

Karyomorphological Studies in Two Species of *Bauhinia* Linn. and Induction of Polyploidy in *Bauhinia acuminata* Linn.

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Received: 15 March 2017/Revised: 26 May 2017/Accepted: 21 June 2017

ABSTRACT- Karyomorphological studies in *Bauhinia acuminata* Linn. and *Bauhinia variegata* Linn. belonging to family *Caesalpinaceae* using leaf tip squash technique and induction of polyploidy in *Bauhinia acuminata* Linn. was carried out. It was observed that in the Karyomorphological studies, both the species have the same number of chromosomes, i.e., $2n=28$ with chromosome length showing symmetric karyotype, with largely submetacentric and metacentric chromosomes. On induction of polyploidy in *B. acuminata* Linn. the result showed that the maximum induction of polyploidy was obtained in the case of seeds treated with 0.10% concentration of colchicine for consecutive 3 days. Karyotype analysis of the induced polyploidy revealed that the chromosome number was obtained $4n=56$, tetraploid. The significance of polyploidy showed, increase in plant height, leaf size and stomata size of the plant under investigation.

Key-words- Karyomorphology, Induction of Polyploidy, Colchicine, Tetraploid

INTRODUCTION

Genus *Bauhinia* has great medicinal value and is the largest genera of the tribe Bauhinieae in the family *Caesalpinaceae* under De Wit's revised system of classification, 1956 which represents more than 300 species. *Bauhinia* genus consisting of trees, climbers and shrubs are distributed in India^[1] of which 12 species are being reported from North Eastern regions of India^[2]. The simple bilobed leaf is an outstanding character of this genus principally responsible for its separate tribal status. This character is not only conspicuous but is universally present in all the species with slight variations in the extent of lobation. Taxonomically it is regarded as a uniform genus by all taxonomists regarding it as a natural assemblage.

In this study, two closely related species of *Bauhinia* of the same family viz., *B. acuminata*- Linn. and *B. variegata* Linn. were selected. Both the plants are ethnomedicinally important plants of Assam^[2].

B. acuminata Linn. commonly called as Mati-katota in

Assamese is not so common species and found in Rani Reserve- Kamrup, North Cachar Hills-North Range, Bhumeswar Hill-Goalpara and often cultivated in gardens of various parts of Assam, India. Commonly known as Dwarf White Orchid Tree is widely cultivated for its ornamental value. These species occur widely in deciduous forest and scrub. Leaves are bilobed, shaped like an ox or cow hoof; long and broad, with the apical cleft. Flowers are fragrant, with five white petals, ten yellow tipped stamens and a green stigma. Fruit is a pod 7.5 to 15 cm long and 1.5 to 1.8 cm broad. Perfect little tree for places, where we don't want anything wild to take over^[3-5].

B. variegata Linn. commonly known as (orchid tree, camel's foot, mountain ebony, Napoleon's hat, paper mulberry, poor man's orchid, bauhinia); locally called Bogakatra, Kurol, Kotora in Assam. Throughout the province, ascending to 2500ft in the Khasi Hills, more common in hilly regions. *B. variegata* Linn. is small to the medium-sized deciduous erect tree. Leaves as broad as long or sometimes broader, usually deeply cordate. Flower clusters are unbranched at ends of twigs, buds not angled, fertile stamens 5. They are capable of growing in a wide range of soils from gravelly, shallow, rocky soil on the hill slopes to sandy loam and loamy soil in the valley.

