RESEARCH ARTICLE

Cytotaxonomical analysis of *Momordica* L. (Cucurbitaceae) species of Indian occurrence

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Abstract

Somatic chromosome number and detailed karyotype analysis were carried out in six Indian *Momordica* species viz. *M. balsamina*, *M. charantia*, *M. cochinchinensis*, *M. dioica*, *M. sahyadrica* and *M. cymbalaria* (syn. *Luffa cymbalaria*; a taxon of controversial taxonomic identity). The somatic chromosome number 2*n* = 22 was reconfirmed in monoecious species (*M. balsamina* and *M. charantia*). Out of four dioecious species, the chromosome number was reconfirmed in *M. cochinchinensis* (2*n* = 28), *M. dioica* (2*n* = 28) and *M. subangulata* subsp. *renigera* (2*n* = 56), while in *M. sahyadrica* (2*n* = 28) somatic chromosome number was reported for the first time. A new chromosome number of 2*n* = 18 was reported in *M. cymbalaria* against its previous reports of 2*n* = 16, 22. The karyotype analysis of all the species revealed significant numerical and structural variations of chromosomes. It was possible to distinguish chromosomes of *M. cymbalaria* from other *Momordica* species and also between monoecious and dioecious taxa of the genus. Morphology and crossability among the dioecious species was also studied. Evidence from morphology, crossability, pollen viability and chromosome synapsis suggests a segmental allopolyploid origin for *M. subangulata* subsp. *renigera.* The taxonomic status of the controversial taxon *M. cymbalaria* was also discussed using morphological, karyological and crossability data.

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Introduction

The genus *Momordica* belongs to the tribe Joliffieae (Schrad.), of the family Cucurbitaceae and is a native of the Palaeotropics (Robinson and Decker-Walters 1997), comprising of about 40 species (Chakravarty 1982), distributed in warm tropics of both hemispheres, chiefly in Africa and with about 10 species in South-East Asia (De Wilde and Duyfjes 2002). According to the latest revision of Indian *Momordica* spp. (Joseph 2005) there are six well identified species of which four are dioecious and two monoecious. The monoecious taxa are *M. charantia* L. (2*n* = 22) and *M. balsamina* L. (2*n* = 22). The dioecious taxa are *M. dioica* Roxb. (2*n* = 28), *M. sahyadrica* (Joseph and Antony 2007) (2*n* = 28), *M. cochinchinensis* (Lour.) Spreng. (2*n* = 28) and *M. subangulata* Blume subsp. *renigera* (G. Don) (De Wilde and Duyfjes 2002) (2*n* = 56). However Joseph (2005) has not mentioned *M. cymbalaria* which is treated under the genus *Momordica* by various authors (De Wilde and Duyfjes 2002).

Momordica dioica and *M. charantia* are ubiquitous in distribution over India except in north-east, whereas *M. subangulata* ssp. *renigera* is restricted to north-east and adjoining north Bengal hills. *M. cochinchinensis* has localized distribution in Andamans and few localities in the eastern and north eastern states. *M. balsamina* is restricted to the arid belt comprising, Rajasthan and Gujarat, while *M. cymbalaria* is distributed in Andhra Pradesh, Karnataka, Madhya Pradesh and Maharashtra. *M. sahyadrica* is a recently described species (Joseph and Antony 2007) and is endemic to the Western Ghats.

Chromosome number has been a valuable character in biosystematics owing to its implications in phylogeny and

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evolution (Steussy 1990). *Momordica* species are not easily amenable to cytological analysis as their chromosomes are very small and do not stain well (Bhaduri and Bose 1947) vis-a-vis the cytoplasm (Trivedi and Roy 1972). In literature, chromosome number of one species of *Momordica* is ascribed to that of another due to misidentification in taxonomical identity. Therefore, the present study was undertaken to document chromosome number and karyotype analysis in all six Indian species of *Momordica* including a new record in *M. sahyadrica* (Joseph and Antony 2007); to reconfirm the existing chromosome status of Indian *Momordica* species and to study the cytotaxonomic relationship of *M. cymbalaria* if any, with other *Momordica* species. Results from this study will also improve our knowledge of understanding on the chromosomal makeup of the species and can be applied to resolve taxonomic disputes of *M. cymbalaria* as well for breeding programmes.

Materials and methods

Chromosomal analysis

Seeds of *M. balsamina*, *M. charantia, M. cochinchinensis, M. dioica*, *M. sahyadrica, M. subangulata* subsp. *renigera*, and *M. cymbalaria* collected from various sources (table 1) were germinated *in vitro* (25°C–30°C). Actively growing root tips were excised between 10.00–11.00 a.m. and pretreated in cold water at 4°C for 12 h. Pretreated root tips were fixed in 1 : 3 acetic-alcohol for 24 h, hydrolyzed in 1 N HCl at 60°C for 12 min, stained in Fuelgen solution for 2–3 h in dark and squashed in 2% aceto-orcein. For meiotic studies, staminate buds were fixed between 10.30–11.30 a.m. in 1 : 3 propionic : alcohol for 24 h, hydrolyzed in 1 N HCl at 60°C for 10 min, stained in Fuelgen for 3–4 h and squashed in 2% aceto-carmine.

