Production and preliminary characterization of inter-specific hybrids derived from *Momordica* species

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A crossing programme involving seven species of *Momordica* and two varieties of *Momordica charantia* was undertaken. Within the sect. *Momordica*, high crossability and pollen fertility was observed in the inter-varietal cross (*M. c.* var. *charantia* × *M. c.* var. *muricata*), whereas low crossability and moderate pollen fertility was observed in the inter-specific cross (*M. charantia* × *M. balsamina*). No crossability barriers were found within the sect. *Cochinchinensis*, except for *M. cochinchinensis* × *M. dioica* and *M. cochinchinensis* × *M. sahyadrica*. *M. dioica* and *M. sahyadrica* showed higher crossability with *M. subangulata* subsp. *renigera* (both directions) and *M. cochinchinensis* (unidirectional). *M. s.* subsp. *renigera* had reproductive compatibility with *M. cochinchinensis* in both directions. *M. sahyadrica* and *M. dioica* showed high crossability in both directions and produced fertile hybrids. Cross between the sect. *Momordica* and *Cochinchinensis* yielded parthenocarpic fruits. *M. cymbalaria* (sect. *Raphanocarpus*) was neither crossable with sect. *Momordica* nor sect. *Cochinchinensis*. The chromosome numbers of the hybrids were as expected from the parental numbers. Based on crossability, a closer relationship was found between two varieties of bitter melon (var. *charantia* and var. *muricata*) and also between *M. charantia* and *M. balsamina*. All dioecious species included in this study appear to be closely related. The result support that recent taxonomic revision of the genus and the gene pool classification provide a base for improvement of *Momordica* species.

Keywords: Fertility estimates, gene pool, *Momordica* species, taxonomic relationship, wide hybridization.

THE genus *Momordica* L. (Cucurbitaceae) comprises 60 species[1] distributed chiefly in Africa, with about ten species in Southeast Asia[2]; out of which seven species (*M. charantia* L., *M. balsamina* L., *M. dioica* Roxb. Ex Willd., *M. cymbalaria* Fenzl ex Naud., *M. subangulata* Blume subsp. *renigera* (G. Don) de Wilde, *M. cochinchinensis* (Lour.) Spreng. and *M. sahyadrica* Joseph & Antony) are reported to occur in India. The genus *Momordica* is unique in India as all the species bear edible fruits esteemed for their taste and medicinal properties. Bitter melon (*M. charantia*) is the most widely cultivated species of this genus in India, Sri Lanka, the Philippines, Thailand, Malaysia, China, Japan, Australia, tropical Africa, South America and the Caribbean. Teasle gourd (*M. s.* subsp. *renigera*) the semi-domesticated vegetable crop is grown commercially in several states of India (West Bengal, Odisha, Assam, Tripura, Andaman and Nicobar Islands) and Bangladesh. Besides these two species, the cultivation of other species is restricted only to specialized agro-geographical pockets of India, mainly by tribal and poor farming communities. These species have a huge potential to be exploited as alternative crops not only for their nutritive value, but also for improving the livelihood of these tribal and poor farming communities.

Inter-specific hybridization is used to improve crops by transferring desirable agronomic characters and some specific traits such as pest and stress resistance from wild species to cultivated species[3]. Moreover, inter-specific crosses in a genus also provide information about the relationship between the taxa. However, in the genus *Momordica*, information on crossability is misleading due to the taxonomic misidentification of species especially in the Indian context. The successful cross reported between *M. dioica* and *M. cochinchinensis*[4] appears to be between *M. dioica* and *M. s.* subsp. *renigera* as the description of the species *M. cochinchinensis* by the authors seems to fit more with *M. s.* subsp. *renigera* as described later[2]. Crossability relationship is a pre-requisite for gene transfer in wide hybridization. An understanding of complete crossability spectrum of *Momordica* spp. may help to achieve this objective. Therefore, the present study was

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Table 1. *Momordica* species included in the present study

Species	Accessions used for hybridization	Sex type and habit	Seeds per fruit	Source/collector
Sect. *Momordica*				
M. charantia var. *charantia*	Pusa Do Mausmi	Monoecious annual	23	Indian Agricultural Research Institute, New Delhi
M. charantia var. *muricata*	WB-41	Monoecious annual	4	Indian Agricultural Research Institute, New Delhi
M. balsamina	IC-467683	Monoecious annual	4	National Bureau of Plant Genetic Resources, New Delhi
Sect. *Cochinchinensis*				
M. dioica	CHSG-28	Dioecious perennial	22	Central Horticultural Experiment Station, Bhubaneswar
M. sahyadrica	IC-550144	Dioecious perennial	28	National Bureau of Plant Genetic Resources (Regional Station), Thrissur
M. subangulata subsp. *renigera*	IC-553771	Dioecious perennial	30	Central Horticultural Experiment Station, Bhubaneswar and National Bureau of Plant Genetic Resources, New Delhi
M. cochinchinensis	IC-553689	Dioecious perennial	30	Central Horticultural Experiment Station, Bhubaneswar
Sect. *Raphanocarpus*				
M. cymbalaria	CYM-1	Monoecious perennial	3	S. Anbu (Former Dean), Horticulture College and Research Institute, Periyakulam

designed involving all the *Momordica* spp. of Indian occurrence to elucidate the cross-compatibility relationship between the cultivated species and the wild species after establishing correct taxonomic identity of the species.

