

On the occurrence, distribution, taxonomy and genepool relationship of *Cucumis callosus* (Rottler) Cogn., the wild progenitor of *Cucumis melo* L. from India

K. Joseph John · Sheen Scariah ·
V. A. Muhammed Nissar · M. Latha ·
S. Gopalakrishnan · S. R. Yadav · K. V. Bhat

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Abstract Recent research point to the Indian wild taxa of *Cucumis callosus* (Rottler) Cogn. as the wild progenitor of melon (*C. melo* L.). Overlapping distribution with cultivated and weedy and feral forms of *melo* and normal fertility of F₁ and BC₁ generations of its cross with cultivated melon indicate its progenitor status. A perusal of herbarium data indicate its natural distribution pattern in the region comprising Vindhya Hills and Aravalli mountain ranges extending northwards to Indo-Gangetic plains and southwards to the Deccan plateau touching rain shadow areas of Western Ghats. Characterised by drought tolerance and field resistance to a host of pests and diseases, it is conspicuously absent in the high rainfall areas of Western Ghats and upper Himalayan region. Based on its morphological distinction and F₁ and BC₁ fertility

with *C. melo*, a subspecific rank within *C. melo* is postulated. First-hand information on its occurrence, distribution and crossability relationship with other Indian taxa of *Cucumis* are given.

Keywords *Cucumis callosus* · Distribution · Gene pool relationship · Progenitor of melon · Taxonomy wild melon

Introduction

Melon (*Cucumis melo* L.) is an important horticultural crop across wide areas of the world and their use is extremely diverse, depending on the type of fruit (Akashi et al. 2002). In India, characterised by different agro-ecological ranges and climatic patterns, extensive variation in cultivated melon types ranging from oriental pickling melon (*C. melo* L. var. *conomon* Thunb.), zarda (*C. melo* L. var. *maltensis* Ser.), snap melon (*C. melo* L. var. *momordica* Roxb.), snake melon (*C. melo* L. var. *flexuosus* (L.) Pangalo) and musk melon (*C. melo* L. var. *cantaloupensis* Heberle) are found besides the feral form, *C. melo* L. subsp. *agrestis* Naud.

Some of the basic questions to be answered regarding the evolution of cultivated plants are their geographic origin, progenitor taxa and region of domestication (Vavilov 1935). Cucumber (*C. sativus* L.) is believed to have originated in India and there is

K. J. John (✉) · S. Scariah · V. A. M. Nissar · M. Latha
National Bureau of Plant Genetic Resources, Regional
Station, KAU PO, Thrissur, Kerala 680656, India
e-mail: josephjohnk@rediffmail.com;
josekattukunnel@gmail.com

S. Gopalakrishnan
Genetics Division, Indian Agricultural Research Institute,
Pusa Campus, New Delhi 110012, India

S. R. Yadav
Shivaji University, Kohlapur 416004, India

K. V. Bhat
National Bureau of Plant Genetic Resources,
Pusa Campus, New Delhi 110012, India

unanimous agreement on that count due to the prevalence of its wild progenitor *C. sativus* f. *hard-wikii* (Royle) W. J. de Wilde et Duyfjes across the country and also due to linguistic evidences (Fuller 2006, 2007). However, when it comes to the origin and domestication of melon, majority opinion favours a sub-Saharan African origin with India as a secondary centre (Kerje and Grum 2000; Luan et al. 2008; Perin et al. 2002; Whitaker and Bemis 1976; Whitaker and Davis 1962). As pointed out by Sebastian et al. (2010), the impressive species richness in Africa sharing the $2n = 24$ chromosome number which is same as melon prompted many to support this view. However, the greatest diversity in cultivated landraces is to be found in Asia (Akashi et al. 2002; Dwivedi et al. 2010; Tanaka et al. 2007). Further, successful recovery of F_1 offsprings could not be achieved from the crosses of *C. melo* with various African species of *Cucumis* (Sebastian et al. 2010). These considerations prompted recent workers to think in favour of an Asian origin of melon and Sebastian et al. (2010) postulated an Indian origin for *Cucumis melo* from *C. callosus* (Rottle) Cogn. et Harms. In fact, Parthasarathy and Sambandam (1989) based on their observations of free compatibility with *C. melo* and normal behaviour of chromosomes during diakinesis, had proposed *C. callosus* of India as the progenitor of *C. melo*.