Karyotype studies

Ten well scattered metaphase plates were selected for karyotyping of each species. Total chromosome length and volume of a karyotype was calculated by applying the formula $\pi r^2 h$ where 'r' and 'h' represent the radius and the length of the chromosome, respectively. Form percentage (F%) of individual chromosome was calculated following the method of (Das and Mallick 1993). Total form percentage (TF%) of a karyotype was the average of sum total F% of a karyotype. Mean values of total genomic chromosome length and total genomic chromosome volume with standard error were calculated.

Morphological study

The tuberous roots of *M. cochinchinensis*, *M. dioica*, *M. sahyadrica*, *M. subangulata* subsp. *renigera*, *M. cymbalaria* and the seeds of *M. balsamina* and *M. charantia* were sown in pots containing mixture of soil, sand and FYM (2 : 1 : 1). Each species (40 plants each of *M. charantia*, *M. dioica,* 10 each of *M. subangulata* subsp. *renigera*, *M. sahyadrica*, five each of *M. cochinchinensis*, *M. cymbalaria*, and *M. balsamina*) was scored for 27 stable morphological traits (table 2). Genetic similarity and cluster analysis were performed using the NTSYS-pc software version 2.0 (Applied Biostatistics, New York, USA).

Crossability study

Hand pollinations were carried out in an insect proof glass house (25°C ± 3°C) of National Phytotron Facility (Indian Agricultural Research Institute, New Delhi, India) during 2007–2009. As anthesis of *M. dioica* occurs in the evening hours, the pollen was collected in the evening, stored at 4°C, and used to pollinate *M. cochinchinensis* and *M. subangulata* subsp. *renigera* and *M. cymbalaria* in the following morning while the pollen of *M. cochinchinensis* and *M. subangulata* subsp. *renigera* and *M. cymbalaria* were collected in the morning, stored at 4°C, and used to pollinate *M. dioica* in evening hours. In other cross combinations, fresh pollen was used for hybridization. Pollen viability of *M. subangulata* subsp. *renigera* was measured microscopically after staining with potassium iodide.

Results

Chromosome number and karyotype analysis

Somatic chromosome number of monoecious species *M. balsamina* and *M. charantia* was observed as $2n = 22$ while the

Table 1. Germplasm accessions, source and chromosome numbers of *Momordica* spp. studied.

Species	Accession no.	Source/site of collection	2*n*
M. balsamina	IC 467683	Regional Station of NBPGR, Thrissur, Kerala	22
M. charantia	Pusa Do Mausmi	IARI, New Delhi	22
M. dioica	CHSG 28	CHES, Bhubaneswar	28
M. sahyadrica	IC-550144	Regional Station of NBPGR, Thrissur, Kerala	28*
M. cochinchinensis	IC-553689	CHES, Bhubaneswar, Orissa	28
M. subangulata subsp. *Renigera*	IC-553771	Regional Station of NBPGR, Thrissur, Kerala	56
M. cymbalaria	CYM-1	Thirunelveli, Tamil Nadu, India	18**

*First report of chromosome number; **new report.

Table 2. Data matrix for numerical study based on morphology.

Character	Character state	1	2	3	4	5	6	7
Habit	0, Annual; 1, perennial	0	0	1	1	1	1	1
Sex expression	0, Monoecious; 1, dioecious	0	0	1	1	1	1	0
Germination	0, Epigeal; 1, hypogeal	0	0	1	1	1	1	1
Roots	0, Nontuberous; 1, tuberous	0	0	1	1	1	1	1
Adventitious root tubers	0, Absent; 1, present	0	0	0	0	1	0	0
Petiole umblical glands	0, Absent; 1, present	0	0	0	0	0	1	0
Foliaceous bract (♂ flower)	0, Absent; 1, present	1	1	1	1	1	1	0
Staminate flowers	0, Solitary; 1, in short racemes	0	0	0	0	0	0	1
Nature of ♂ flower bud bract	0, Protective; 1, nonprotective	1	1	0	0	0	0	1
Anthesis	0, Morning; 1, evening	0	0	1	0	0	0	0
Leaf lobing	0, Deeply lobed; 1, angled	0	0	0	0	1	0	1
Flower size	0, Small; 1, large	0	0	0	0	1	1	0
Corolla colour	0, Yellow; 1, milky white	0	0	0	0	0	0	1
Bull's eye nectar guide	0, Absent; 1, present	0	0	0	0	1	1	0
Stigma colour	0, Green; 1, yellow	0	0	1	1	1	1	0
Anther symmetry	0, Symmetrical; 1, asymmetrical	0	0	0	0	0	0	1
Fruit type	0, Fleshy; 1, dry	0	0	0	0	0	0	1
Fruit shape	0, Ovoid-oblongoid; 1, pyriform	0	0	0	0	0	0	1
Fruit ridges	0, Absent; 1, present	0	0	0	0	0	0	1
Fruit spines	0, Absent; 1, present	0	0	1	1	1	1	0
Tubercles on fruit surface	0, Absent; 1, present	1	1	0	0	0	0	0
Fruit colour at ripening	0, Orange red; 1, green	0	0	0	0	0	0	1
Placenta colour on ripening	0, Red; 1, white	0	0	0	0	0	0	1
Aril on ripening	0, Wet slimy; 1, dry granular	0	0	0	0	0	0	1
Intensity of bitterness	0, Extreme; 1, light/less/nil	0	0	1	1	1	1	0
Seed sculpturing	0, Present; 1, absent	0	0	0	0	0	0	1
Seed margin	0, Dented; 1, smooth	0	0	0	0	0	0	1