Materials and methods

The hybridization experiment comprised of 8 × 8 diallel crossing scheme and the accessions used to produce the hybrids in the present study are given in Table 1. Hand pollination was carried out to produce hybrids in an insect-free glasshouse (temperature 25 ± 3°C) at the National Phytotron Facility in the Indian Agricultural Research Institute, New Delhi and in an experimental farm at the Central Horticultural Experiment Station, Bhubaneswar. Fresh pollen was used for all the combinations except for the cross-combination involving *M. dioica* due to its evening anthesis, unlike other *Momordica* species. An estimate of crossability was made based on the proportion of the number of fruits harvested with viable seeds with the number of pollinations attempted and was classified[5] as low (0.01–0.25), medium (0.26–0.50) and high (0.51–1.0). The seeds obtained from the hybridization of the parents were classified according to their external morphology (A, Typical seeds with embryo and cotyledon developed; B, Seeds with rudimentary embryo and papery cotyledon; C, Seeds with normal seed coat devoid of embryo and cotyledon). The putative hybrid seeds were germinated in trays containing well-decomposed farmyard manure (FYM) at a temperature of 25–30°C. The seedlings were raised in shade net house in earthen pots containing soil : FYM : sand (2 : 1 : 1). Putative hybrids were compared with their parents for morphological characters like leaf lobing, colour of the petals, presence of black colour spot in the petals, calyx colour and size, fruit shape and size. Self and backcross pollination by either parent was made on the hybrids. Chromosome count and pollen fertility were estimated as described earlier[6].

Results

Crossability and seed germination

Out of 56 cross-combinations, 13 crosses were identified as of hybrid origin based on their general morphology, pollen fertility and cytological studies. Attributes like fruit set, seed set, seed germination and pollen fertility of successful hybrids were studied to estimate the species relationship (Table 2).

In the sect. *Momordica*, two botanical varieties of bitter melon (var. *muricata* and var. *charantia*) exhibited high crossability (0.97) and the seeds of the parental cross had high germination (Figure 1; Table 2). Cross between *M. balsamina* and *M. charantia* was not successful when the former was used as seed parent. The reciprocal cross was partially successful with low crossability (0.06), though most of the seeds were hollow and few seeds even had shrivelled embryos with papery cotyledons. Nevertheless, the F_1 progeny had a fair number of fertile pollen (60.20%).

Table 2. Fruit set, seed set and germination in successful inter-specific/varietal crosses involving *Momordica*

Combination	Crossability proportion	Seed set (number/fruit)*			Germination (%)	Pollen stainability (%)
		A	B	C		
Sect. *Momordica* (two species; two varieties; six combinations)						
M. charantia var. *charantia* × *M. charantia* var. *muricata*	0.97	20	0	0	100	85.40
M. charantia var. *muricata* × *M. charantia* var. *charantia*	0.85	3	0	0	100	83.70
M. charantia var. *charantia* × *M. balsamina*	0.06	0.14	7	0	50	60.20
Sect. *Cochinchinensis* (four species; 12 combinations)						
M. dioica × *M. sahyadrica*	0.75	20	0	2	78	70.70
M. dioica × *M. subangulata* subsp. *renigera*	0.53	4	0	2	10	10.98
M. dioica × *M. cochinchinensis*	0.60	10	0	0	40	00.85
M. subangulata subsp. *renigera* × *M. dioica*	0.65	22	0	3	70	07.66
M. subangulata subsp. *renigera* × *M. sahyadrica*	0.84	23	0	2	48	13.36
M. subangulata subsp. *renigera* × *M. cochinchinensis*	0.02	3	0	0	40	18.10
M. sahyadrica × *M. subangulata* subsp. *renigera*	0.50	9	0	1	20	15.24
M. sahyadrica × *M. dioica*	0.81	12	0	2	80	68.40
M. sahyadrica × *M. cochinchinensis*	0.65	8	0	1	62.5	01.15
M. cochinchinensis × *M. subangulata* subsp. *renigera*	0.05	5	0	0	25	12.50
Sect. *Raphanocarpus* (only one species, intersectional crosses were not successful)						

*A, Typical seeds with embryo and cotyledon developed; B, Seeds with rudimentary embryo and papery cotyledon; C, Seeds with normal seed coat devoid of embryo and cotyledon.