Over the past decade, based on the synthesis of a large quantum of new archaeological and genetic evidences, archaeobotanists have postulated the process of domestication as a protracted and diffuse process, progressing parallel in different locations around the Near East (Allaby et al. 2010; Fuller et al. 2011), as against the earlier notion of a focused, single process in the Near East, where the whole package of 'founder crops' emanating from a core area at essentially the same time (Abbo et al. 2010; Lev-Yadun et al. 2000). Archaeological evidences suggest that melons were widespread in Egypt, Arabia, India, and China by 2000 BC and in all likelihood, its cultivation started in more than one region with at least two domestication events, one in India and the other in the Near East (most likely Egypt) (Zohary and Hopf 2000), and probably three (Egypt, India/Pakistan, Lower Yangtze) (Fuller (2012). Of particular interest is the recovery of seed remains (by flotation) of *Cucumis* sp. (comparable to *C. prophetarum* L., or perhaps *C. trigonus* Roxb. = *C. callosus*) from two

Southern Neolithic sites in Deccan Plateau of South India (1,800–1,200 cal. BC) (Fuller et al. 2001), though not confirmed by direct AMS dating. However, archaeological reports may be of limited value for pinpointing areas and the probable routes of melon or cucumber domestication, as precise identification of fossil seeds of cucumber and melon are extremely difficult (Sebastian et al. 2010).

In the most well quoted revision of *Cucumis* (Kirkbride 1993), *C. callosus* is treated as a synonym of *C. melo*. He followed Jeffrey (1980) in arriving at such a conclusion. However, all the earlier botanists like Gamble (1919), Naudin (1859), Roxburgh (1832) recognise this entity as a distinct species. The veteran botanist CB Clarke (1879) observes that Naudin stressed the perennial nature of the root as a distinguishing character from *C. melo*. However, he merged the entity *C. pubescens* Willd. (presently equated with *C. melo* subsp. *agrestis*) with *C. trigonus* Roxb. Kurz (1877) who studied Bengal (India) plants separated *C. trigonus* characterised by solitary peduncles from *C. pubescens* with clustered peduncles and made the latter a variety of *C. melo*. This concept of Kurz (1877) prevails even to this date in the Indian botanical circles. Verma and Pant (1985) treated *C. trigonus* as synonym of *C. callosus*.

Chakravarthy (1959; 1982) and Mathew (1983) retained its separate species status from that of *C. melo* and *C. melo* subsp. *agrestis*. Mathew (1998) has given illustrations of the plant based on his study of the taxa in the Flora of Tamilnadu Carnatic. Narrating the history of taxonomic treatments of *C. callosus*, Nesom (2011) aptly states that "diversity and ambiguity of interpretation are widespread". Hence, its treatment as a synonym of *C. melo* (Kirkbride 1993), without even assigning a subspecific rank, warrants a re-examination of its specific status.

A sound knowledge of the morphological and biological features and correct taxonomic identities is a prerequisite for successful use of germplasm in cucumber and melon breeding (Kristkova et al. 2003; Renner and Schaefer 2008). Wild species are rich reservoir of useful genes which are not present in cultivated gene pool (Tanksley and McCouch 1997). It is in this context that we have carried out an investigation into the morphology, taxonomy, distribution, stress tolerance and biosystematic relationship of this less known but potential wild melon from India.

Materials and methods

Seeds of *Cucumis callosus* (IC 550203, IC 550180, IC 550196 and IC 550202) were obtained from NBPGR Regional Station, Jodhpur and raised in pots along with all other *Cucumis* taxa of Indian occurrence under insect-proof net house during post-monsoon (October–April) season in 2008–2009, 2009–2010 and 2010–2011. We have also studied live specimens in the field. Intra-specific classification as adopted by Jeffrey (2001) and Pitrat et al. (2000) was followed for cultivated melon classification. All the accessions were morphologically characterised using the descriptor and descriptor states developed by NBPGR (Mahajan et al. 2001) and modified following Kirkbride (1993) and Kristkova et al. (2003). Herbarium survey was carried out at Central National Herbarium, Kolkata (CAL), Calicut University Herbarium (CALI), Botanical Survey of India, Pune (BSI) and Madras Herbarium, Coimbatore (MH) and a total of 108 sheets of *Cucumis* were examined. Herbarium label information was used to derive latitude and longitude of the locality. This along with passport data information was used to plot distribution map using DIVA–GIS software.

