International Journal of Botany Studies
ISSN: 2455-541X
Impact Factor: RJIF 5.12
www.botanyjournals.com
Volume 2; Issue 5; September 2017; Page No. 46-49

Karyomorphological investigation into the white seeded *Abrus precatorius* L. in Bangladesh

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Abstract
Cytological investigation was done in white seeded *Abrus precatorius* L. intended to ascertain the chromosome number and karyotype. Somatic chromosome count of this variety was found to be 2n=22. Chromosomes were small in size, ranging from 2.10 to 0.90 µm. Resting chromosomes were simple chromocenter type with 15-18 heteropycnotic bodies. TF% and asymmetry index (AI) was 38.36 and 3.38 respectively. The haploid complement was grouped into three morphological categories *viz.* metacentric, submetacentric and sub-telocentric. The centromeric formula was 3m+3nm+4sm+1st. According to Stebbins (1971) classification the karyotype was of 2B type.

Keywords: white seeded *Abrus precatorius*, karyotype, resting chromosome, Bangladesh

Introduction

The genus *Abrus* Adanson is included in the family Fabaceae under the tribe Abreae, which is distributed in the tropical regions of both hemispheres. In Bangladesh two species *viz. Abrus precatorius* L. and *Abrus pulchellus* Wall. *Ex* Thw. have been reported (Naderuzzaman, 2009) [14]. Between the two species, *A. precatorius* is found to be grown in almost all parts of the country but the distribution of *A. pulchellus* is restricted to Chittagong, Cox's Bazar and Sylhet regions (Naderuzzaman, 2009) [14]. *A. precatorius* is more common than *A. pulchellus* and the former can be easily identified by its bright scarlet seeds with black spots at hilum. *A. pulchellus* is black seeded without any spots on seed coat with 22 chromosomes in somatic cells (Naderuzzaman, 2009) [14]. Chromosome number of red seeded form was determined as 2n=22 (Gill and Husaini, 1986; Yeh *et al.* 1986; Kumari and Bir, 1990; Agbagwa, 2011) [7, 22, 10, 2]. Another variety of *A. precatorius* having white seed has been collected and identified. But there is no information about the white seeded *A. precatorius* in Bangladesh. This plant has however been mentioned in some reports published in India (Biswas, 1973; Pillay *et al.*, 2005; Parthyusha *et al.*, 2010) [4, 18, 15] depicting its medicinal properties but no karyological information is known so far.

Both red and white seeded plants are perennial climbers with profuse branching. Young branches are delicate but older ones are rather tough. Flowers bloom in compact raceme. In case of red seeded *A. precatorius*: calyx lobes shallow, dented and green, corolla light pinkish stamen 9, unequal. Fruit a pod, seeds are red with a black spot at the hilum. Flowering and fruiting in July to September (Naderuzzaman, 2009) [14]. In white seeded *A. precatorius*: calyx lobes shallow, dented green, corolla white, stamen 9, unequal. Fruit a pod, seeds are white with a brown spot at the hilum. Flowering and fruiting in October to December (personal observation). Different parts of both red and white seeded *A. precatorius* hold ethnomedical, ethnopharmacological and toxicological potentials (Biswas, 1973; Anam, 2001; Acharya, 2004; Moshi *et al.* 2005; Pillay *et al.*, 2005; Parthyusha *et al.*, 2010; Garaniya and Bapodra, 2014) [4, 3, 1, 13, 18, 15, 6].

Except for flower and seed colour and flowering time, there was no visual difference between red and white seeded *A. precatorius*. Therefore, the present study was aimed to determine the chromosome number and karyotype of white seeded *A. precatorius*.