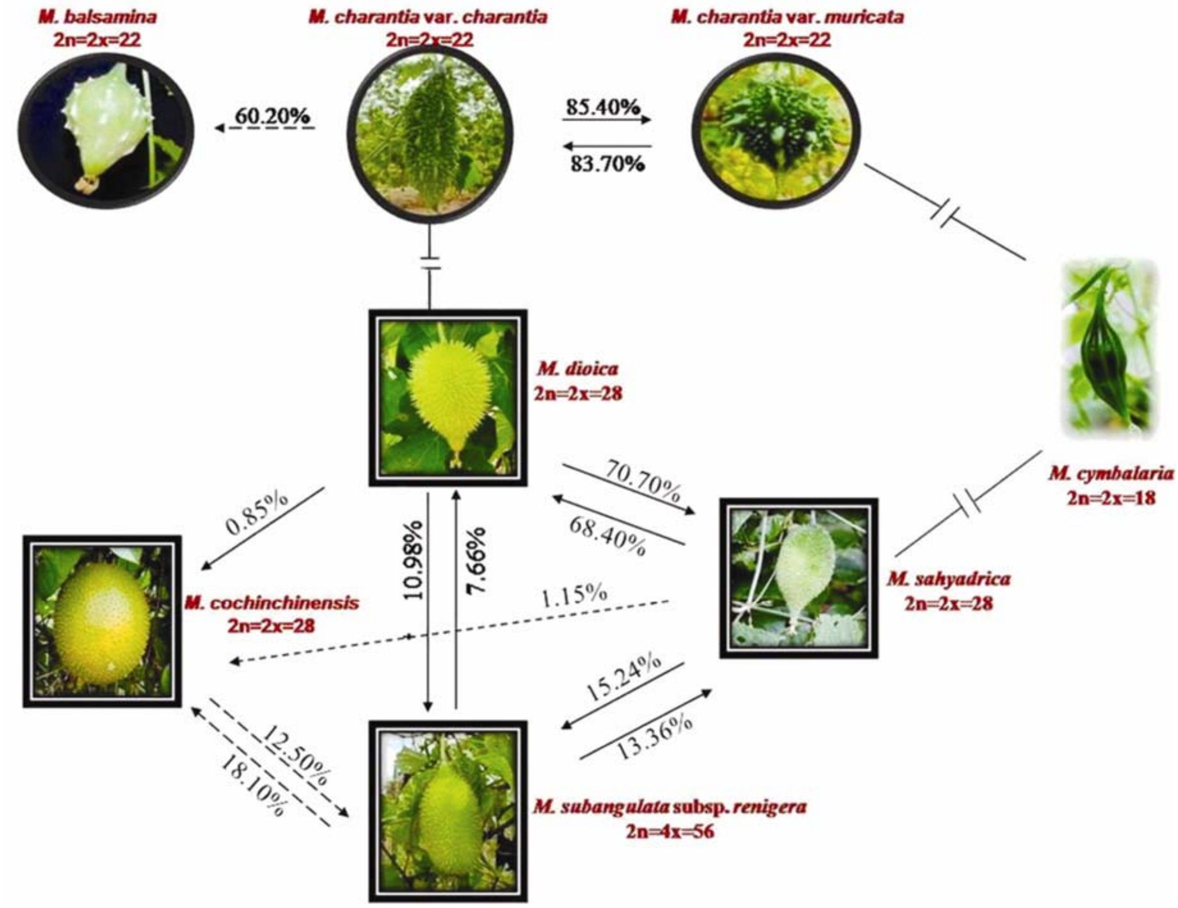

Figure 1. Crossing relationships among seven species of *Momordica*. → indicates crossing direction from female to male. Double side block (—||—) indicates that fruit set/fruits set with viable seeds were not obtained. Dashed line (----) indicates partially cross-compatible (< 50% fruits with viable seeds set). Solid line (→) indicates completely cross-compatible (> 50% fruits with viable seeds set). Numbers above the arrows represent percentage of pollen fertility.

