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## Karyotype analysis of *Thespesia lampas* (Cav.) Dalz. & Gibs. from Bangladesh

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### Abstract

Somatic karyotype analysis was carried out in *Thespesia lampas* (Cav.) Dalz. & Gibs., a member of the plant family malvaceae. Somatic chromosome number of this species was confirmed to be  $2n=26$ . Individual chromosome length was ranged from 1.88  $\mu\text{m}$  to 1.00  $\mu\text{m}$  and total length of the haploid complement was 18.96  $\mu\text{m}$ . According to Stebbins classification (1971) the karyotype was 2B symmetric type and TF% was 35.89. The centromeric formula formula of haploid complement was  $1m+6nm+5sm+1s$ .

**Keywords:** *Thespesia lampas*, Chromosome number, Karyotype analysis, Bangladesh

### Introduction

Malvaceae is one of the most important families among angiosperms consisting of 82 genera and 1,500 species (Essiott and Iwok, 2014). The genus *Thespesia* Sol. Ex Corr. represented by 17 species distributed throughout the tropical East Africa, Asia and Australia. In Bangladesh, it occurs in the sal forest of Mymensingh and Gazipur district and Chittagong hill tracts (Rahman, 2009) [5]. Two species of *Thespesia* have been reported from Bangladesh namely, *T. populnea* and *T. lampus* (Rahman, 2009) [5]. The taxonomic position of *T. lampus* (synonyms: *Hibiscus lampus* Cav.; *H. callosus* Blume; *Azanza lampus* (Cav.) Alefeld) within Malvaceae is undecided and needs better investigation. It is closely related to section *Azanza* DC. in the genus *Hibiscus* L.. *Thespesia lampas* is highly variable in leaf form and hairiness and many subdivisions have been proposed based on this characters. Karyotype analysis has been used for resolving the taxonomic and evolutionary tribulations in many plant species (Samaddar *et al.* 2012; Young *et al.*, 2012) [7, 6]. In this article chromosome karyotype of *T. lampus* is described in detail.

### Material and Methods

Mature seeds were collected from Bangladesh Forest research Institute (BFRI), Chittagong and planted in the medicinal plant conservatorium, Department of Botany, University of Chittagong. Morphology of seeds of this plant is shown in Figure -1A.

Mitotic metaphase was examined from germinated root tip cells. First growing healthy roots were pretreated with saturated solution of Para-dichlorobenzene (PDB) for 3 h in room temperature. After pre-fixation the roots were fixed in 1:3 (v/v) Carnoy's solution for 24 hours at 4 °C. The roots were then preserved in 70% (v/v) alcohol for long time preservation at 4 °C in refrigerator. Roots were hydrolyzed in 1 N HCl 10 seconds at 60 °C. After thorough washing, roots were immersed into 2% (w/v) aqueous solution of iron alum for 5-10 minutes. Then the roots were further washed with distilled water for 3-4 times. Before squashed in a drop of 0.5% (w/v) acetocarmine, roots were stained in 0.5% (w/v) aqueous solution of haematoxylin for 15-20 minutes. The prepared slides were then examined under a Optica Vision 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.

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

Chromosomes were measured based on five well separated metaphase cells. Classification of chromosomes was done following the nomenclature of Levan *et al.*, (1964) [4].