1, *M. charantia*; 2, *M. balsamina*; 3, *M. dioica*; 4, *M. sahyadrica*; 5, *M. subangulata* subsp. *renigera*; 6, *M. cochinchinensis*; 7, *M. cymbalaria*.

taxon of controversial identity (*M. cymbalaria*) showed $2n = 18$ (table 1). The meiotic analysis at diakinesis/metaphase I also showed $2n = 9$II in *M. cymbalaria* (figure 1). Out of the dioecious species studied, somatic chromosome number varied from $2n = 28$ (*M. cochinchinensis, M. dioica* and *M. sahyadrica*) to $2n = 56$ (*M. subangulata* subsp. *renigera*). The chromosomes in all the species were found to be small in size (table 3).

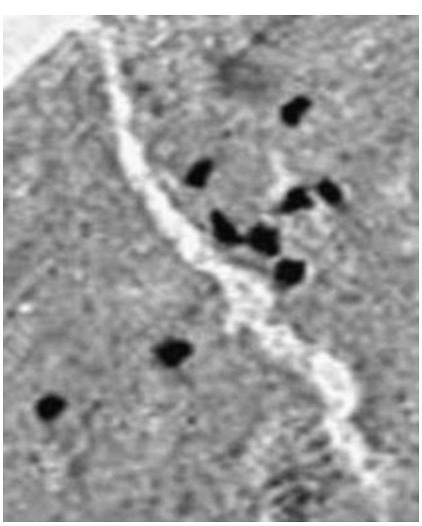

Figure 1. Pollen mother cell of *Momordica cymbalaria* showing $2n = 9$II.

On the basis of the size of the chromosome and the position of the constrictions, a number of chromosome types were found to be common with the genotypes studied, though they differed from each other in the minute structural details of the karyotype. A general description of the representative types of chromosomes is given below. Type A, chromosomes are medium sized with two constrictions in nearly median to median and nearly sub-median to sub-median in position, respectively. Type B, medium to small sized chromosomes with nearly median to median primary constrictions. Type C, chromosomes are medium to small chromosomes with sub-median to nearly terminal primary constrictions.

Detailed karyotype analysis of all the seven species showed numerical and structural alterations of chromosomes. Though all three types of chromosomes were present in all the studied genotypes, numerical differences were most prominent among them. The karyotype formula of all the genotypes revealed definite differences in the chromosome structure. Type A, B and C chromosomes were present in all genotypes but the dose difference of each type of

Table 3. Karyotype data of different species of *Momordica.*

Species	No. of SCC	Karyotype formula	Chromosome length (μm)			Chromosome volume (μm^3)		Form %
			Range	Total	Mean	Total	Mean	
M. balsamina	2	2A+10B+10C	0.65–1.98	28.61 ± 1.25	1.30	14.73 ± 0.45	0.67	38.55
M. charantia	2	2A+8B+12C	0.87–1.72	29.04 ± 0.78	1.32	14.95 ± 0.36	0.68	40.21
M. dioica	2	2A+16B+10C	0.85–2.17	38.53 ± 0.95	1.38	19.84 ± 0.24	0.71	43.88
M. sahyadrica	2	2A+18B+8C	0.73–1.83	37.53 ± 0.35	1.34	19.32 ± 0.53	0.69	44.42
M. cochinchinensis	2	2A+14B+12C	1.16–2.03	46.05 ± 1.15	1.64	23.71 ± 0.55	0.85	42.56
M. subangulata subsp. *renigera*	4	4A+14B+38C	0.52–1.26	51.88 ± 1.40	0.93	26.71 ± 0.42	0.48	32.26
M. cymbalaria	2	2A+12B+4C	1.68–3.59	50.00 ± 1.45	2.62	25.75 ± 0.55	1.43	42.89

SCC, number of secondary constricted chromosome.