Direct and reciprocal crosses were attempted with *C. melo* L. var. *conomon* Thunb., *C. melo* L. var. *momordica* Roxb., *C. melo* L. var. *maltensis* Ser., *C. melo* L. subsp. *agrestis* Naud., besides *C. sativus* L., *C. prophetarum* L., *C. silentvalleyi* (Manilal, T. Sabu et P.J. Mathew) Ghebret. et Thulin, *C. indicus* Ghebret. et Thulin and *C. hystrix* Chakrav. Hybrid fruit set and growth were monitored and F₁ and BC₁ generations raised in successful crosses. Reaction to various biotic and abiotic stresses were recorded under natural epiphytotic conditions in the characterisation plot.

Results and discussion

As there is still no unanimous agreement on specific status of *C. callosus*, its morphology was studied in detail along with cultivated and feral forms of *melo*. The results are presented in Table 1 and Fig. 1.

C. callosus can be distinguished from other entities of the genus by its tuberous tap root, deeply lobed and upwardly curved strong yellowish green coloured leaf lamina, drooping branches, visibly white long hairy

tomentose ovary, u-shaped curved pedicel of female flowers, brilliant greenish yellow coloured corolla, round or obovoid fruit with ten prominent white longitudinal stripes and thick shining epicarp.

Morphological key to distinguish *C. callosus* from other subcategories of *C. melo*

Based on comparative study of morphology a key is presented for easy identification of the taxa.

Leaves deeply 5–7 lobed, male flowers solitary, roots tuberous, perennials—*C. callosus*

Leaves unlobed (or moderately lobed), male flowers in fascicles, roots non-tuberous, annuals

Stem slender, flowers with diameter below 4 cm, pedicel slender, fruits very small below 50 g, often bitter, seeds below 5 mm in length, weedy and feral

C. melo subsp. *agrestis*

Stem robust, flowers with diameter above 4 cm, pedicel robust, fruits large and above 50 g, often non bitter, seeds more than 6 mm in length, cultivated

C. melo subsp. *melo*

Distribution

Chakravarthy (1959) reported its historical distribution between 40° N and 40° S of the Equator comprising India, Pakistan, Iran, Afghanistan and parts of North Africa running eastwards to Malaysia, China, and Australia. However, Oliver (1979) did not make any mention of it in his ‘Flora of Tropical Africa’. In India, it has been found to occur naturally in the wild state in whole of Deccan Plateau and Indo-Gangetic plain comprising the states of Tamil Nadu, Karnataka (barring coastal Karnataka and Western Ghats), Maharashtra, Madhya Pradesh, Uttar Pradesh, Jharkhand, Chattisgarh, Orissa, West Bengal, Punjab, Haryana, Assam and Himachal Pradesh (Fig. 2). However, it is conspicuously absent in the high rainfall humid regions comprising Western Ghats and Eastern Himalayas. We have examined all the herbarium sheets listed by Chakravarthy (1959) in his monograph of Indian Cucurbitaceae. Contrary to his

Table 1 Comparative morphology of *C. callosus*, *C. melo* cultivars and *C. melo* subsp. *agrestis*

S. no.	Character	<i>C. callosus</i>	<i>C. melo</i> cultivars	<i>C. melo</i> subsp. <i>agrestis</i>
Qualitative characters				
1	Life span	Perennial	Annual	Annual
2	Tap root	Tuberous	Non tuberous	Non tuberous
3	Stem type	Slender	Robust	Moderately slender
4	Branching	Less, drooping	Branching, procumbent	Highly branching, procumbent
5	Petiole	Slender	Robust	Moderately robust
6	Leaf lobing	Deeply lobed	Unlobed	Unlobed
7	Leaf lamina shape	Sub-orbicular	Sub-orbicular/more or less reniform	Sub-orbicular
8	Leaf pubescence	Hirsute	Villose	Villose/sub hairsute
9	Male flowers	Solitary	Fascicles of 4–9	Fascicles of 3–5
10	Petal colour	Light greenish yellow	Bright yellow	Bright yellow
11	Female flower pedicel	Looped, U shaped	Slightly curved	Slightly curved
12	Fruit shape	Round/obovoid	Polymorphous	Oblong or turbinate
13	Ovary pubescence	Tomentose	Pubescent/puberulent	Pubescent
14	Seed funicle	Mucronate	Acute	Acute
15	Seed size	Medium, bulged	Big	Small
16	Shelf life of fruits	Longer duration (12 months or more)	Short duration(few days) (3–6 months for var. <i>conomon</i>)	Short to medium duration (varying)
17	Fruit pulp taste	Bitter	Sour	Bitter/sour
18	Dispersal	Difficult as fruit wall is intact	Easy as fruit wall crack or disintegrate	Moderately easy
Quantitative characters				
1	Leaf length	6–7.5 cm	6.5–9.5 cm	6–7.5 cm
2	Flower diameter	2 cm	4–6 cm	2–4 cm
3	Pedicel length	0.5–1 cm	1–2.5 cm	Up to 1 cm
4	Calyx tube	2–4 mm	6–8 mm	3–5 mm
5	Anther length	2 mm	3–4 mm	1.5–3 mm
6	Fruit length	3.5–5 cm	7–20 cm	2.5–6 cm
7	Fruit diameter	3–5.2 cm	8–15.5 cm	2.8–4.5 cm
8	Fruit circumference	12.4–13.2 cm	22.3–43 cm	8–11.3 cm
9	Single fruit weight	13–21 g	150–1,250 g	10–30 g
10	Flesh thickness	0.4–0.5 cm	1.4–3.5 cm	0.4–0.6 cm
11	Seed length	6.61–7.22 mm	7.8–9.37 mm	4.18–4.94 mm
12	100 seed weight	1.6–1.7 g	1.7–1.9 g	1.3–1.6 g