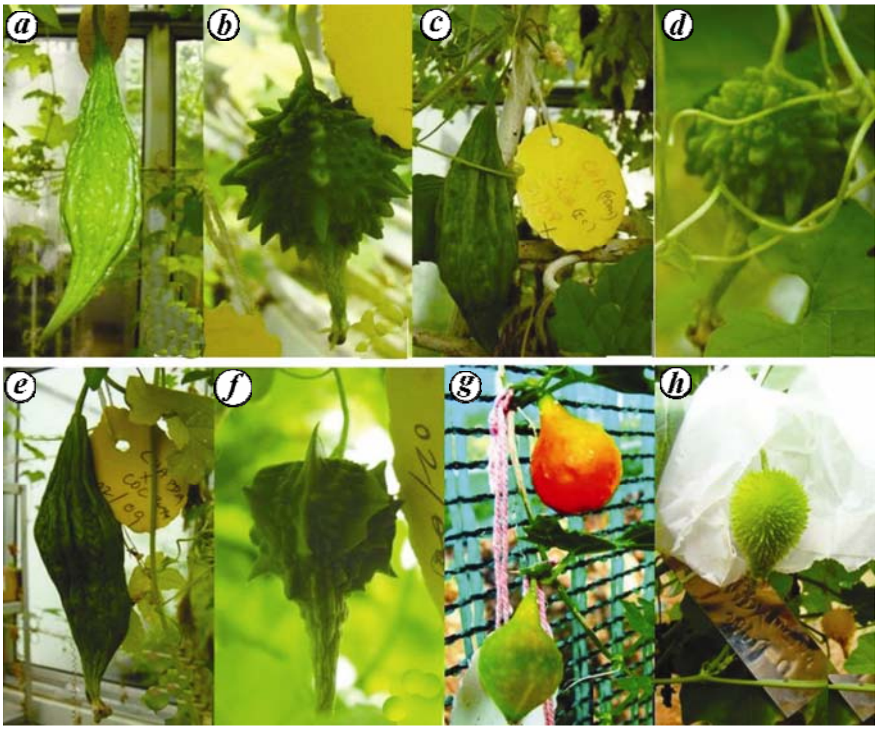

Figure 2. Fruits of parental cross (parthenocarpic/stenospermocarpic fruits) between varieties of bitter melon and dioecious species. ***a***, *M. charantia* var. *charantia* × *M. dioica*; ***b***, *M. charantia* var. *muricata* × *M. dioica*; ***c***, *M. charantia* var. *charantia* × *M. subangulata* subsp. *renigera*; ***d***, *M. charantia* var. *muricata* × *M. subangulata* subsp. *renigera*; ***e***, *M. charantia* var. *charantia* × *M. cochinchinensis*; ***f***, *M. charantia* var. *muricata* × *M. cochinchinensis*; ***g***, *M. balsamina* × *M. cochinchinensis*; ***h***, *M. dioica* × *M. charantia*.

In the sect. *Cochinchinensis*, except for the crosses *M. cochinchinensis* × *M. dioica* and *M. cochinchinensis* × *M. sahyadrica*, all the other combinations produced viable seeds. High crossability and seed germination was observed in the cross involving diploid species of this sect. (Table 2). The tetraploid *M. s.* subsp. *renigera* recorded high crossability and seed germination with *M. dioica* and *M. sahyadrica*, whereas low crossability and seed germination was recorded with *M. cochinchinensis*. The sect. *Raphanocarpus* is represented in India only by *M. cymbalaria* which was not crossable with any species of other sect.

The inter-sect. crosses involving *M. charantia* with *M. dioica*, *M. s.* subsp. *renigera*, *M. cochinchinensis*; and *M. balsamina* with *M. cochinchinensis* produced ample parthenocarpic fruits. Interestingly, in the reciprocal cross-combination *M. charantia* induced parthenocarpic fruit set only in *M. dioica* (Figure 2). In the cross-combination *M. charantia* × *M. cochinchinensis*, the seeds had abnormally large cotyledons causing the seed coat to split open (Figure 3). However, these seeds failed to germinate.

Morphology and chromosome number of hybrids

The morphological difference among the hybrids and parents in intra-sect crosses of sect. *Momordica* was not evident though the fruit size and shape was intermediate. In sect. *Cochinchinensis*, morphologically, the hybrids appeared to be intermediate between the parents. The fruit shape, size, colour of the petals and calyx were intermediate between the parents. Hybrids involving *M. s.* subsp. *renigera* and *M. cochinchinensis* as one of the parents, inherited black spots on the petals. The lobed leaf character was inherited consistently by hybrids in all crosses in sect. *Cochinchinensis* (Figures 4 and 5). The mitotic chromosome number in the F_1 hybrids was $2n = 22$ (*M. charantia* × *M. balsamina*); $2n = 28$ (*M. dioica* × *M. sahyadrica, M. dioica* × *M. cochinchinensis*, *M. sahyadrica* × *M. cochinchinensis*) and $2n = 42$ (*M. s.* subsp. *renigera* × *M. dioica, M. s.* subsp. *renigera* × *M. cochinchinensis*, *M. s.* subsp. *renigera* × *M. sahyadrica*) in the respective crosses.

Fertility of hybrids

Pollen stainability (Table 2) as well as fruit and seed set in the hybrids following self-pollination and backcross-pollination by either parental species has been studied (Table 3).

The inter-varietal cross of *M. charantia* had high pollen stainability, which confirmed the high fruit set and seed set upon self- and backcross-pollination with either of the parents. Though the inter-specific hybrid between *M. charantia* and *M. balsamina* showed moderate pollen