Leaves make good fodder and eaten by sheep, goats, and cattle. The main uses of *Bauhinia* plant are as fuel calorific value is 4800 kcal/kg^[6]. The mature seeds and young pods of *Bauhinia* are eaten, cooked and pickled in the native

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DOI: 10.21276/ijlssr.2017.3.4.20

countries. The *Bauhinia* leaves extract is being used for medicinal purposes including anti-inflammatory, antifungal, antipyretic, analgesic, antispasmodic, antitumor and antimicrobial activities [7]. The stems, roots, and leaves are also used for the treatment of several diseases, especially in pain, diabetes, infections, ulcer, jaundice, leprosy and also utilized as folk medicines [8]. Phytochemical study of the bark extract revealed the presence of flavonoids which have anticarcinogenic activity. The plant extract of *Bauhinia variegata* Linn. due to the presence of β -sitosterol exhibited a significant hypolipidemic effect, reduced the obesity as well as decreased the levels of cholesterol, triglyceride, VLDL cholesterol (lipid profile) [9]. Lectins (glycol proteins) from *Bauhinia* seeds have been reported to possess antitumor activity [10]. The *Bauhinia* seeds are known to be a good source of protein, vitamin A, and minerals. It also contains a significant amount of oil as compared to soya bean and cotton seeds nutritionally viable for human health [11].

Bauhinia is a plant of great economic importance. Since, some anticarcinogenic properties have been found especially in *B. variegata* Linn. thus this species serves as a raw material for lead molecules in anti-carcinogenic drug development. As such, it needs conservation and detailed chromosome study would aid in creating germplasm.

MATERIALS AND METHODS

This study was conducted in the Department of Botany, Handique Girls' College, Guwahati (Assam), India in the duration of six months from January- June 2016. Seeds of *Bauhinia acuminata* Linn. were collected from the Handique Girls' College Campus, Guwahati, Assam and the seeds of *Bauhinia variegata* Linn. were collected from Amsoi Hills, Nagaon, Assam and were raised under the department botanical garden.

Method of Cytological investigation

For detail karyotypic studies, karyotypes were prepared from the somatic chromosomes. For cytological studies, leaf tip squash technique was done. Very young leaves were collected from the *B. acuminata* Linn. and *B. variegata* Linn. between 7:00 a.m. to 7:30 a.m.; washed in double distilled water and pre-treated with saturated solution of paradichlorobenzene at a suitable temperature for 3 hours at $4^{\circ}\text{C}\pm 2^{\circ}\text{C}$. Pre-treated young leaves were fixed in a suitable fixative such as Carnoy's Fluid-II (1:3:6; Glacial acetic acid: Chloroform: Ethanol) for 24 hours to 28 hours at room temperature. After fixation, the leaves were thoroughly washed with 70% ethanol and finally, they were stored and used for cytological work. For the preparation of slides of leaves were firstly stained with acetic orcein: nHCL (9:1) mixture and warmed over the flame for 10–15 mins and kept for 2–3 hours at room temperature. Single leaf tip was taken on a drop of 45% acetic acid on a slide. Only the dividing tip region was taken discarding the other tissue. Coverslip was placed over the tip and squashed by

applying uniform pressure with the thumb through a piece of blotting paper, a gentle tapping is done with matchstick after passing it lightly over the flame and finally sealed with paraffin for further studies [12]. The temporary slides thus prepared were observed under the compound microscope at a magnification of 1000x using oil immersion (10 \times 100x, oil immersion). This procedure was standardized through trial and error method.

Well, scattered metaphase plates were selected for karyomorphological analysis of the chromosomes. Perfectly stained chromosomes were photographed using trinocular microscope N400-M, CMOS camera with an image analysis system. The drawings of the chromosomes were made with the help of camera lucida apparatus. Idiograms were then constructed on tracing paper.

Following parameters of the chromosomes were considered:

(i) Length of the long arm (ii) Length of the short arm (iii) Total length of the chromosome (iv) Volume of the chromosome (v) Relative length and (vi) Centromeric position.

On the basis of the length, the chromosomes were categorized under the following type.

Type A= 3.00 μm and above, Type B= 2.50 μm to 2.90 μm , Type C= 2.00 μm to 2.49 μm , Type D= 1.00 μm to 1.90 μm , Type E= 0.01 μm to 0.99 μm .

The volume of an individual chromosome was calculated as Chromosome Volume (V) = $\pi r^2 h$.