observations, the sheets identified by him as *C. callosus* from Kerala and Lakshadweep are actually *C. melo* subsp. *agrestis*. Also, we have collected and studied live samples from Lakshadweep from the localities mentioned by him, but these populations are invariably *C. melo* subsp. *agrestis*.

A total of 33 sheets in Botanical Survey of India, Kolkata (CAL) were labelled as this entity, which include a few old sheets without precise locality details. Some of them are *C. melo* subsp. *agrestis*. The typical ones are cited below for further reference of users.

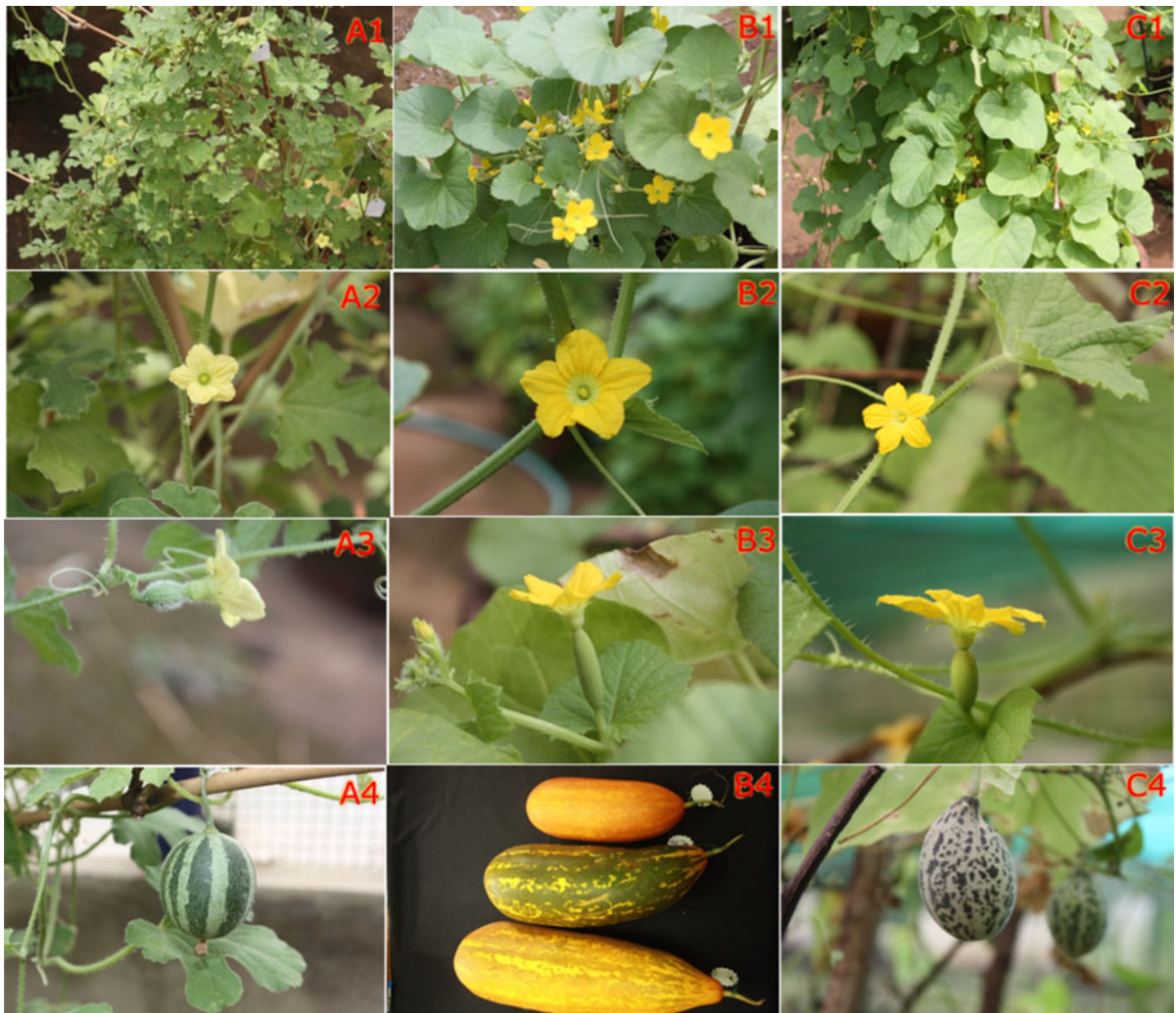


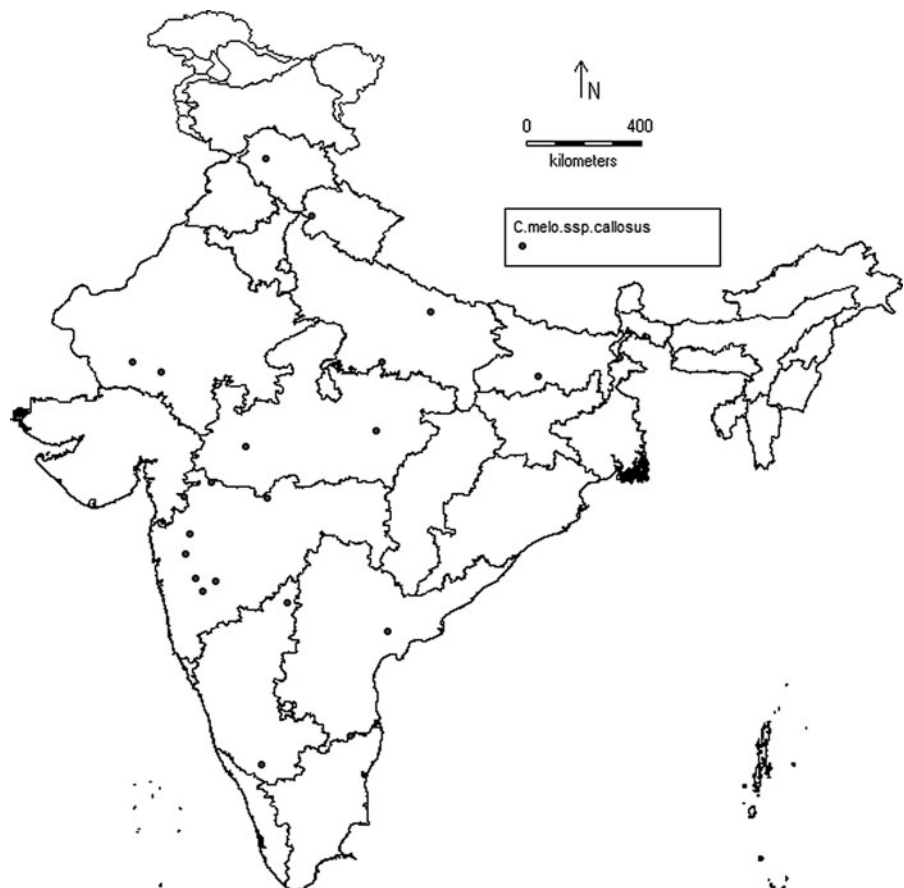
Fig. 1 Comparative morphology: A1–A4: habit, male flower, female flower and fruit of *C. callosus*; B1–B4: habit, male flower, female flower and fruit of *C. melo* var. *conomon*; C1–C4: habit, male flower, female flower and fruit of *C. melo* subsp. *agrestis*

Specimen examined and confirmed

Pillai P, 32884, Rajgir WLS, Bihar, 13.09.03; Subbarao GV, 46764, Anandakonda, Chittore, Andhra Pradesh, 27.12.1975; Hooker, 39585, Chilka Lakeside, Ganjam, Orissa, 10.08.1913; Kumarsen, 130407, Kangra, Himachal Pradesh, 28.09.1894; R K Mohan, 255, Rallapadu, Prakasam District, Andhra Pradesh, 19.10.1983; G King, 182070 (Ac), Dehradun, Uttar Pradesh, 24.03.47; AS Bell, 182067(Ac), Banda, Uttar Pradesh, 1902; P Mukerjee, 182081(Ac), Indore, Madhya Pradesh, 30.08.1889; S Kurz, 182080 (Ac), Ranigunj, West Bengal. s.n.