chromosome found prominent (figure 2). Two numbers of type A chromosomes were present in all the species except in the tetraploid species, *M. subangulata* subsp. *renigera* ($2n$ = 56), where two pairs of secondary constricted chromosomes were present. Variation of types B and C chromosomes was very prominent. Lowest number (8) of type B chromosomes was present in *M. charantia* and highest (18) in *M. sahyadrica*. High number of sub-median to nearly terminal type C chromosomes (38 in number) was present in *M. subangulata* subsp. *renigera*. Type C chromosomes were the most numerous in all the genotypes that varied from 4 in *M. cymbalaria* to 38 in *M. subangulata* subsp. *renigera*. Detailed analysis of the somatic complements and the different genomic characteristics showed genotype specific variations in chromosome structure (table 3). The total genomic chromosome length varied from 28.61 μm in *M. balsamina* to 51.88 μm in *M. subangulata* subsp. *renigera* and the total genomic chromosome volume also varied accordingly. However, average chromosome length was minimum (0.93 μm) in the tetraploid species *M. subangulata* subsp. *renigera* and maximum (2.62 μm) was found in *M. cymbalaria.* The centromeric index in the chromosomes of all the genotypes varied from 32.26% in *M. subangulata* subsp. *renigera* to 44.42 in *M. sahyadrica.*

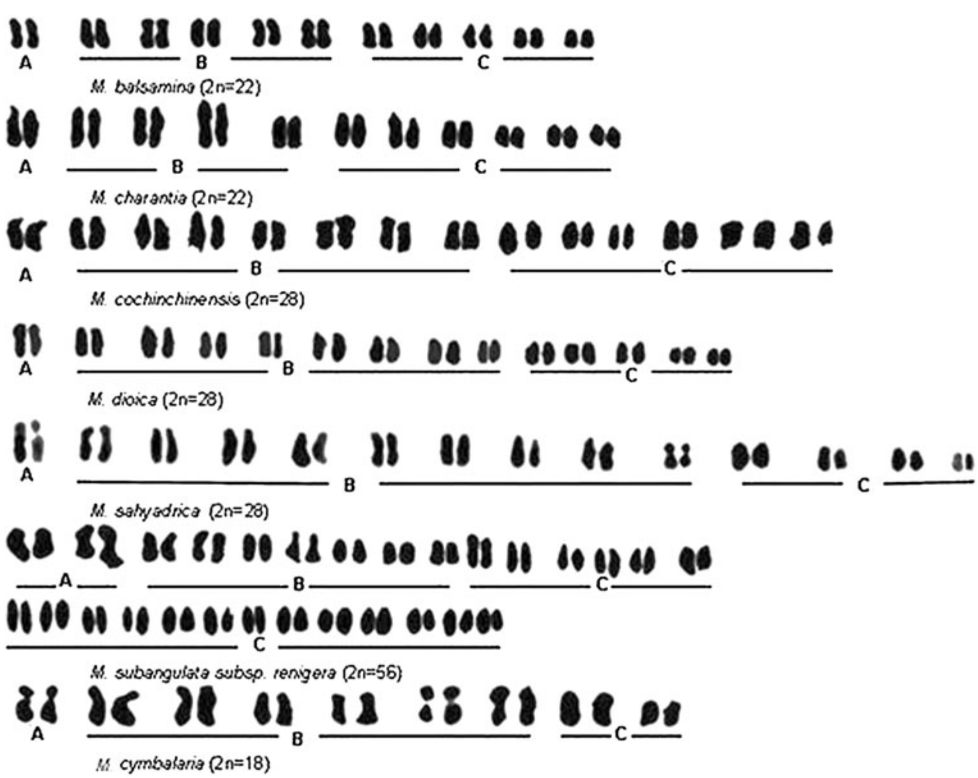

Figure 2. Karyograms of different species of *Momordica*.

Significant variations in chromosome length, volume and TF% were observed among the studied species of *Momordica*.

Morphological studies

Momordica spp.: Annual or perennial; leaves, entire or deeply lobed (3–7); flowers, monoecious or dioecious, solitary/pseudo raceme, medium to big size, with foliaceous bracts, corolla creamish yellow to bright yellow coloured, stigma green to pale green (in monoecious species) or yellow (in dioecious species). Fruits, medium to big sized (5–800 g), fleshy, green when unripe and orange red when ripe, ovoid oblongoid (figures 3, a–f), ornamented with soft spines, warts or tubercles, splits from base into three valves. Seeds, enclosed in orange red sarcotesta (aril), sculptured, margins often undulate and dentate.