Where, r= radius of the chromosome h= length of the whole chromosome. The total chromosome volume was then expressed by adding the volumes of all the chromosomes of the complement [17].

Induction of Polyploidy in *B. acuminata* Linn.

Induction of polyploidy was undertaken in *B. acuminata* Linn. using colchicine as the polyploidizing agent. For this purpose, four different concentrations as 0.05%, 0.1%, 0.25%, 0.50% were used. For preparing each of these concentrations 0.05 gm, 0.1 gm, 0.25 and 0.50gm colchicine powder were dissolved in 100cc of double distilled water respectively.

For induction of polyploidy in *B. acuminata* Linn. seeds were treated with colchicine solutions. For this purpose, mature seeds were collected and properly dried prior to colchicine treatment. These seeds were soaked in double distilled water for 6 hours. The pre-soaked seeds were used in each treatment. Treatments were started from 8 a.m. in the morning to colchicine with the chromosome divisional cycle. The seeds were treated with 0.05%, 0.1%, 0.25% and 0.50% colchicine solution for 3 consecutive days with 12 hours interval. After treatment, the seeds were washed thoroughly with double distilled water to eliminate the residual effects of the chemical if any. Treated seeds and stem cuttings were then planted in earthen pots filled with a mixture of well-prepared soil, farmyard manures and

sand in the ratio of 3:1:1. Root and shoot initiation started after 8 and 15 days of treatment respectively. Plants are risen, where tested morphologically as well as cytologically for polyploidy. Cytological assays comprise of chromosome count in the root meristematic cell. Root tip squash was prepared and Karyomorphological analysis was done. Morphological assay for induced polyploidy plant height, leaves size, petiole length, internode length and size of stomata its frequency. These parameters were studied in the treated plants, when they become 45 days and elder and were compared with those of normal diploid plants.

For the study of the stomata, epidermal peels of the lower epidermis of the leaves were taken out by mechanical stripping stained with safranin and finally mounted in 4% glycine by taking in a clean slide. The slide, thus prepared was observed under the compound microscope (10× 40x). The number of stomata and epidermal cells was counted. The stomatal frequency and stomatal index were calculated using the following formula.

$$\text{Stomatal Frequency (S.F)} = \frac{S}{E} \times 100$$

$$\text{Stomatal Index (S.I)} = \frac{S}{E+S} \times 100$$

where, S=Number of stomata

E= Number of the epidermal cell

The length and breadth of stomata were measured with an ocular micrometer (10×40x magnification). Lower epidermal peels were microphotograph using trinocular microscope-N400-M, CMOS camera 5M with image analysis system.

RESULTS

Cytological and karyotypic investigations in *B. acuminata* Linn. and *B. variegata* Linn. were showed that both the species consist of $2n=28$ chromosomes (Table 1). On the basis of the length the chromosomes, it was observed that

B. acuminata Linn. six chromosomes belong to type A and twenty-two chromosomes belong to type B and the chromosome formula can be written as: $A_6 + B_{22} + C_0 + D_0 + E_0 = 2n = 28$. Depending on the position of their centromere, the karyotypic formula can be written as $M_6 + Sm_{22} = 28$. However, in *B. variegata* Linn., it was observed that twenty-four chromosomes belong to type D and four chromosomes to type E. So the chromosome formula can be written as: $A_0 + B_0 + C_0 + D_{24} + E_4 = 2n = 28$ and karyotypic formula can be written as $M_4 + Sm_{24} = 28$.

