromosomes = 7.30 μm

Table 2: Karyotype details of metaphase chromosomes of *A. pulchellus*

Chromosome pairs	Chromosome arms (μm)		Total length (μm)	Arm ratio (r =L/S)	Relative length (%)	Centromeric position
	Long arm (L)	Short arm (S)				
1	0.74	0.36	1.10	2.1	6.90	Submetacentric
2	0.60	0.60	1.20	1.0	7.51	Metacentric
3	0.50	0.50	1.00	1.0	6.30	Metacentric
4	0.94	0.46	1.40	2.0	8.80	Submetacentric
5	0.54	0.31	0.85	1.7	5.32	Submetacentric
6	0.65	0.35	1.00	1.8	6.30	Submetacentric
7	0.50	0.50	1.00	1.0	6.30	Metacentric
8	0.47	0.23	0.70	2.0	4.40	Submetacentric
9	0.30	0.20	0.50	1.5	3.13	Metacentric
10	0.40	0.30	0.70	1.3	4.40	Metacentric
11	0.61	0.32	0.93	1.9	6.13	Submetacentric
12	0.60	0.10	0.70	6.0	4.40	Acrocentric
13	0.58	0.12	0.70	4.8	4.40	Acrocentric
14	0.60	0.10	0.70	6.0	4.40	Acrocentric
15	0.59	0.11	0.70	5.4	4.40	Acrocentric
16	0.30	0.30	0.60	1.0	3.75	Metacentric
17	0.52	0.08	0.60	6.5	3.75	Acrocentric
18	0.40	0.20	0.60	2.0	3.75	Submetacentric
19	0.25	0.25	0.50	1.0	3.13	Metacentric
20	0.42	0.08	0.50	5.3	3.13	Acrocentric
21	0.33	0.17	0.50	1.9	3.13	Submetacentric
22	0.25	0.25	0.50	1.0	3.13	Metacentric

Total length of haploid complement of *A. pulchellus* = 15.98 μm

shortest arm is 0.08 μm. Chromosome 17 had the highest L/S arm ratio. The total haploid length of *A. pulchellus* is 15.98 μm. Details of the karyotype are shown in Table 2.

Though the size grouping in the 2n = 22 of *A. precatorius* is not reflected in the 2n = 44 of *A. pulchellus*, there seem to be a doubling of the morphological types in *A. precatorius* i.e., metacentric, submetacentric and acrocentric in *A. pulchellus*.

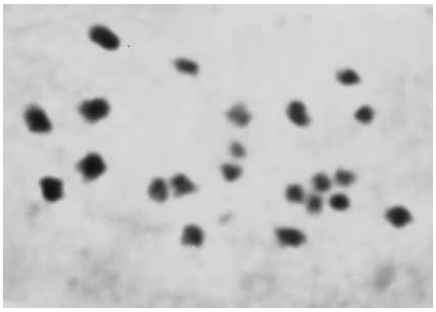

Fig. 1: Photomicrograph of somatic metaphase chromosomes of *A. precatorius*

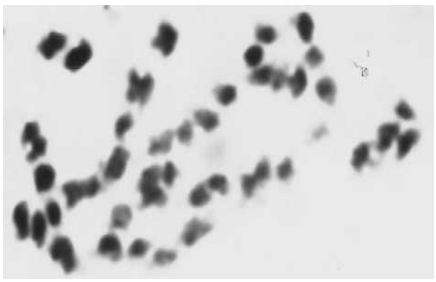

Fig. 2: Photomicrograph of somatic metaphase chromosomes of *A. pulchellus*

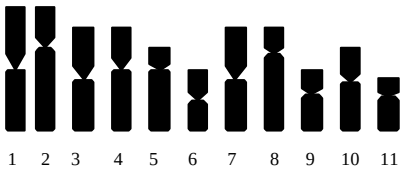

Fig. 3: Ideogram of chromosome complement of *A. precatorius*

DISCUSSION

There are no known reports on the karyotype of *Abrus* species. Except, Agbagwa and Okoli (2005) who reported metacentrics, submetacentics and acrocentrics as morphological types of the 44 chromosomes of *A. pulchellus*, previous cytological studies on *Abrus* (Borgen, 1980; Gill and Husaini, 1986; Yeh *et al*., 1986; Chatterjee *et.al*., 1989; Kumari and Bir, 1990) gave only the chromosome numbers. Gill and Husaini (1986) and Chatterjee *et al*. (1989) reported n = 12 for *A. pulchellus* which implies 2n = 24. However, this study shows that in *A. pulchellus* n = 22 and 2n = 44 (Fig. 2). The basic chromosome number (x) for *A. pulchellus* is therefore 11 and not 12 as previously reported. For *A. precatorius,* chromosome number of 22 (n = 11) observed in this study as shown in Fig. 1 agrees with the findings of Yeh *et al*. (1986) and Kumari and Bir (1990). Thus for this species (*A. precatorius*), n = x = 11. Obviously *A. pulchellus* is a polyploidy which agrees with Agbagwa and Okoli (2005).

Karyotype essentially reveals differences in sizes of chromosome complements and their centromeric positions. This study has shown that both *A. precatorius* and *A. pulchellus* possess metacentric, submetacentric and acrocentric chromosomes. No telocentric chromosomes were identified in both species. The doubling of the morphological types observed in the karyotype of *A. precatorius* 2n = 22 in *A. pulchellus'* 2n = 44 (Fig. 3, 4) may suggest that the polyploid *A. pulchellus* was derived from *A. precatorius*. Consequently, *A. precatorius* is the ancestral taxon. The occurrence of several structural chromosome forms between the two species is a pointer to the fact that many structural changes occur within the genus. This may explain why the sizes of chromosomes in *A. pulchellus* were not exactly double that of *A. precatorius* even when they have the same number of morphological forms in each case. Interestingly, the total length of haploid complement of *A. precatorius* (7.30 μm) is almost half that of *A. pulchellus* (15.98 μm). Thus the two species can be said to have strong karyotypic relationship.

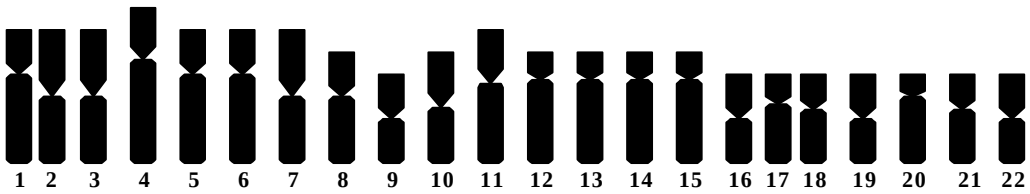

Fig. 4: Ideogram of chromosome complement of *A. pulchellus*

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