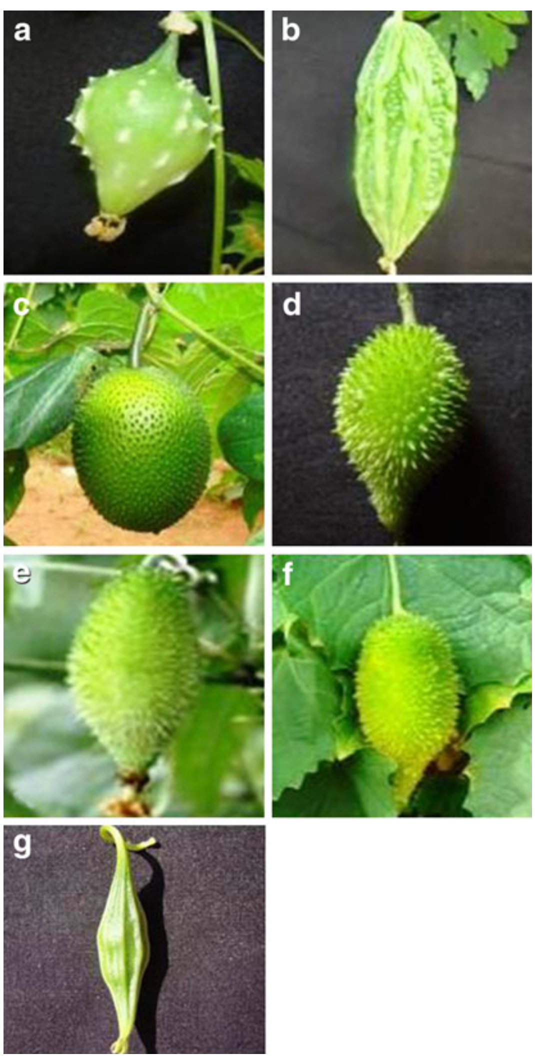

Figure 3. Fruits of *Momordica* spp. (a) *M. balsamina*, (b) *M. charantia*, (c) *M. cochinchinensis*, (d) *M. dioica*, (e) *M. sahyadrica*, (f) *M. subangulata* ssp. *renigera*, (g) *M. cymbalaria*.

***Momordica cymbalaria (syn. L. cymbalaria)*:** perennial; leaves, angled (3–5); flowers, monoecious, lacks foliaceous bract; male flowers borne in short racemes, corolla milky white, stigma pale green. Fruits, very small (< 1 g), dry dehiscent, light green to green when unripe and green when ripe, pyriform, ribbed (figure 3 g), splits into irregular crumps. Seeds, lack orange red arils, seed surface and margins smooth.

The phenogram (figure 4) based on 27 qualitative characters grouped seven species into three main clusters, where Jaccard's similarity coefficient ranged between 0.05–0.90 (table 4). Cluster I included monoecious species (*M. charantia* and *M. balsamina*); cluster II included dioecious species (*M. dioica*, *M. sahyadrica*, *M. cochinchinensis*, and *M. subangulata* subsp. *renigera*) and cluster III contained *M. cymbalaria*. Cluster II was divided into two sub groups wherein *M. dioica*, *M. sahyadrica* were linked at high level and *M. cochinchinensis*, *M. subangulata* subsp. *renigera* clustered together.

Crossability studies

All possible hybrid combinations involving three dioecious species (*M. dioica*, *M. cochinchinensis*, *M. subangulata* subsp. *renigera*) set fruits with the exception of *M. cochinchinensis* × *M. dioica*. More than 50% fruits were obtained in combinations involving *M. dioica* and *M. subangulata* subsp. *renigera*. Forty per cent fruit set was observed in *M. dioica* × *M. cochinchinensis*. After over 50 attempts to obtain hybrids between *M. subangulata* subsp. *renigera* and *M. cochinchinensis*, one fruit was eventually obtained in direct crosses and two fruits were obtained in reciprocal crosses (table 5). All attempts (> 50) to cross *M. cymbalaria* with *Momordica* species available in India failed (data not shown).

Discussion

Chromosome number study

Chromosome numbers are reported for the first time in *M. sahyadrica* ($2n = 28$). Somatic chromosome number of $2n = 22$ was observed in the monoecious taxa (*M. balsamina* and *M. charantia*) and $2n = 28$ was observed in dioecious taxa (*M. dioica* and *M. cochinchinensis, M. sahyadrica*). Thus monoecious and dioecious species (excluding *M. cymbalaria*) fall into two distinct numerical groups of $x = 11$ and $x = 14$, respectively. The species of the genus *Momordica* of Indian occurrence can be classified into two distinct groups or subgenera, different in their basic chromosome numbers and sex expression: (i) contains basic number of 11 which includes monoecious taxa (*M. charantia* and *M. balsamina*); and (ii) contains basic number of 14 which includes dioecious taxa (*M. dioica*, *M. subangulata*, *M. sahyadrica* and *M. cochinchinensis*).