Karyotype of a species gives a picture of its chromosomal variation, both numerical and structural, within the complement which could be an important parameter to evaluate the evolutionary trends. Karyotype analysis could also be helpful for the determination of taxonomic affinities among different groups of plants (Maurat *et al.*, 2005; Pavlov *et al.*, 2008; Yilmaz *et al.*, 2009; Agbagwa, 2011; Samaddar *et al.* 2012; Hossen *et al.*, 2016) [12, 17, 23, 2, 8]

Material and Methods

Germplasm of this plant was collected from a road side bush in Koira upazila, Khulna, adjacent to Sundarban and is being maintained in the medicinal plant garden of Botany department, University of Chittagong, Bangladesh. Morphology of flowers and seeds of this plant are shown in Figure -1 (a, b). Analysis of somatic chromosomes was done from the fast dividing root tip cells by examining the mitotic metaphase chromosomes. Mature sun dried seeds were germinated on two layered wet filter paper taken in petri dishes. The petri dishes were maintained in the laboratory in dark chamber at room temperature. Germinated roots of ca. 1.0-1.5 cm were collected at 10.00-10.30 a.m. in saturated solution of para-dichlorobenzene (PDB) for 2-3 hours and kept at 4º C in refrigerator. After pre-fixation the roots were fixed in 1:3 (v/v) Carnoy's solution for 24 hours at 4º C. Roots

were hydrolyzed in 1 N HCl and 45% glacial acetic acid (1:1) for 30 seconds at ca. 60º C. After a thorough washing, roots were immersed into 2% (w/v) aqueous solution of iron alum for 5 minutes. Before squashed in 0.2% (w/v) acetocarmine, roots were stained in 0.5% (w/v) aqueous solution of haematoxylin for 20 minutes. The prepared slides were then examined undera Optica Vison Pro microscope and well separated chromosome plates were photographed with Optica Microscope camera (5MP) fitted on Optica Vision Pro Microscope at a magnification of 1000x (10x eyepiece and 100x objective) using oil immersion. Camera lucidia drawings of well spread metaphase chromosomes were taken with help of light microscope installed in the Plant Biotechnology Laboratory of Chittagong University.

Chromosomes were characterized calculating the following parameters: (1) shortest (SC) and longest (LC) chromosome length; (2) ratio of longest and shortest chromosome (LC/SC); (3) mean long arm (p) length and mean short arm (q) length; (4) total length of chromosome (CL); (5) proportion of chromosomes with arm ratio more than 2:1 (6) mean centromeric indes (CI= length of short arm/total length of chromosome x 100) (7) TF% (Huziwara, 1962) [9]; (8) Stebbins (1971) [20] qualitative classification for the determination of asymmetry (9) AI= asymmetry index (Paszko, 2006) [16]; (9) karyotypic formula (Table 1 and 2).

Measurement of chromosomes was done based on five well separated metaphase cells. Resting nuclei and prophase chromosome types were determined according to Tanaka (1971) [21] and classification of chromosomes was prepared following the nomenclature of Levan *et al.*, (1964) [11].

Table 1: Detail Karyotype analysis of haploid complement of white seeded *A. precatorius.*

Chromosome pairs	Chromosome length (µm)		Total length of chromosome (µm)	Arm ratio (q/p)	Centromeric index (CI)	Centromeric type
	Long arm (q)	Short arm (p)				
1	1.400	0.800	2.200	1.75	36.364	Submetacentric
2	0.900	0.900	1.800	1.00	50.000	Metacentric
3	1.260	0.520	1.780	2.42	29.213	Submetacentric
4	0.870	0.870	1.740	1.00	50.000	Metacentric
5	0.940	0.600	1.540	1.57	38.961	Nearmetacentric
6	0.720	0.720	1.440	1.00	50.000	Metacentric
7	0.960	0.460	1.420	2.09	32.394	Submetacentric
8	0.760	0.500	1.260	1.52	39.683	Nearmetacentric
9	0.980	0.260	1.240	3.77	20.968	Subtelocentric
10	0.730	0.480	1.210	1.52	39.669	Nearmetacentric
11	0.730	0.270	1.000	2.70	27.000	Submetacentric

Table 2: Range of chromosome length, ratio of longest and smallest chromosome, mean long arm length, mean short arm length, mean chromosome length, centromeric index, asymmetric index (Paszko, 2006), Stebbins asymmetry type (1971), TF% and karyotypic formula of white seeded *Abrus Precatorius*.