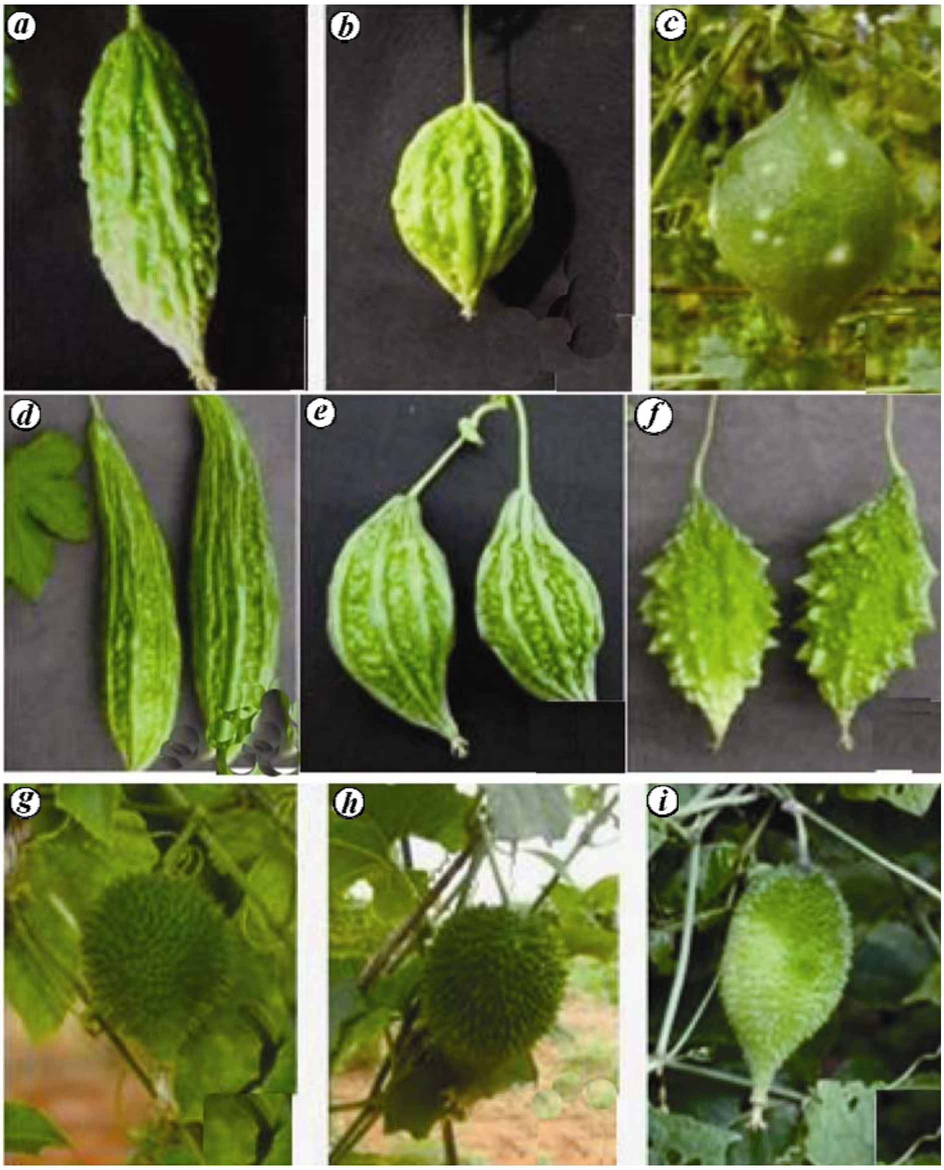

Figure 3. Fruits of *M. charantia* (var. *charantia* and var. *muricata*), *M. balsamina*, *M. dioica*, *M. sahyadrica* and their hybrids. ***a***, *M. charantia*; ***b***, *M. charantia* × *M. balsamina*; ***c***, *M. balsamina*; ***d***, *M. charantia* var. *charantia*; ***e***, *M. charantia* var. *charantia* × *M. charantia* var. *muricata*; ***f***, *M. charantia* var. *muricata*; ***g***, *M. dioica*; ***h***, *M. dioica* × *M. sahyadrica* and ***i***, *M. sahyadrica*.

fertility (60.20%), selfing produced lower fruit set (33.33%) and seed set (3 seeds/fruit). Except in the hybrids between *M. dioica* and *M. sahyadrica*, self- and backcross-pollination failed to set any fruits in diploid hybrids of the sect. *Cochinchinensis*. The range of pollen fertility in the triploid hybrids as estimated by pollen stainability was 7.66% (*M. s.* subsp. *renigera* × *M. dioica*) to 18.10% (*M. s.* subsp. *renigera* × *M. cochinchinensis*) (Table 2; Figure 6). Self-pollination among the respective triploid hybrids did not yield any fruits. Moreover, the backcross progenies could be obtained only in the triploid hybrids involving *M. s.* subsp. *renigera* and *M. dioica* though the fruit set and seed set was very low (Table 3).

Discussion

The presented data on the production, viability and the pollen fertility of F_1 hybrids allow an estimation of the degree of relatedness of the species studied. The cultivated variety of bitter melon (var. *charantia*) crossed readily with the wild variety (var. *muricata*). The F_1 plants produced flowers with > 80% stainable pollen and selfed flowers produced fruits with abundant seeds. Such high fruit set and fertility is due to the fact that the wild bitter melon (*M. c.* var. *muricata*) does not differ much from the cultivated bitter melon (*M. c.* var. *charantia*) except for the miniature size of fruits and seeds and many natural intermediate types are known[7]. The data also