A perusal of the herbarium and passport data indicate that germplasm collection trips for this taxa should centre around the open sunny localities in the districts of Coimbatore, Salem, Tirunelveli, Ramnad, Chengulpet, Sivaganga and Rajapalayam in Tamil Nadu, Prakasam, Krishna, Chittur, Nellore, Rengareddi, Medak and Hyderabad in Andhra Pradesh, Bellary in Karnataka, Dhule, Thana and Pune in Maharashtra, Ganjam in Orissa, Ranigunj, Jalsuka and Sunderbans in West Bengal, Indore and Jabalpur in Madhya Pradesh, Raipur in Chattisgarh, Dehradun in Uttaranchal, Banda, Gonda, Agra, Baraich and Saharapur in Uttar Pradesh, Jodhpur in Rajasthan, Rajgir

Fig. 2 Distribution of *C. callosus* in India



and Sahibgunj in Bihar, Sutlej river bank in Punjab and Palamau and Ranchi in Jharkhand.

Ecology and phenology

It grows on various types of soils; black cotton soil, clayey loam, gravely soil and sandy loam with slightly alkaline pH. It grows well in the arid environment with low relative humidity. The plant is very common throughout on sandy soils, prostrate or climbing on field hedges (Bhandari 1978). Subterranean sprouts emerge from rootstocks with pre-monsoon showers and come to flowering and fruiting from July to December in Deccan Plateau, East India and Indo-Gangetic plains. Seeds germinate with pre-monsoon showers and takes around 60–70 days for flower initiation. Anthesis takes place between 05.00 AM to 05.30 AM. Ideal time for pollination for maximum fruit set is between 07.00 and 09.00 AM.

Unlike other *Cucumis* species, the fruits continue to remain on vines for months together without any abscission. However, seeds extracted from 90 days old fruits were found to be fully viable, indicating a requirement of less than 90 days for physiological seed maturity.

Stress tolerance

We have observed its potential for tolerance to extreme drought, growing and reproducing for many months, when potted plants were kept in a rain-proof polyhouse without any extraneous supply of water. It also survived a severe epidemic of Spidermite (*Tetranychus neocaledonicus*) in 2009. Earlier workers had reported its resistance to fruit fly and *Fusarium* wilt (Chelliah and Sambandham 1971; Sambandam and Chelliah 1972). It was found consistently resistant to fruit fly (*Bactrocera daucus*) under field conditions as

Table 2 Details of successful crosses of *Cucumis callosus*

S. no.	Parents	Flowers pollinated (no.)	Fruit set (no.)	Fruit set (%)	Seeds
1	<i>C. callosus</i> × <i>C. callosus</i>	23	15	65.23	Filled, viable
2	<i>C. melo</i> ^a × <i>C. callosus</i>	95	14	14.73	Filled, viable
3	<i>C. callosus</i> × <i>C. melo</i> ^a	18	1	6 ^b	Filled, viable
4	<i>C. melo</i> ^a × <i>C. melo</i> ^a	37	20	54	Filled, viable.
5	<i>C. melo</i> ^a × <i>C. melo</i> var. <i>agrestis</i>	39	11	28.21	Filled, viable
6	<i>C. melo</i> var. <i>agrestis</i> × <i>C. melo</i> ^a	158	59	37.34	Filled, viable
7	<i>C. melo</i> var. <i>agrestis</i> × <i>C. melo</i> var. <i>agrestis</i>	30	8	27	Filled, viable
8	<i>C. sativus</i> ^a × <i>C. callosus</i>	28	8	28.57	Unfilled, not viable
9	<i>C. callosus</i> × <i>C. sativus</i> ^a	54	0	0	No fruit set

^a Cross include subcategories of the species

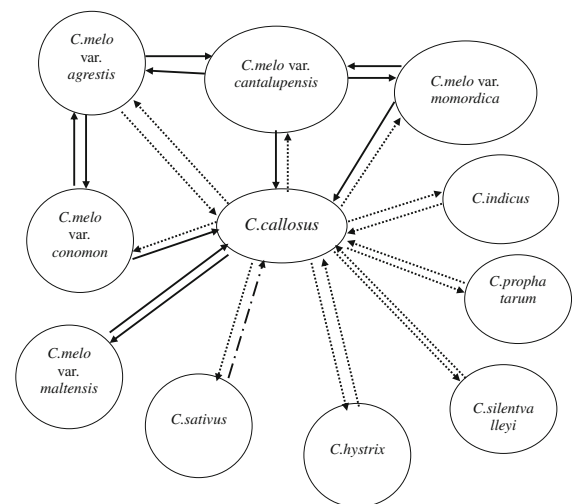
^b Successful fruit set only with var. *maltensis* (Zarda)

