INVESTIGATION OF *BAUHINIA BLAKEANA* DUNN. A HYBRID SPECIES

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ABSTRACT

Bauhinia blakeana Dunn. is a hybrid belonging to Family Caesalpiniaceae, tribe Cercideae. Studies of Morphological characters, cytological characters and phytochemical screening of leaf aterials of *B. blakeana* and it sparental species have been carried out. The putative parental species shared many morphological similarities with *B. blakeana.* Average chiasmata per bivalent were intermediate between *B. purpurea* and *B. variegata.* Two common flavonols viz. kaempferol, quercetin (Quercetin-3-rhamnosides) were detected in all the species while isorhamnetin was detected in *B. blakeana* and *B. purpurea.* Apigenin was present in all the species. Flavonols (spots 7, 18) were common in *B. blakeana* and *B. purpurea* and (spot 5) in *B. blakeana* and *B. variegate* while (spot 10) was common in *B. purpurea* and *B. variegata.* Results of the present study showed complementation of their putative parental species.

Key Words: *Bauhinia* species, Phytochemicals, Chromatography, flavonoids, Meiosis

INTRODUCTION

The species *Bauhinia blakeana* Dunn.was first described by the father of French Mission in 1908. They discovered this beautiful ornamental tree at Pok Fu Lam, Hong Kong. Since 1865, it has been used as Hong Kong's floral emblem. It is sterile and considered as a hybrid (Lau Ramsden and Saunders, 2005). This sterility had led to the suggestion that it is probably of hybrid origin with *B. purpurea* L. and *B. variegata* L. as the most likely candidates as parental species. The putative parental species share many morphological similarities with *B. blakeana* including pollen morphology (Larsen, 1975), chromosome counts of n=14 and 2n=28 have furthermore been reported for all three species (Sharma and Raju, 1968; Choudhary and Choudhary, 1988; Kumari and Bir, 1989).

Morphologically distinct species having the same number and similar chromosomal behavior may not completely normal meiotic pairing due to intra-chromosomal alterations. Behavior of chromosomes during meiosis provides an account for the better understanding of inter-relationship among species.

The role of biochemical systematic in the study of hybridizing population and the analysis of past hybridization and introgression was demonstrated by Alston and Turner (1963), Smith and Levin (1963), Torres and Levin (1964) and Garber and Strommaes (1965). The chemical work on hybridization was well reviewed by Harborne and Turner (1984). Heywood (1976) indicated that the chromatographic pattern of flavonoids has proved extremely valuable in the analysis of hybridization for example in *Baptisia* and *Asplenium* (fern). The application of flavonoids in taxonomic study has been reported in Leguminosae (Alston and Turner, 1963). Harborne (1973) indicated that flavonoids can be used as taxonomic markers because they posses structural variability, chemical stability, widespread distribution in the plant kingdom and easy and rapid identification.

The present study aims to determine the hybrid origin and the parentage of *B. blakeana.* A range of approaches are adopted, including morphological variation, meiotic study and phytochemical study of flavonoids.

MATERIALS AND METHODS

Frequent field trips had been made to survey the area for collecting plant materials. Identification of each species from fresh materials was done with the help of standard floras and monograph. Morphological and floral characters were studied.

For meiotic studies young flower buds of selected three species were fixed between 9.30 A.M to 10.30 A.M in 1:3 aceto-alcohol then they were stored under refrigeration. Few drops of $FeCl_3sol^n$ was used as mordant. The buds were pricked by needle for easy penetration of fixative. Before staining the materials were washed with 70% ethanol. Anthers from the bud were warmed in 2% acetocarmine for 20-25 minutes. Then anthers were taken on slides and squashed with 2% acetocarmine stain. Prepared slides were sealed with paraffin wax and used for meiotic studies.

For flavonoids studies, 5 grams of mature and healthy leaves were collected from each species. After air drying, the phenolic compounds were extracted from these leaves at room temperature in different solvents like 70% Ethanol, Petroleum Ether, Acetone and Methanol using standard procedures. Isolation and purification of the compounds were done by repeated chromatography using Whatman 3 mm chromatography paper. Characterization of compound was carried out following standard technique (Markham, 1982). The purified compounds were taken in ethanol and their UV and visible light spectrum were measured with Spectrophotometer. Further, to know the position of substitution, spectral shifts after the addition of standard diagnostic chemicals were determined.