Range SC-LC (µm)	Ratio of LC/SC chromosome	q (µm) mean (±SD)	p (µm) mean (±SD)	CL (µm) mean (±SD)	CI mean (±SD)	AI	<2:1=0.64 l/s=B	TF (%)	Karyotypic formula
2.20-1.00	2.20	0.93±0.22	0.58±0.048	2.77±0.35	37.65±9.8	3.38	2B	38.36	3m+3nm+4sm+1st

Result and discussion

The somatic chromosome number of this plant was confirmed to be 2n=22 (Figure 1c). The resting nuclei were found to be simple chromocenter type with darkly stained 18-20 heteropycnotic bodies (Fig. 2a). It was evident that mitotic prophase chromosomes were of proximal type and condensation of chromocenters was observed to be initiated from proximal region to distal region of both arms (Fig. 2b). The karyotype consists of eleven chromosome pairs. Of the eleven basic chromosomes 3 were metacentric, 3 near metacentric, 4 submetacentric and 2 were subtelocentric. Length of chromosomes ranged from 0.90 µm to 2.10 µm. Arm ratio was measured as 1.00 to 3.77. Total length of the haploid complement was 16.63µ. Centromeric indices varied between 20.97 to 50.00 (Table-1). TF% was 38.36 (Table-2). None of the chromosomes had secondary constrictions. The ratio of the longest and the smallest chromosome lengths were more than 2:1 and the frequency of chromosomes having arm ratio less than 2:1 was 0.64. Thus the karyotype was 2B symmetric type. Asymmetry index (AI) was calculated as 3.38 (Table 2). In the present article karyomorphological analysis of white seeded *A. precatorius* is described in detail. It is pertinent to mention here that no previous report in connection to karyotype analysis was found. The earliest report of chromosome number of red seeded *A. precatorius* was 2n = 22 (Fedrovo, 1969) [5]. Later on it was confirmed by the endeavor of Yeh *et al.* (1986) [22], Kumari and Bir (1990) [10] and Agbagwa (2005, 2011) [2]. The somatic chromosome number was found similar in both red and white seeded plants. Agbagwa (2011) [2] analyzed detail karyotype of an African red seeded *A. precatorius* and reported 4 metacentric, 4 submetacentric and 3 acrocentric pairs in the diploid complement with small size chromosomes (range: 1.00 µm to 0.04 µm). In our experiment the diploid complement of white seeded *A. precatorius* was clearly dominated by metacentric and near-metacentric chromosomes and the length of chromosomes was comparatively larger. The number of acrocentric chromosom was two pairs in the diploid complement of white seeded plant but it was three pairs in African red seeded plant. Therefore, the karyotype of red seeded plant was more asymmetric than that of white seeded ones. Further studies on karyotype of red seeded plants of Bangladesh will illuminate the overall evolutionary trends in red and white seeded *A. precatorius*.