Thrissur region is a hotspot for fruit fly on various cucurbits. Fruits remain intact on vines for more than 3 months and this trait may contribute to extended shelf life. Fruit skin is intact without any crack and was dominant in the F₁ and F₂ of crosses with snap melon (*C. melo* var. *momordica*), which is characterised by rupture of fruit skin and softening of edible tissues. Fruit flesh is not mealy or granular, but firm. The pulp and flesh are highly bitter.

Crossability relationships

The cross between *C. callosus* and other taxa of Indian occurrence is presented in Table 2. Among 195 crosses attempted, cross compatibility was observed only in the crosses involving different taxa of *C. melo*. Fruit set without viable seeds was observed when *C. callosus* was used as a pollen parent with different taxa of *C. sativus*. In the crosses involving *C. callosus* with different species of *Cucumis* studied other than *C. sativus* and *C. melo* no fruit set was observed. The crossability relationship of *C. callosus* with other taxa studied is presented in crossability polygon (Fig. 3).

A perusal of Table 2 and Fig. 3 clearly shows that *C. callosus* falls in the primary gene pool of *C. melo*. However, even with hand pollination at optimum stigmatic receptivity, direct crosses yielded only 15 % fruit set and reciprocal only 6 % as compared to 65 % in the case of selfing (assisted pollination). Reciprocal crosses with *C. melo* var. *conomon*, var. *momordica* and var. *cantaloupensis* failed to set fruits; but, var.



CROSSABILITY POLYGON OF *CUCUMIS CALLOSUS* WITH OTHER TAXA OF *CUCUMIS*

- Indicate the direction of cross
- Freely crossable with the production of fertile F₁ (Fruit set and seed set)
- Not crossable, no fertile F₁ (No fruit set and seed set)
- - - - Formation of fruits, but unfilled seeds

Fig. 3 Crossability polygon of *C. callosus* with other taxa

maltensis produced fully developed mature ripe fruit with 232 healthy seeds. We haven't come across any natural hybrids of *C. melo* × *C. callosus* and vice versa during the past 3 years when both were grown side by side. Similarly, direct (27 flowers) and reciprocal (97 flowers) crosses with *C. melo* subsp. *agrestis* failed to set fruits. Pollinator specificity and other barrier mechanism, if any, need to be further investigated. Contrary to this, the entities *melo* and

agrestis show more intimate gene transfer in nature. Natural hybrids, intermediate between cultivated and wild/feral, are met with occasionally in farmers' field and often they are bitter, contaminating the main produce. This again reiterates our stand that *C. callosus* even while falling within the primary gene pool of *C. melo*, is distinct from both cultivated melon and feral *agrestis* whereas *melo* and *agrestis* forms are much closer. Crossability studies indicate its placement in the primary gene pool of *C. melo* under the broader biological species concept of *C. melo*. F₁ and BC₁ of *C. melo* var. *conomon* and *C. callosus* were found to be fully fertile, the F₁ being intermediate between parents for quantitative traits (Fig. 4). Details of the performance of F₁ and BC₁ will be published elsewhere.

Kirkbride (1993) refers to Australian forms of *C. melo* with highly dissected leaves and unusual pubescence on the female flower hypanthium and suggested a need for further biosystematic studies to understand and accommodate the variation. The material mentioned may be *C. callosus* in all probability. Kirkbride (1993) recognised *agrestis* and *melo* as two subspecies of *Cucumis melo*. Our candidate taxa is certainly wide apart from both subspecies *melo* and subsp. *agrestis*. But cultivated *melo* and wild and feral *agrestis* forms are morphologically much closer

to each other except for plant and fruit size. This similarity indicates the divergence of cultivated *melo* from *C. callosus* with this latter entity as a common ancestor for both *melo* and *agrestis*. Morphological variation parallel to that of cultivated *melo* is observed in wild and weedy *agrestis*. During the course of domestication, wild traits like bitterness, small fruit size, long maturity periods, hard flesh and resistance to biotic and abiotic stress could have been lost.