RESULS AND DISCUSSION

A total of three species of *Bauhinia* have been studied for their patterns of variations in morphological characters. They can be distinguished by their habit, leaf size, shape, number of veins; flower size, petal colour, petals shape, number of fertile stamens, flowering period and fruit sets. *B. purpurea, B. variegate* and *B. blakeana* were trees. *B. variegata* was deciduous when flowering (Bailey, 1941,Little & Wadsworth, 1964). The leaf of *Bauhinia* was quite unique, it was bilobed. The division was one- fourthof their length in *B. purpurea* and one- third of their length in *B. variegata* and *B. blakeana*. Number of leaf veins was 13 in *B. blakeana,* 9-13 in *B. purpurea* and 9-11 in *B. variegata.* Size of leaves differ among these species.

Flower buds showed great diversity among species. Acutely angled buds were present in *B. purpurea,* sharply angled buds were in *B. blakeana* and buds not angled in *B. variegata* were also reported by Smith A.C (1985). Flowers are important distinctive features. From present study, shape, size and color of *B.blakeana* was intermediate between *B. purpurea* and *B. variegata* (Plate 1-3).Flowering periods of three species overlapped.*B. purpurea* flowered from November to January, *B. blakeana* flowered from November to March and *B. variegata* flowered from December to April.

TABLE 1: Meiotic Chromosomes of the *Bauhinia* species

Name of the plant	Number of PMC analyzed	Mean number of bivalents per PMC			Mean number of chiasmata per PMC	Mean number of Terminalised chiasmata	Average chiasmata per bivalent	Coeffcient of terminalization
		Total	Ring	Rod				
B. blakeana	50	14	7.52	6.49	16.90	12.2	1.20	0.72
B. purpurea	50	14	7.58	6.44	16.74	13.2	1.19	0.78
B. variegata	50	14	7.42	6.60	17.07	14.9	1.21	0.87

Meiotic metaphase and diakinesis studies in the present investigation revealed 14 bivalents in all the three species (Plates 4, 7, 8, 9).

Some irregularities such as some chromosomes were off the equator (Plate-5) at metaphase II in *B. blakeana*. At anaphase II several lagging of chromosomes were observed (Plate 6). Mean number of chiasmata per PMC, Average chiasmata per bivalent of *B. blakeana* were shown intermediacy between *B. purpurea* and *B. variegata.* Mean number of ring and rod bivalents were intermediate between two species. Variation in their chromosomes was tabulated in (Table-1).

TABLE 2: Chromatographic and spectral properties of flavonoid spots of *Bauhinia* species

Spots	Flourscence			Solvent System: Rfx100						Spectral Max inEtOH	Identity
	Visible Light	UV	UV+NH_3	BAW	15% AcOH	30% AcOH	Water	Phenol	Forestal		
1	Bl	Bl	Bl	76	51	--	16	34	--	249, 376	Q-3-Rhamnoside
2	Bl	Bl	YG	7	49	--	--	--	--	----	--
3	Nc	Bl	Yl	66	41	--	17	27	39	251, 373	Quercetin
4	Bl	Bl	Yl	18	6	--	--	--	--	----	--
5	Yl	Yl	BY	88	64	--	23	42	49	258, 363	Kaempferol
6	Bl	Bl	Yl	79	--	13	--	--	27		--
7	Bl	Bl	GY	68	10	--	--	26	43	252, 371	Quercetin
8	Yl	GY	GY	74	60	--	13	--	28	224	Glucose?
9	Yl	Yl	Yl	71	44	--	17	--	39	249, 368	Q-3-Rhamnoside
10	Bl	Bl	Yl	49	56	28	--	39	64	251, 372	Q-3-rutinoside
11	Yl	Yl	Yl	15	29	--	--	33	26		--
12	Yl	Yl	Bl	82	65	12	31	49	61	259, 364	Kaempferol
13	Yl	BY	BY	72	64	--	--	39	--	----	--
14	GY	BY	BY	77	47	61	--	69	56	255, 367	Isorhamnetin
15	Nc	B	B	19	7	--	--	--	--	----	--
16	B	B	B	89	33	--	--	92	84	248, 347	Apigenin
17	GY	B	B	86	--	--	28	66	44	----	--
18	RB	B	B	74	47	37	20	31	40	253, 369	Q-3-Rhamnoside
19	GY	B	B	93	--	--	40	89		258, 344	Apigenin
20	BY	R	R	7	59	--	--	--	--		--
21	RB	R	R	36	19	47	--	31	29		--
22	GY	RB	RB	79	45	67	--	71	57	253, 368	Isorhamnetin
23	Nc	RB	BY	33	21	69	--	25	33	----	--