Induction of polyploidy in *Bauhinia acuminata* Linn. revealed, all the concentrations of the colchicine was not equally effective in inducing polyploidy. Colchicine solution having concentration 0.10% was found to be most effective. Karyomorphological analysis of the induced polyploidy revealed the chromosome number to be 56 while in the case of the diploid the somatic chromosome number was found to be 28 (Table 1). Out of these 56 chromosomes, a notable difference between the longest and the shortest chromosome was also seen. A general increase in the chromosome size was noticed when compared with those in the diploid. The length of the 56 chromosomes was measured and found to vary from 0.70 μm to 2.4 μm whereas, those in the case of the diploid varied from 1.00 μm to 1.5 μm . It has been observed in induced polyploidy that since a lot of chromosomes are present, depending upon their length the chromosomes. Four chromosomes were categorized under type C, a total of fifty chromosomes were observed under type D out of which forty-six chromosomes were categorized under subtype d' and six chromosomes were under subtype d'' and two chromosomes are categorized under type E. Based on this the chromosome formula and karyotypic formula can be written as: $C_4 + D_{50}(d'_{42} + d''_8) + E_2 = 4n = 56$ and $M_4 + Sm_{50} + St_2 = 4n = 56$ respectively.

The microphotograph, camera lucida diagram, karyotype and idiogram of the chromosomes are represented in Fig. 1 (A), (B), (C), and (D) respectively.



(a)

(b)

(c)

(A)