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## Results and Discussion

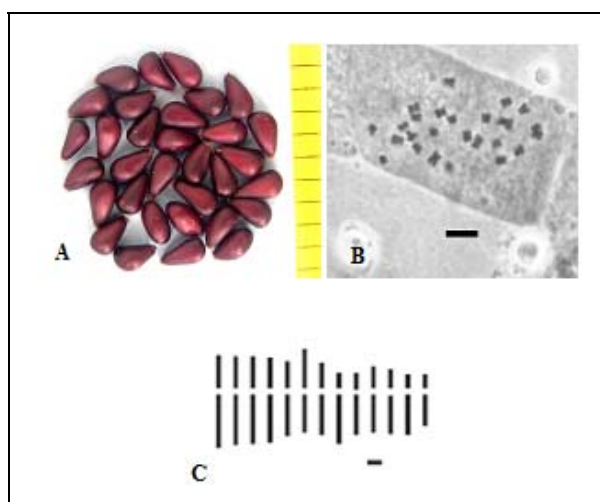
The somatic chromosome number of this plant was found to be  $2n=26$  (Fig. 1B). The karyotype consists of thirteen chromosome pairs. Of the thirteen basic chromosomes 1 was metacentric, 6 near metacentric, 5 sub-metacentric and 1 was sub-telocentric. Length of chromosomes ranged from  $1.00\mu\text{m}$  to  $1.88\mu\text{m}$ . Arm ratio was measured as 1.00 to 3.18. Centromeric indices varied between 23.91 to 50. TF% was 35.97. Total length of the haploid complement was  $18.96\mu\text{m}$ . None of the chromosomes had secondary constrictions (Fig. 1C). 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 0.61. Thus the karyotype was 2B symmetric type.

Previous chromosome count and karyotype study of *Azanza lampua* Cav. (Syn. *H. lampus* Cav.) was carried out by Bhatt and Dasgupta (1976)<sup>[1]</sup> and confirmed the somatic number  $2n$

= 28. They reported haploid chromosome complement with 10 pairs of sub-median 3 pairs of sub-terminal and 1 pair with median centromere. The complement was devoid of satellite chromosome. The total length of the chromosome complement was  $59.40\mu\text{m}$  and ranged from  $3.06\mu\text{m}$  to  $1.44\mu\text{m}$ . Rahman (2009)<sup>[5]</sup> however, mentioned the chromosome number of *Thespesia lampas* (Cav.) Dalz. & Gibs. (syn. *Azanza lampua* Cav.) to be  $2n=26$ . In the present investigation, the chromosome count of *T. lampus* was confirmed to be  $2n=26$ . According to centromeric position the thirteen basic chromosomes were categorized into 1 metacentric, 6 near metacentric, 5 sub-metacentric and 1 sub-telocentric. None of the chromosomes had secondary constrictions. Length of chromosomes ranged from  $1.00\mu\text{m}$  to  $1.88\mu\text{m}$ . Total length of the haploid complement was  $18.96\mu\text{m}$ . TF% was 35.97 indicated less symmetrical karyotype.

**Table 1:** Karyomorphological analysis of *T. lampus* (Cav.) Dalz. & Gibs.

Chromosome pairs	Mean long arm ( $\mu\text{m}$ ) (q)	Mean short arm ( $\mu\text{m}$ ) (p)	Total chromosome Length ( $\mu\text{m}$ )(p+q)	Centromeric index	Arm ratio	Centromeric type
1	1.15	0.73	1.88	38.83	1.58	nm
2	1.06	0.68	1.74	39.08	1.56	nm
3	1.05	0.68	1.73	39.31	1.54	nm
4	1.11	0.55	1.66	33.13	2.02	sm
5	0.90	0.71	1.61	44.10	1.27	nm
6	0.78	0.78	1.56	50.00	1.00	m
7	0.93	0.52	1.45	35.86	1.79	nm
8	1.05	0.33	1.38	23.91	3.18	st
9	0.93	0.34	1.27	26.77	2.74	sm
10	0.76	0.49	1.25	39.20	1.55	nm
11	0.81	0.44	1.25	35.20	1.84	sm
12	0.88	0.30	1.18	25.42	2.93	sm
13	0.73	0.27	1.00	27.00	2.70	sm



**Fig 1:** *Thespesia lampas* (Cav.) Dalz. & Gibs (A) Seed morphology, (B) Somatic chromosome (scale bar:  $5\mu\text{m}$ ), (C) Idiogram of genomic chromosome (scale bar:  $0.3\mu\text{m}$ )

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