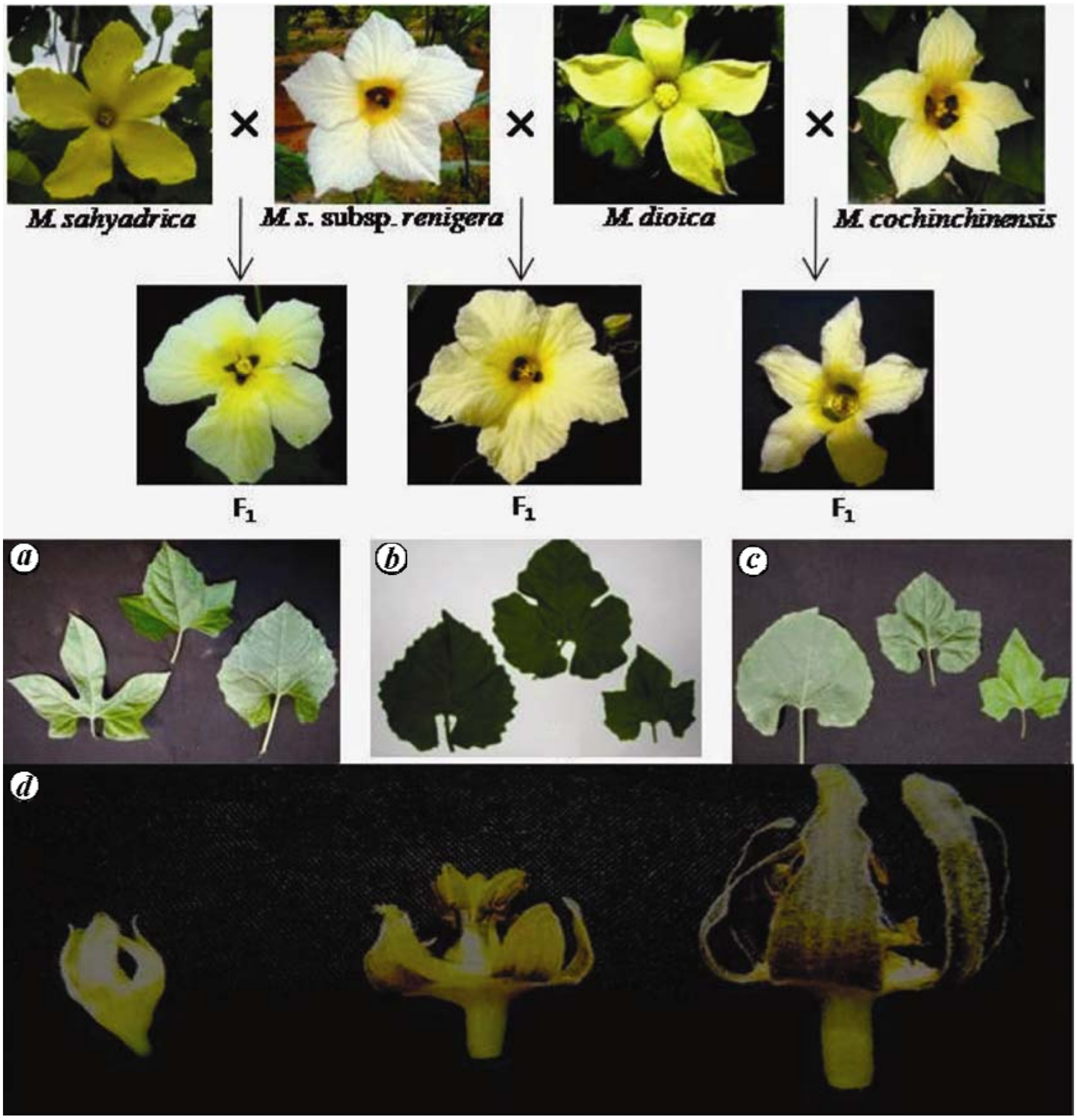

Figure 4. Leaves, flowers and calyx of *Momordica* spp. and their hybrids. ***a***, *M. cochinchinensis* (left), *M. s.* subsp. *renigera* (right) and their hybrid (centre); ***b***, *M. s.* subsp. *renigera* (left), *M. dioica* (right) and their hybrid (centre); ***c***, *M. s.* subsp. *renigera* (left), *M. sahyadrica* (right) and their hybrid (centre); ***d***, *M. dioica* (left), *M. s.* subsp. *renigera* (right) and their hybrid (centre).

Figure 5. Seeds of hybrid between *M. charantia* × *M. cochinchinensis* showing abnormal cotyledon.

Table 3. Fruit and seed set of the hybrids following self-pollination and backcross-pollination by either parental species

Hybrid	Self-pollination			Pollinated by maternal parent			Pollinated by paternal parent		
	P	F	S	P	F	S	P	F	S
M. c. var. *charantia* × *M. c.* var. *muricata*	10	100	16	10	100	18	10	100	18
M. c. var. *muricata* × *M. c.* var. *charantia*	10	100	10	10	100	10	13	100	15
M. c. var. *charantia* × *M. balsamina**	6	33.33	3	–	–	–	–	–	–
M. dioica × *M. sahyadrica*	10	100	14	10	100	11	10	100	16
M. sahyadrica × *M. dioica*	10	100	21	10	100	20	10	100	20
M. dioica × *M. s.* subsp. *renigera* (4x)	45	0	0	63	0.03	1.5	170	0.18	1.33
M. s. subsp. *renigera* (4x) × *M. dioica*	139	0	0	52	7.69	2	167	1.80	1.50
M. sahyadrica × *M. s.* subsp. *renigera* (4x)	50	0	0	50	0	0	50	0	0
M. s. subsp. *renigera* (4x) × *M. sahyadrica*	50	0	0	50	0	0	50	0	0
M. cochinchinensis × *M. s.* subsp. *renigera* (4x)	20	0	0	20	0	0	20	0	0
M. s. subsp. *renigera* (4x) × *M. cochinchinensis*	50	0	0	50	0	0	25	0	0
M. dioica × *M. cochinchinensis*	20	0	0	205	1.46	0	80	0	0
M. sahyadrica × *M. cochinchinensis**	20	0	0	–	–	–	–	–	–

*Backcrossings were not attempted. P, Number of pollinations; F, Percentage of seeded fruits; S, Mean number of seeds/fruit.