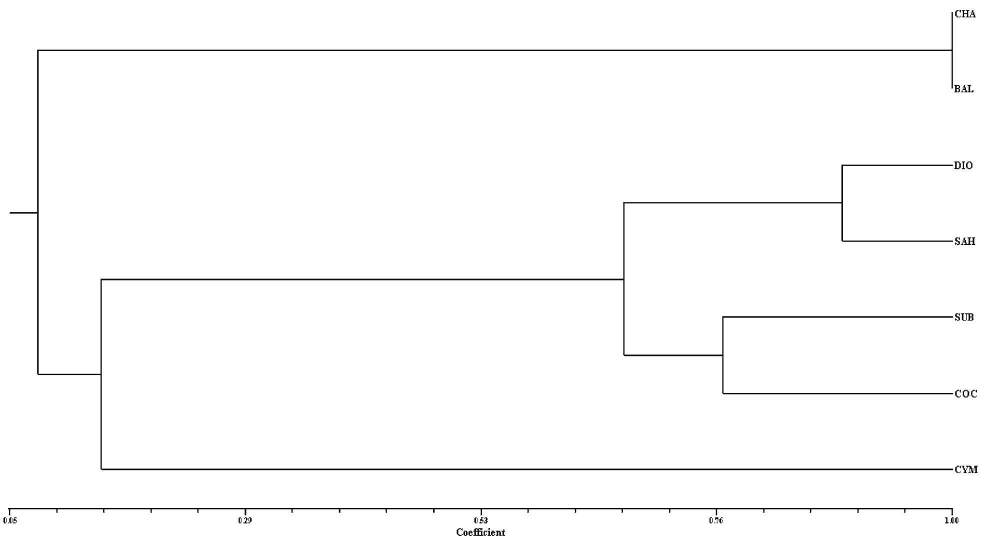

Figure 4. Phenogram showing relationships of *Momordica* species derived from UPGMA cluster analysis using 27 characters. CHA, *M. charantia*; BAL, *M. balsamina*, DIO, *M. dioica*, SAH, *M. sahyadrica*; SUB, *M. subangulata* subsp. *renigera*, COC, *M. cochinchinensis*, CYM, *M. cymbalaria.*

The findings are consistent with those published previously for all taxa (McKay 1930; Whitaker 1933; Richharia and Ghosh 1953; Sen and Datta 1975; Jha *et al*. 1989) except for *M. sahyadrica* and *M. cymbalaria*. Chromosome numbers are reported for the first time in *M. sahyadrica* ($2n = 28$). In this study, we also report for the first time $2n = 18$ in *M. cymbalaria* and confirmed this number in contrast to varying reports of $2n = 22$ (Beevy and Kuriachan 1996) and $2n = 16$ (Mehetre and Thombre 1980). Chromosome number of $2n = 4x = 56$ was reported in *M. subangulata* subsp. *renigera* contrary to the earlier report $n = 14$ (De Sarkar and Majumdar 1993). The chromosome number of the cultivated teasel gourd (*M. dioica*) from Tripura and Bangladesh was reported as $2n = 4x = 56$ (Sinha *et al*. 1997; Cho *et al*. 2006). However, field and herbarium studies conducted on teasel gourd (Bhat karela, Assam kakrol) reported that the cultivated dioecious *Momordica* specimen of northeast India is actually *M. subangulata* subsp. *renigera* and not *M. dioica* (Joseph *et al*. 2007). Hence, reports of Sinha *et al*. (1997) and Cho *et al*. (2006) of $2n = 4x = 56$ deserve to be ascribed to *M. subangulata* subsp. *renigera* and not to *M. dioica* in conformity with our findings.

Karyotype analysis

Karyotype analysis showed distinct numerical and structural alterations of chromosomes in all species. However, all the species recorded for their common type of chromosomes types A, B and C suggested a common ancestry of all the species. It was observed that two numbers of secondary constricted chromosomes type A were found in all the diploid species except in tertaploid species *M. subangulata* subsp. *renigera* ($2n = 56$) where four numbers of secondary

Table 4. Pairwise Jaccard's similarity coefficient based comparisions among different accessions of *Momordica* spp.

	CHA	BAL	DIO	SAH	SUB	COC	CYM
CHA	1.000						
BAL	1.000	1.000					
DIO	0.091	0.091	1.000				
SAH	0.100	0.100	0.889	1.000			
SUB	0.071	0.071	0.615	0.667	1.000		
COC	0.077	0.077	0.667	0.727	0.769	1.000	
CYM	0.056	0.056	0.136	0.143	0.167	0.125	1.000

BAL, *M. balsamina*; CHA, *M. charantia*; COC, *M. cochinchinensis*, DIO, *M. dioica*, SAH, M. *sahyadrica*, SUB, *M. subangulata* subsp. *renigera*, CYM, *M. cymbalaria*.

Table 5. Crossability among the tetraploid species (*M. subangulata* subsp. *renigera*) and its presumed parents (*M. dioica* and *M. cochinchinensis*).

Combination	No. of pollination	Fruit set (no./fruit)	Seed set (no./fruit)	No. of seeds sown	No. of seeds germinated
DIO × COC	5	2 (40%)	9.7	10	4
DIO × SUB	15	8 (53%)	3.5	10	1
SUB × DIO	40	26 (65%)	22.38	10	7
SUB × COC	57	1 (1.75%)	3	5	2
COC × DIO	128	–	–	–	–
COC × SUB	38	2 (5.26%)	4.5	8	2

DIO, *M. dioica*, COC, *M. cochinchinensis*, SUB, *M. subangulata* subsp. *renigera*.