Abbreviation: Nc= Indicates no colour in visible light; (--) = Rf values variable, so not included; (--) = Uncertain chemical identity; BAW = n-butanol, acetic acid, water;AcOH = Aqueous acetic acid; EtOH= Ethanol; TLC= Thin layer chromatography; Bl= Bright yellow; Yl= Yellow; GY= Greenish yellow; RB= Reddish brown; R= Reddish; B= Brown; BY= Blackish yellow.

Twenty four chromatographic spots were identified in this study. They are flavones (spots 16, 19), flavonols (spots 1, 3, 5, 7, 9, 10, 12, 14, 18, 22) and twelve unknown spots (Table-2). Two common flavonols were detected in the taxon studied. They were quercetin and kaempferol.

Plate 1

Plate 2

Plate 3

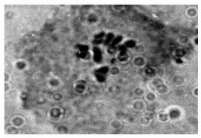

Plate 4

Plate 7

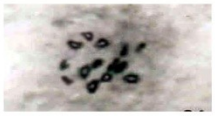

Plate 8

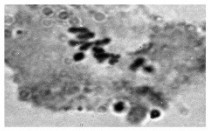

Plate 5

Plate 6

Habit : Plate 1 *B. blakeana*; Plate 2 *B. purpurea*; Plate 3 *B. variegata*

Plate 4,5,6 : Meiotic chromosomes and laggard cell of *B. blakeana*

Plate 7: Meiotic Metaphasic chromosomes of *B. purpurea*

Plate 8: Meiotic Metaphasic Chromsomes of *B. variegata*

The presence of derivatives of quercetinviz.Quercetin-3-rhamnosides was recorded in the hybrid and its two putative parental species. Flavone, apigenin was recorded in all the three species (Table-3). Flavonols (spots 7, 18) were common in *B. blakeana* and *B. purpurea* and (spot 5) in *B. blakeana* and *B. variegate* while (spot 10) was common in *B. purpurea* and *B. variegata.* Presence of isorhamnetin was recorded in *B. blakeana* and its putative species *B. purpurea.* Presence of Kaempferol and Quercetin in *B. purpurea* and *B. variegata* and isorhamnetin in *B. purpurea* agree with the finding of previous report (Santosh *et al.,* 1998). Distribution of various flavonoids in different species are tabulated in Table-4.

CONCLUSION

Above data shows that the morphological intermediacy is not evident, however, in many of the morphological characters of *B. blakeana,* only few are shown to be intermediate between the putative parents. Most of the intermediate characters are derived length/width ratios and therefore reflect differences in shape rather than size. But meiotic study shows most of the characters of the hybrid *B. blakeana* are intermediate between its putative parental species. Phytochemical analysis provides rather equivocal evidence for hybridization. The morphological, cytological and phytochemical data therefore indicate that it is clearly feasible for *B. purpurea* and *B. variegata* to interbreed.

TABLE 3: Distribution of chromatographic spot types in*Bauhinia* species

Species	Spot types
B. blakeana	1, 5, 6, 7, 11, 14, 18, 19, 22, 23
B. purpurea	2, 7, 8, 9, 10, 12, 15, 18, 21
B. variegata	3, 4, 5, 10, 13, 16, 17, 20

TABLE 4: Distribution of compounds in *Bauhinia* species

Species	Quercetin	Kaempferol	Isorhamnetin	Apigenin
B. blakeana	+	+	+	+
B. purpurea	+	+	+	+
B. variegata	+	+	-	+

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