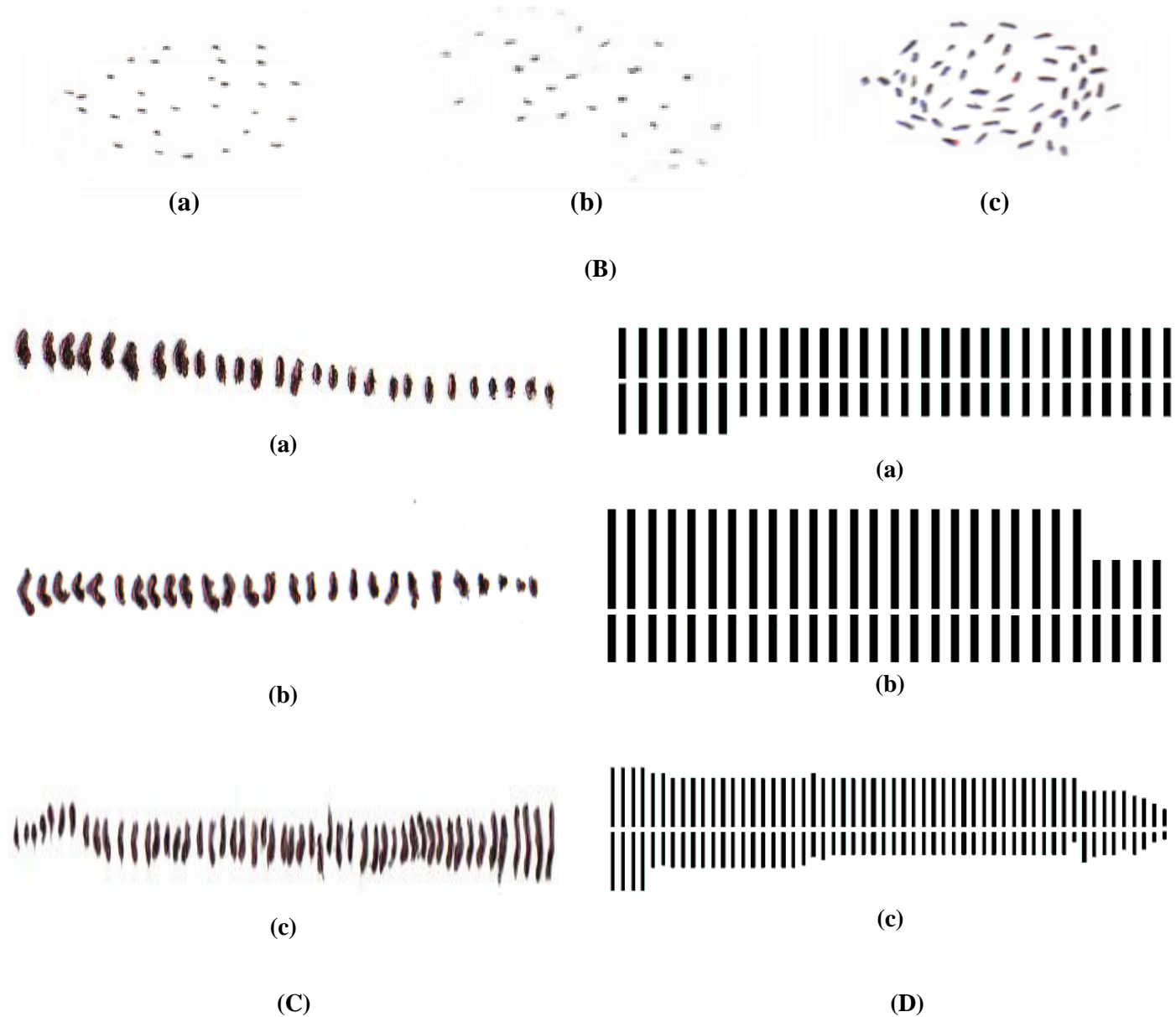


Fig. 1: (A) Microphotograph of somatic chromosomes at metaphase stage (15× 100x oil immersion); (B) Camera lucida diagram of somatic chromosomes at metaphase stage (10 × 100x oil immersion); (C) Karyotype; (D) Idiogram (a) *B. acuminata* (b) *B. variegata* (c) Induced polyploidy in *B. acuminata*

Table 1: Summary of the karyotype in the normal diploid (2n) and induced tetraploid (4n) of *B. acuminata* Linn.

Taxa	Chromosome No. (2n)	Range of chromosome					Types of chromosome	Karyotype formula
		Length	Relative length	Radius	Volume	Arm ratio		
<i>B. acuminata</i> Linn.	28	1.00–1.50	50.00–60.00	0.40	0.50–0.90	1.00–1.50	A ₆ +B ₂₂	M ₆ +Sm ₂₂ 2n=28
<i>B. acuminata</i> Induced polyploidy	56	0.70–2.4	51.00–80.00	0.50	1.25–1.50	1.00–4.00	C ₄ +D ₅₀ (d' ₄₂ +d'' ₈)+E ₂	M ₄ + Sm ₅₀ + St ₂ =4n=56

DISCUSSION

A detailed survey was undertaken to ascertain the uses of these plants for treatment of various by the ethnic people of North Eastern Region of India. Both the plants are ethno-medicinally important plants of Assam^[2]. In the present investigation, it has been found that both the species are grown in different parts of the North Eastern regions of India and are variously used by the ethnic people of the region. The flower is used to cure diarrhea and the leaves and flowers are eaten in North-Eastern regions of the country^[8]. Naga uses the plant as an antidote to certain toxins and poisons^[13]. *B. acuminata* Linn. had high SPF value with antioxidant, antibacterial property and can be used as an efficient agent for UV radiation hazards^[14]. Contains a significant amount of oil as compared to soya bean and cotton seeds nutritionally viable for human health^[11]. Lectins (glycol proteins) from *Bauhinia* seeds have been reported to possess antitumor activity^[10]. The presence of flavonoids (anticarcinogenic activity)^[15]. Anti-inflammatory, anti-fungal, anti-pyretic, analgesic, anti-spasmodic, anti-tumor and antimicrobial activities^[7]. Good source of protein, vitamin A and minerals^[11]. Keeping these important medicinal aspects in mind, commercial cultivation of such remarkable and useful plants as a raw material for the development of new drug formulations in the treatment of life-saving ailments in the near future may not be ruled out.

Such a significant plant deserves greater attention, conservation, and protection.

Karyomorphology

The importance of karyotype analysis in distinguishing plant species is well known. Karyomorphology and chromosome number of a variety or species are useful in its identification as also in establishing the evolutionary relationships among related species^[16]. Karyotype suggests primitive and advanced features. In the present investigation, a comparison has been made between the karyotypes of two species of *Bauhinia* Linn. through leaf tip squash method may, therefore, be considered as the first attempt through this technique to study the detailed cytological aspects.