constricted chromosomes were present. Recent evidence based on genome analysis has indicated segmental allopolyploid origin of *M. subangulata* subsp. *renigera* (Bharathi *et al*. 2010). Two pairs of secondary constricted chromosomes are probably derived from the diploid progenitor species. Variation of type B and C chromosomes was very prominent; however, changes in type C chromosomes were more prominent in all the species. It was evident that high number of type C chromosomes in *M. subangulata* subsp. *renigera* might be due to the break and reunion of metacentric chromosomes and its duplication in the natural process of evolution. Total chromosome length also support this result as there were not much significant increase of chromosome size than the diploid species *M. cochinchinensis* ($2n = 28$) or *M. cymbalaria* ($2n = 18$) chromosomes. Average chromosome length and volume was minimum (0.93 μm and 0.48 μm^3 respectively) in the tetraploid species *M. subangulata* subsp. *renigera* and maximum (2.62 μm) was found in *M. cymbalaria*. High numbers of sub-median to nearly terminal type C chromosomes were present in *M. subangulata* subsp. *renigera*. The centromeric index in the chromosomes of all the genotypes varied from 32.26% in *M. subangulata* subsp. *renigera* to 44.42% in *M. sahyadrica*. Among the studied species of *Momordica*, a significant variations in chromosome length, volume and TF% were observed. Chromosome size was considerably uniform between the two groups of *Momordica* with the exception of *M. subangulata* subsp. *renigera* which had smaller

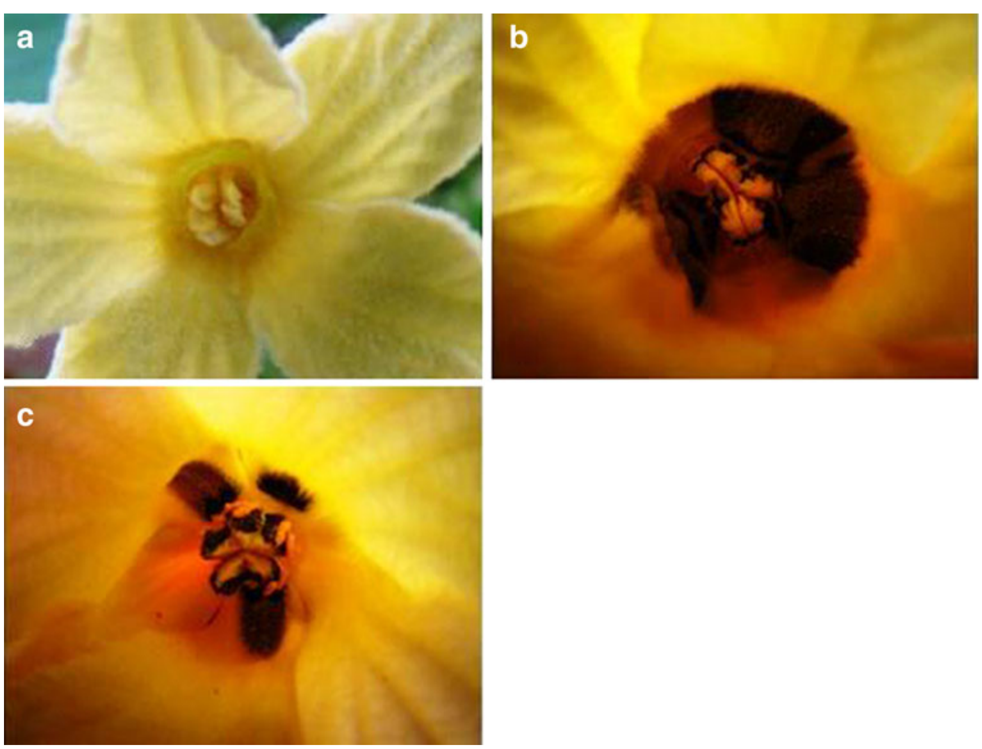

Figure 5. Flowers of dioecious *Momordica* (a) *M. dioica*, (b) *M. cochinchinensis*, (c) *M. subangulata* subsp. *renigera*.

chromosomes than other dioecious species and *M. cymbalaria* which had larger chromosomes than other *Momordica* spp. The size of somatic metaphase chromosomes fall within the range of 0.52–3.59 μm in all the species studied. The smallest length of 0.52 μm was found in *M. subangulata* subsp. *renigera* while the longest chromosome (3.59 μm) was found in *M. cymbalaria*. The ratio of the longest to the shortest chromosome ranged from 1.68 (*M. cochinchinensis*) to 2.94 (*M. balsamina*).

Evolution of M. subangulata subsp. renigera

Based on the published evidence of plant description, photographs, domestication and distribution of the species, it is inferred that *M. subangulata* subsp. *renigera* was identified as tetraploid as compared to *M. dioica* (Roy *et al*. 1966; Sen and Datta 1975; Ali *et al*. 1991; Hossain *et al*. 1996) and sometimes as *M. cochinchinensis* (Vijay and Jalikop 1980; Mohanty *et al*. 1984; Shadeque and Baruah 1984; Handique 1988; Ram *et al*. 2002; Rasul *et al*. 2004). *M. subangulata* subsp. *renigera* was even described as a new taxonomic entity as *M. hybrida* (Mondal *et al*. 2006). An autotetraploid constitution of this species was suggested (Roy *et al*. 1966; Sen and Datta 1975) due to high frequency of quadrivalents during meiosis. Though Roy *et al*. (1966) suggested an autotetraploid origin; the number of quadrivalents reported varied between three and six. Sinha *et al*. (1997) also reported that the number of bivalents was significantly higher than those of trivalents and quadrivalents with only occasional presence of 28 bivalents.