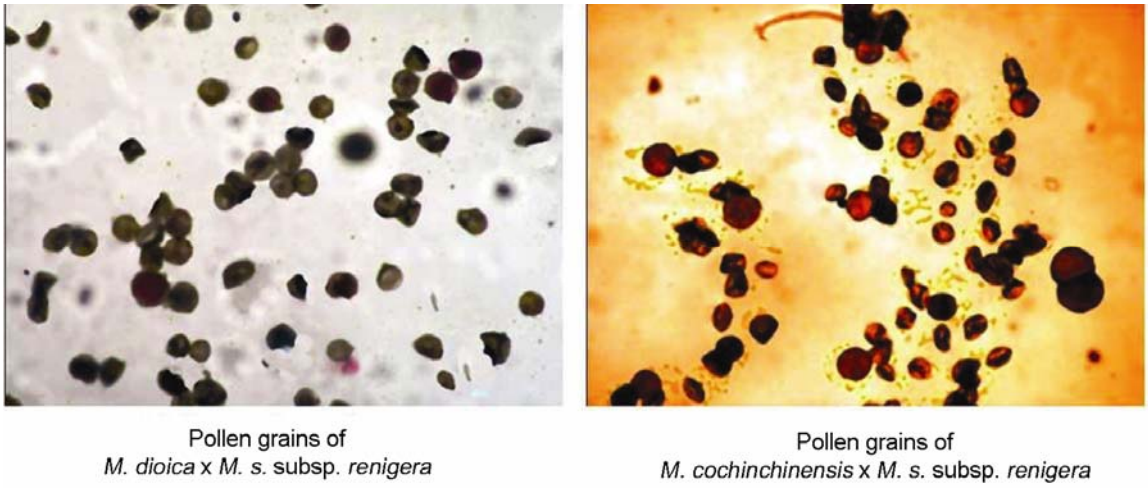

Figure 6. Pollen of F_1 hybrid between *M. cochinchinensis* × *M. s.* subsp. *renigera* and *M. dioica* × *M. s.* subsp. *renigera*.

sustain the fact that the wild variety (*M. c.*var. *muricata* syn. with *M. c.*var. *abbreviata* Ser.) is the progenitor of cultivated bitter melon[8,9]. The high crossability holds the potential for improvement of cultivated bitter melon from the ample variation available within the wild variety carrying fruit-fly tolerance[10].

Fertile hybrids when formed are usually from the crosses between 'closely related' species[11]. However, *M. charantia* has been reported to be closer to *M. angolensis* and *M. balsamina* to *M. involucrata*[12] and *M. foetida*[13], but the African species (*M. angolensis*, *M. involucrata* and *M. foetida*) were not included in the present study. It was difficult to obtain hybrids between *M. charantia* × *M. balsamina* as reported by Singh[14]. However, the sole hybrid obtained was moderately fertile, which may be due to its high bivalent frequency with normal meiotic behaviour[14]. These results coupled with morphological[15], karyo-morphological[6,14,16] and molecular[17] results reinforce the viewpoint that *M. charantia* and *M. balsamina* are intimately related, but distinct and probably stabilized by reproductive isolation due to fertilization barriers.

High fruit set (on selfing and backcrossing) and fair pollen stainability of F_1 hybrids between *M. dioica* and *M. sahyadrica* indicated that these two species are closely related. It is supported by the fact that *M. dioica* is considered to be the progenitor of *M. sahyadrica*[18] and *M. dioica* shows closer morphological similarity with *M. sahyadrica*[19]. *M. sahyadrica* and *M. dioica* were crossable with *M. cochinchinensis* as a female parent only and the pollen fertility of F_1 hybrid also was very low (0.85–1.15%), which indicated that *M. dioica* and *M. sahyadrica* are partially related to *M. cochinchinensis* as reported based on morphology[20] and meiotic pairing[21].

M. s. subsp. *renigera* was the only species which had reproductive compatibility in both the directions with

M. cochinchinensis and among the triploid hybrids backcross progeny could be obtained only in the hybrid between *M. dioica* and *M. s.* subsp. *renigera*, demonstrating the close relationship among these species. The triploid hybrids between diploids (*M. dioica* and *M. cochinchinensis*) and tetraploid (*M. s.* subsp. *renigera*) were nearly sterile/partially fertile. However, *M. dioica* and *M. cochinchinensis* are suggested as putative parents of *M. s.* subsp. *renigera* through morphological and cytological evidence[20,21]. This low fertility may be due to pure cytological reasons and the relationships, not because of their low relativity. The result is further supported by the diversity analysis of plastid, mitochondrial and nuclear DNA, wherein *M. dioica*, *M. cochinchinensis* and *M. s.* subsp. *renigera* are nested within a clade[12]. The accessions of *M. cochinchinensis* are more robust in vegetative as well as reproductive characters and their potential use in improvement is yet to be explored.