The low genetic variability in cultivated melon was emphasised by many authors (Neuhausen 1992; Shattuck-Eidens et al. 1990). Incorporation of genes from wild species have been advocated for broadening the genetic base of melon/cultivated crops and transfer of several useful traits related to abiotic and biotic stress tolerances. A wider distribution across 75,000 km² stretch encompassing diverse agro-ecological zones touching Himalayan foothills to Eastern Ghats, Aravalli and Vindhya Mountains and arid Deccan plateau indicates possibility for collection of variability in this wild taxa. Utilisation of this taxa in melon improvement will lead to the development of varieties with extreme drought tolerance, resistance to fruit fly, powdery mildew, Fusarium wilt and spider-mite, long shelf life, extended harvest time and non cracking of skin and mesocarp. Most of the problems

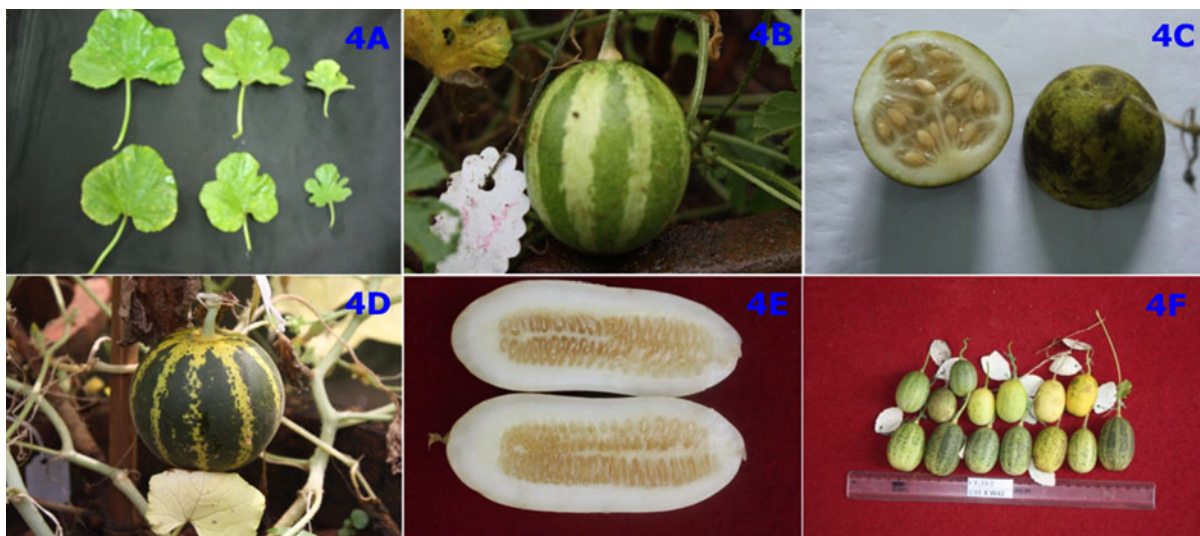


Fig. 4 Successful crosses of *C. callosus* with *C. melo* cultivars and *C. melo* subsp. *agrestis*: **a** Leaf of *C. melo*, F₁ hybrid and *C. callosus*; **b** Fruit of cross (*C. callosus* × *C. melo* var. *maltensis*); **c** Cross section of crossed fruit (*C. callosus* × *C. melo* var.

maltensis); **d** F₁ of *C. melo* var. *conomon* × *C. callosus*; **e** Longitudinal section of crossed fruit (*C. melo* var. *conomon* × *C. callosus*); **f** F₁ of *C. melo* var. *momordica* × *C. callosus*

encountered in wide hybridisation like non-adaptability of wild taxa, non-synchronous flowering, and pre and post fertilization barriers are not observed in *C. callosus*–*melo* hybridisation programme. However, correct taxonomic delineation and identification is of importance to facilitate the use of these wild species in crop improvement programmes. Assigning a distinct taxonomic status is also important from conservation point of view, lest the entity may not get adequate representation in *ex situ* gene banks.

Conclusions

Our studies on morphology of *C. callosus* support the viewpoint of Sebastian et al. (2010) for a separate taxonomic status for it. However, crossability and F₁ fertility with *C. melo* does not permit a separate species status, different from that of *melo*. Based on this study we conclude that *C. callosus* is a distinct taxa easily distinguishable from cultivated *C. melo* and wild *C. melo* subsp. *agrestis*. However, as the cross derivatives with *melo* are fertile, it deserves to be assigned only a subspecific rank within *C. melo*.

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