Based on morphological characteristics, *M. subangulata* subsp. *renigera* ($2n = 4x = 56$), has been described as an allopolyploid derived from hybridization between *M. dioica* ($2n = 28$) and *M. cochinchinensis* ($2n = 28$) followed by spontaneous chromosome doubling (Mondal *et al*. 2006). *M. subangulata* subsp. *renigera* shares certain characteristics with *M. dioica* as well as with *M. cochinchinensis*. *M. subangulata* subsp. *renigera* resembles *M. cochinchinensis* in anthesis time, shape and colour of petal spot (figure 5), petal venation, filament and thecae colour, receptacle and seed shape (figure 6). It also resembles *M. dioica* in its bifid anther and absence of petiolar umbilical glands. However, based on genome analysis, Bharathi *et al* (2010) have indicated a segmental allopolyploid origin of *M. subangulata* subsp. *renigera* with considerable homology between the genomes of *M. dioca* and *M. cochinchinensis*, the putative progenitors of *M. subangulata* subsp. *renigera*. *M. dioica* also showed good crossability with *M. cochinchinensis* and *M. subangulata* subsp. *renigera*.

General chromosomal characteristics

Momordica cymbalaria (syn. *Luffa cymbalaria*) which is taxonomically and genetically remote from other species is not discussed here with other *Momordica* species and is described in later part of this paper. The close relationship between the two monoecious species (*M. charantia* and *M. balsamina*) and also between two dioecious species (*M. dioica* and *M. sahyadrica*) were reflected in the morphological similarities between their chromosomes. Roy *et al*. (1966) reported that *M. dioica*, a perennial dioecious species, differs from *M. charantia* and *M. balsamina* in chromosome number as well as through its markedly asymmetrical karyotype. Chromosomes in the polyploid *M. subangulata* subsp. *renigera* were generally more condensed than those of the presumed parental diploids (*M. dioica* and *M. cochinchinensis*). The fact that established polyploid species or variants have smaller chromosome than their diploid counterparts already had been described by Darlington (1956).

Taxonomic status of M. cymbalaria (syn. L. cymbalaria)

Momordica cymbalaria showed $2n = 18$ chromosomes in somatic metaphase analysis. The number was further confirmed in meiosis wherein clear nine bivalents were observed during diakinesis/metaphase I. This count differ from Mehetre and Thombre's (1980) meiotic count of $n = 8$ and Beevy and Kuriachan's (1996) mitotic count of $2n = 22$. Taxonomic position of *M. cymbalaria* has always been uncertain. The relationship of this taxon with other species was studied based on plant, seed and chromosome morphology (table 2). *M. cymbalaria* has a different chromosome number of $2n = 18$ while other *Momordica* species (chromosome number known so far) have $2n = 22$, 28. The

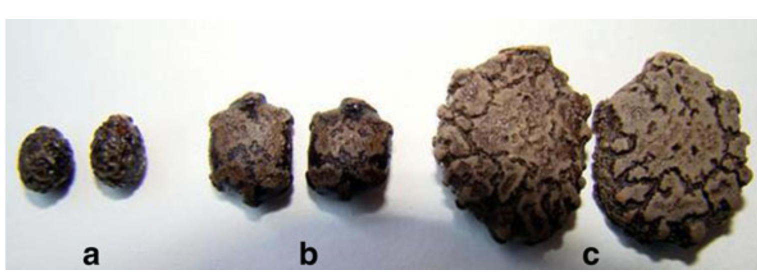

Figure 6. Seeds of dioecious *Momordica* (a) *M. dioica*, (b) *M. subangulata* subsp. *renigera*, and (c) *M. cochinchinensis*.

phenogram also indicates that only *M. cymbalaria* is distantly related to other species. This was evident before initiating the numerical analysis, for *M. cymbalaria* differs from the other species in a large number of conspicuous characters including male flowers borne in short raceme, possession of milky white corollas, minute-scar-like bracts, dry dehiscent fruits, smooth and shiny seeds. In addition, there is no fruit set even after a number of attempts to cross with all the *Momordica* spp. available in India (table 5). The chromosome length of *M. cymbalaria* ranged from 1.68–3.59 μm (table 3) with an average of 2.62 μm while in other species ranged from 0.52–2.17 μm with an average of 0.93 μm (*M. subangulata* subsp. *renigera*)–1.64 μm (*M. cochinchinensis*) and such a difference of having long chromosomes could not entirely be due to artifacts or condensation of the chromosome. Total chromosome length of *M. cymbalaria* is higher (50 μm) than other diploid species of *Momordica* ($n = 11, 14$) though the chromosome number is least ($n = 9$). Hence, we agree with the view of Chakravarty (1959) that this species may not be grouped under *Momordica*. However, we are not suggesting that this species can be grouped with *Luffa* as he did, because we have not studied its relationship with other *Luffa* species. The chromosome numbers reported here together with other approaches in the future may prove useful in further elucidating the taxonomic relationship of *M. cymbalaria* with other *Momordica* species.

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