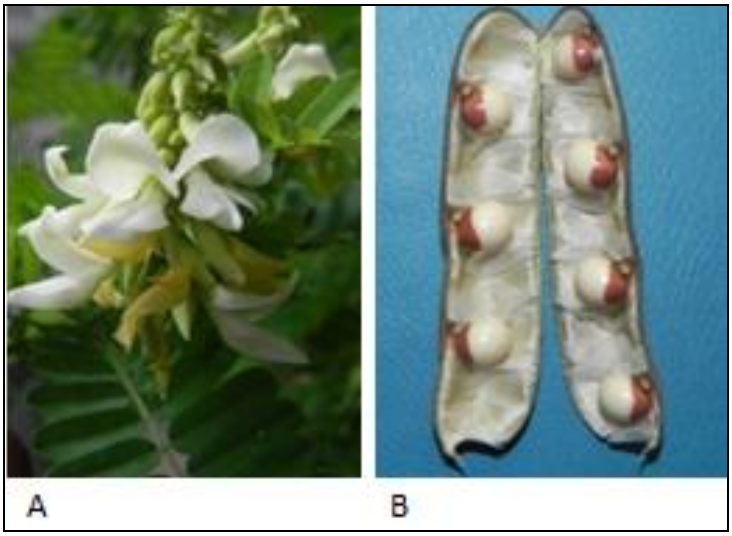

Fig 1: Photographs of flower and seed morphology of white seeded *A. precatorius*. a. Flowers in inflorescence. b. Splited pod bearing mature seeds (white seeds with brown spot at hilum).

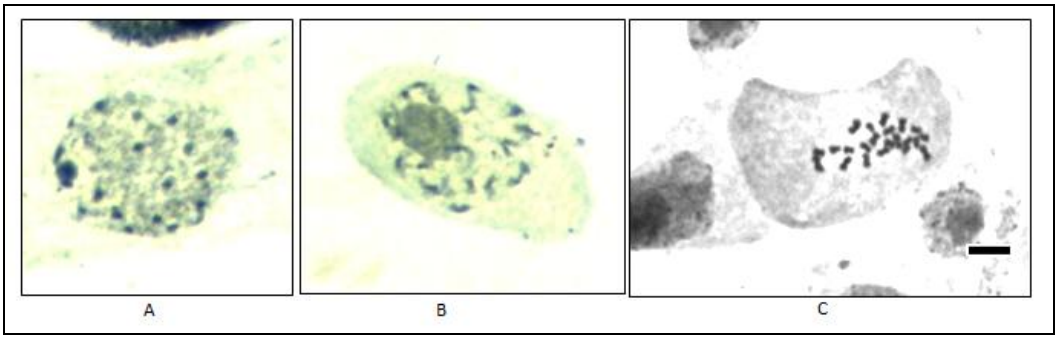

Fig 2: Photomicrographs of resting nucleus, somatic prophase and metaphase chromosomes of white seeded *A. precatorius*. a: Simple chromocenter type resting nucleus, b: Proximal type mitotic prophase chromosomes, c: Somatic metaphase chromosomes. Bar = 5µm.

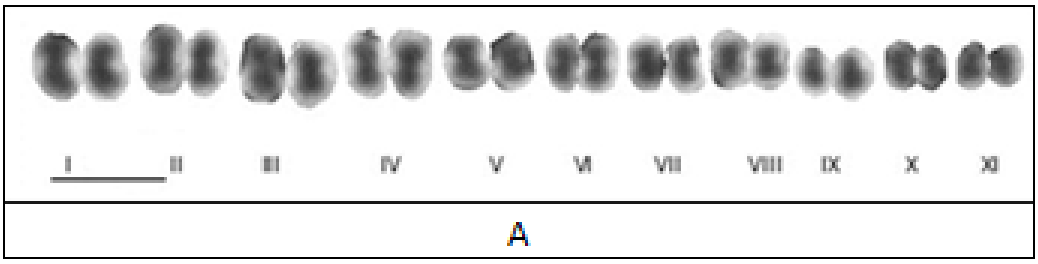

Fig 3a: Idiogram of haploid complement of white seeded *A. precatorius.* Bar = 5µm.

Acknowledgements
We gratefully acknowledge the financial support provided by the Ministry of Science and Technology, People's Republic of Bangladesh (Project No. 05/251/2013-2014).