M. cymbalaria (sect. *Raphanocarpus*) was not crossable with any species of other sect. because it is genetically isolated from other Asian species and is reported to be closer to the African species like *M. humilis*, *M. kirkii*, *M. boivinii* and *M. sessilifolia*[12] and to *M. cabraei*[13]. Bharathi *et al.*[6,17] also highlighted its distinctness from other *Momordica* species of Indian occurrence based on molecular and karyo-morphological evidence. It is possible that *M. cymbalaria* originated along with other African species from a progenitor species different from the dioecious *Momordica* species of Indian occurrence[17].

Morphology of the hybrids was generally intermediate between their parental species. The colour of the petals and calyx was intermediate between the parents. However, characters like lobed leaves and black spot on the petals, wherever present in a cross-combination, were expressed invariably in F_1 hybrids. Resemblance to either of the parents and intermediate character expression was attributed to dominance and partial dominance gene action respectively. The mitotic chromosome numbers in the interspecific hybrids were as expected from the numbers of their parents.

There have been few attempts at crosses between different sections of the genus *Momordica*. Crosses have been made between sect. *Momordica* (*M. charantia*, *M. balsamina*) and sect. *Cochinchinensis* (*M. dioica*) to seek possibilities of transferring desirable attributes of the latter (especially the ‘bitterless’ trait) to the former, but none succeeded[22], indicating the lack of genetic affinity between them. Later studies on the reason for their incompatibility indicated poor pollen germination and inhibition of growth of pollen tubes in the upper part of the style before reaching the embryo sac for effective pollination[16]. Obstruction in transfer of male gamete and fertilization has also been attributed to abnormal behaviour of pollen tubes and heavy deposition of callose at the pollen tube tips[23]. *M. charantia* and *M. balsamina* failed to cross with spiny fruited species (*M. dioica*, *M. sahyadrica*, *M. cochinchinensis*, *M. s.* subsp. *renigera*) indicating that they are genetically distant and had evolved along a separate line diverging from spiny fruited species. The production of parthenocarpic fruits (Figure 2) in the cross between sect. *Momordica* and sect. *Cochinchinensis* needs to be studied further for their practical utility.

Five major patterns of crossing behaviour emerged from the results of the crossing experiments in *Momordica* spp. of Indian occurrence in the present study. (i) Cross-compatibility with pollen fertility (*M. charantia* var. *charantia* × *M. charantia* var. *muricata*, and *M. dioica* × *M. sahyadrica*). (ii) Partial compatibility with pollen fertility (*M. charantia* × *M. balsamina*). (iii) Cross-compatibility with pollen sterility [between diploid species (*M. dioica*, *M. sahyadrica*, *M. cochinchinensis*) and tetraploid species (*M. s.* subsp. *renigera*)]. (iv) Partial compatibility with pollen sterility (*M. dioica* × *M. cochinchinensis* and *M. sahyadrica* × *M. cochinchinensis*). (v) Cross incompatibility between sections.

Further, the present study has brought out the closer relationship between *M. charantia* and *M. balsamina*; *M. dioica* and *M. sahyadrica*, *M. dioica* and *M. s.* subsp. *renigera* and *M. cochinchinensis* and *M. s.* subsp. *renigera*. Our conclusions about the genetic relatedness of the species sustain the recent phylogeny of *Momordica* species based on three genomes[12]. The poor germination of F_1 seeds, unsatisfactory growth and flowering in F_1 seedlings and partial to complete sterility in F_1 indicate the rather limited potential of inter-specific hybrids from these species in conventional crop improvement. In many cases, this sterility was associated with meiotic abnormalities and was a large obstacle that followed hybridization and hindered fertilization. However, F_1s can be backcrossed with cultivated species and after few generations of backcrossing cytologically stable derivatives with desired selected traits can be obtained. The transfer of traits depends on the degree of homology between the two genomes. Another alternative is the production of amphiploids by doubling the chromosomes of interspecific F_1 hybrid by colchicine treatment.

Gene pool classification

Gene pool serves as a tool for conceptualizing the ability of plant population to cross with the conspecific population and those of other species[24]. Based on the present study and available literature on inter-specific hybridization, the following gene pool classification of the cultivated/semi-domesticated species is proposed.

M. charantia: The primary gene pool can be divided into two subclasses, I & II. Gene pool I is formed by its various commercial cultivars and land races as well as populations of the wild taxa var. *muricata*. Based on the next level of compatibility of *M. balsamina* with *M. charantia*, it is placed in primary gene pool II. The dioecious species (*M. dioica*, *M. sahyadrica*, *M. s.* subsp. *renigera*, *M. cochinchinensis*) represent the tertiary gene pool.

M. dioica: None of the dioecious species is reproductively isolated from the others completely. The primary gene pool is represented by its land races/varieties and *M. sahyadrica*. Its secondary gene pool includes *M. cochinchinensis* and *M. s.* subsp. *renigera* whereas all the monoecious species are included in the tertiary gene pool.

M. s. subsp. *renigera*: As it is a tetraploid species and the rest are diploids, the hybrid progeny are triploid and sterile. Therefore, the primary gene pool does not involve any species; the secondary gene pool includes the rest of the diploid dioecious species and the tertiary gene pool is formed by monoecious species.

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