In both *B. acuminata* and *B. variegata* the chromosome number is constant with $2n=28$ and chromosome morphology is grossly identical in all cells. The cytological study reveals that the karyotypes are symmetric which signifies primitive characters. A gross resemblance in the nature of the karyotype in rather short chromosomes with gradation in size but with no abrupt size difference in the complements. A detailed graphical representation summarising the complete karyotypic analysis, shown in Fig. 2.

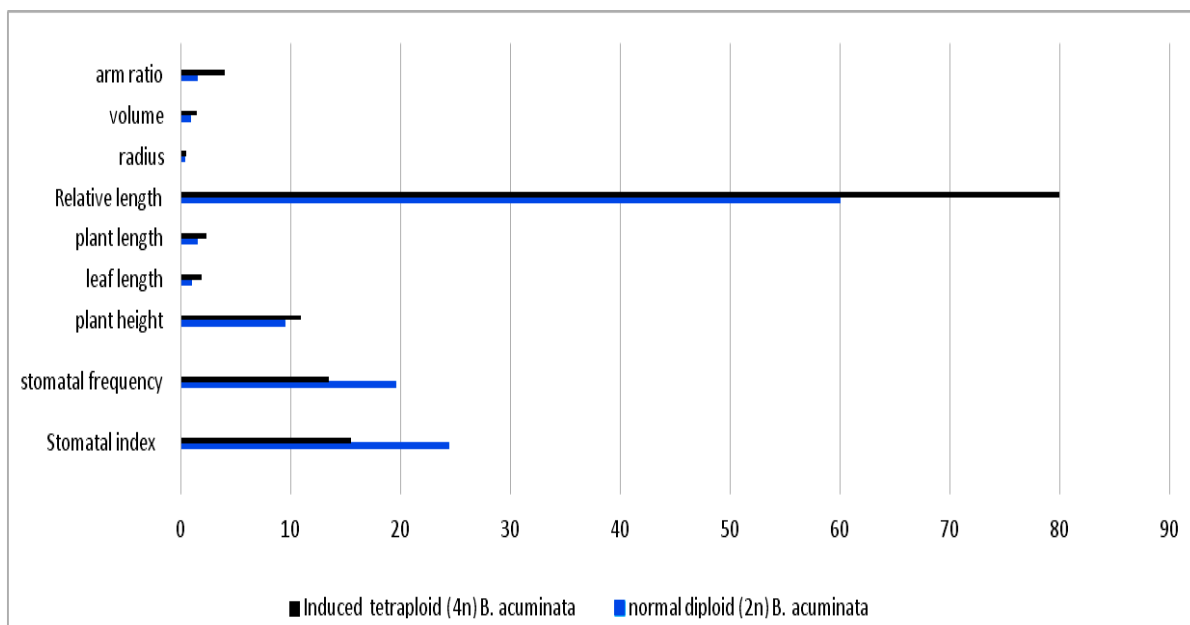


Fig. 2: Graphical representation of the comparative analysis in the normal (2n) diploid and induced (4n) tetraploid in *B. acuminata* Linn.

Polyploidy

Karyotype studies on induced tetraploid revealed the somatic chromosome number to be $4n=56$ whereas, in the normal diploid plant it was found to be $2n=28$. A general decrease in the chromosome size was noticed when compared with those in the diploid. The doubling of the chromosomes produced significant increases in the plant height, leaf size and stomata size (Fig. 3). The findings of

the present study revealed that the ploidy level greatly influenced the size of the stomata. The significant reduction in the stomatal frequency/sq.mm observed in these polyploidy plants is indicative of the consequence of stomatal expansion caused by an increase in their sizes (Table 2).