References

1. Acharya D. Medicinal plants for curing common ailments in India. Pos. Health. 2004; 102:28-30.
2. Agbagwa IO. Karyotype analysis on two *Abrus* Adanson Papilionaceae species in Nigeria. Int. J. Bot. 2011; 7(1):118-121.
3. Anam EM. Anti-inflamatory activity of compounds isolated from aerial parts of *Abrus precatorius* Fabaceae. Phytomedicine. 2001; 8:24-27.
4. Biswas KP, Ghosh E. Bharatya Banaoshadhi in Bengali, Calcutta University Press, 48, Hazra Road, Bullygunge, Calcutta. 1973; 2:298-300.
5. Fedrovo AA. Chromosome numbers of flowering plants. Academy of Sciences of USSR, Moscow. 1969; 926.
6. Garaniya N, Bapodra A. Ethno botanical and Phytophrmacological potential of *Abrus precatorius* L.: A review. Asi. Pac. J. Trop. Biomed. 2014, 27-34.
7. Gill LS, Husaini SWH. Cytological observation in Leguminosae from Southern Nigeria. Willdenowia. 1986; 15:521-527.
8. Hossen R, Sultana SS, Alam SS. Cyto-Molecular Characterization of Three *Tacca* Species from Bangladesh. Cytologia. 2016; 81(4):371-377.

9. Huziwara Y. Karyotype analysis in some genera of Compositae VIII. Further studies on the chromosomes of Aster. Amer. J. Bot. 1962; 49:116-119.
10. Kumari S, Bir SI. Karyomorphological evolution in Papilionaceae. J. Cytol. Genet. 1990; 25:173-219.
11. Levan A, Fregda K, Sandber AA. Nomenclature on centromeric position on chromosomes. Heredities. 1964; 52:201-220.
12. Maurat E, Deniz Y, Zeki A. Morphology, pollen, seed structure and karyological study on *Astragalus ovalis* Boiss. And Balansa Sect.Ammodendron in Turkey. Intl. J. Bot. 2005; 1:74-78.
13. Moshi MJ, Kagashe GA, Mbwambo ZH. Plants used to treat epilepsy by Tanzanian traditional healers. J. Ethnopharmacol. 2005; 97:327-336.
14. Naderuzzaman ATM. *Abrus*. In: Ahmed ZU, Begum ZNT, Hasan MA, Khonder M, Kabir SMH, Ahmed M, Ahmed ATA, Rahman AKA, Haque EU eds. Encyclopedia of Flora and Fauna of Bangladesh, Vol.8, Angiosperm: Dicotyledons Fabaceae-*Abrus*. Asiatic Society of Bangladesh, Dhaka. 2009, 1-3.
15. Parthyusha P, Subramanian MS, Sivakumar R. Pharmacognostical studies on white and red forms of *Abrus precatorius* Linn. Ind. J. Nat. Prod. Res. 2010; 1 (4):476-480.
16. Paszko B. A critical review and a new proposal of karyotype asymmetry indices. Plant Syst. Evol. 2006; 297: 141-145.
17. Pavlova D. Karyotypes of plants growing on serpentines in Bulgaria. Cariyologia. 2008; 61(3):237-244.
18. Pillay VV, Bhagyanathan PV, Krishnaprasad R, Rajesh RR, Vishnupriya N. Poisoning due to White Seed Variety of *Abrus precatorius*. J. Assoc. Phy. India. 2005; 53:317-319.
19. Samadder T, Nath S, Halder M, Sil B, Roychowdhury D, Sen S, *et al.* Sumita J. Karyotype analysis of three important traditional Indian medicinal plants *Bacopa monnieri*, *Tylophora indica*, and *Withania somnifera*. Nucleus 2012; 55(1):17-20.
20. Stebbins GL. Chromosomal evolution in higher plants. Edward Arnold, London, 1971.
21. Tanaka R. Types of resting nuclei in Orchidaceae. Bot. Mag. Tokyo. 1971; 84:118-122.
22. Yeh MS, Yuasa H, Maekawa F. Chromosome number in the Leguminosae. Sci. Rep. Res. Ins. Evol. Biol. 1986; 3:57-71.
23. Yilmaz A, Esra M, Fatma U, Hasan A. Karyomorphological study on six *Trigonella* L. species Leguminosae in Turkey. Caryologia. 2009; 62:89-94.