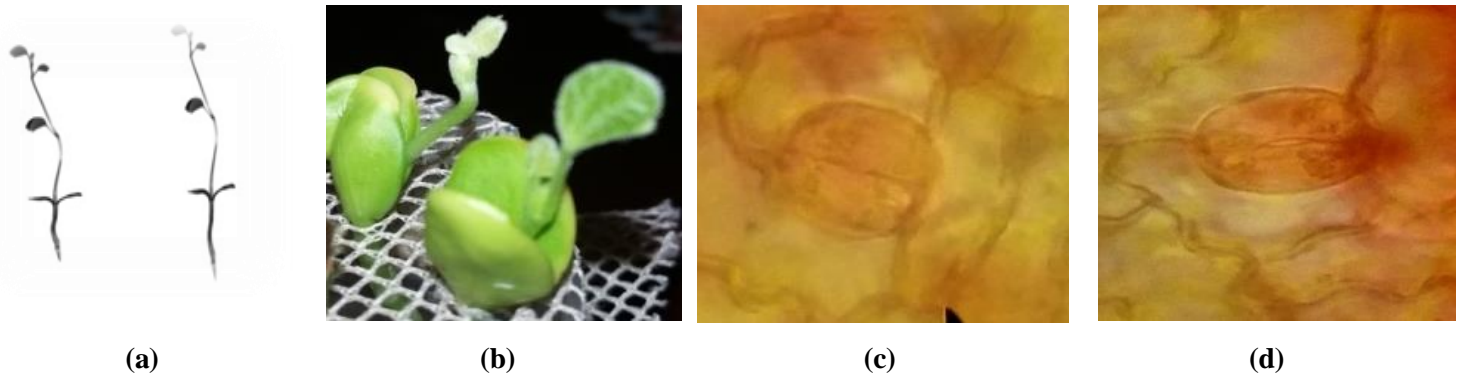


Fig. 3: Morphology of plant, leaves and stomata in normal diploid (2n) and induced tetraploid (4n) in *B. acuminata* Linn.

(a) Photograph showing plant height in normal (2n) and induced tetraploid (4n)

(b) Photograph showing leaf size in normal (2n) and induced tetraploid (4n) seedling after 8-10 days of germination

(c) Photograph showing stomata in normal diploid (2n)

(d) Photograph showing stomata in induced tetraploid (4n)

Table 2: Details of stomatal characters in normal diploid (2n) and induced tetraploid (4n) of *Bauhinia acuminata* Linn.

Ploidy level	Stomatal index	Frequency of stomata/sq.mm	Length of stomata (L) (μm)	Breadth of stomata (B) (μm)	Area of stomata (L×B) (sq. μm)
Diploid	24.44	19.64	10.30±0.25	7.85 ± 0.04	10.30 × 7.85
Polyploid	15.55	13.46	22.50±0.23	16.50 ± 0.20	22.50 × 16.50

CONCLUSIONS

This study represented a detailed karyomorphological study undertaken in *B. acuminata* Linn. and *B. variegata* Linn. for their genetic improvement. Concerning their importance and utility in the production of flavonoids, lectins, proteins, vitamin A, minerals and a significant amount of oil as compared to soya bean and cotton seeds, *ex-situ* conservation measures should be initiated for protection, preservation, and regeneration of such remarkable medicinal plants. Further investigations into the chemical constituents of these plants may definitely lead to the development of new drugs. Induction of polyploidy increases induction of polyploidy increases the number of chromosomes and usually brings about an increase in the size of the affected cells and various degrees of changes in their functions. These polyploids can be useful for breeding programs and production of higher yields of secondary metabolites like flavonoids for economic, medicinal purpose and for driving evolutionary processes.

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**How to cite this article:**

Basumatari M, Das BN: Karyomorphological Studies in Two Species of *Bauhinia* Linn. and Induction of Polyploidy in *Bauhinia acuminata* Linn. Int. J. Life Sci. Scienti. Res., 2017; 3(4):1223-1229. DOI:10.21276/ijlssr.2017.3.4.20

Source of Financial Support: Nil, **Conflict of interest:** Nil