Chapter 6
Cucumis

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6.1 Introduction

The genus *Cucumis* L. is one of the important genera of flowering plants. It includes cucumber (*C. sativus* L.) and melon (*C. melo* L.), two of the most economically important and widely cultivated vegetable crops in the world (Pitrat et al. 1999). However, cucumber and melon suffer from a range of devastating fungal, bacterial, viral, and insect diseases (Whitaker and Davis 1962). The wild *Cucumis* species are of economic interest because they are a reservoir of potentially useful genes such as biotic stress resistances. A comprehensive knowledge of these wild species is of great importance for conservation and utilization of genetic resources that can be employed for cucumber and melon improvement.

6.2 Basic Botany of the Genus *Cucumis*

6.2.1 Taxonomy

Cucumis belongs to the family Cucurbitaceae, subfamily Cucurbitoideae, and is currently placed in the tribe Benincaseae (Jeffrey 2005). According to Kirkbride (1993), the genus *Cucumis* is represented by 32 species, among which two species of *Cucumis*, *C. sativus* L. (Cucumber) and *C. melo* L. (melon), are of great commercial importance. Besides cucumber and melon, the species *C. anguria* (West Indian gherkin) and *C. metuliferus* (African horned cucumber) are commercially cultivated in several areas as well (Garcia-Mas et al. 2004). Other wild species originating mostly from arid and/or semi-arid regions of Africa are cultivated as ornamental plants (e.g., *C. dipsaceus* – "hedgehog gourd" and *C. myriocarpus* – "gooseberry gourd") (Rubatzky and Yamaguchi 1997).

Taxonomy of *Cucumis* was first described by Linnaeus in 1753 (Ghebretinsae et al. 2007). According to this classification, the genus *Cucumis* contains seven species, all of which were cultivated or economically useful. There have been a number of taxonomic placements of *Cucumis* since the work of Linnaeus (Pangalo 1950; Jeffrey 1962, 1967, 1980, 1990; Kirkbride 1993; Schaefer 2007).

The most comprehensive placement of *Cucumis* was proposed by Kirkbride (1993). On the basis of his investigations, the genus *Cucumis* was divided into two subgenera with different geographical origin and basic chromosome numbers. Subgenus *Melo* (30 spp., $n = 12$) was originated in Africa and was partitioned into two sections (*Melo* and *Aculeatosi*), whereas subg. *Cucumis* (two spp., $n = 7$) was originated in Asia. A detailed taxonomic depiction of the genus *Cucumis* elaborated by Kirkbride (1993) is given in Table 6.1. However, this taxonomic treatment was challenged by the rediscovery of *C. hystrix*, a wild *Cucumis* species of Asian origin possessing 24 chromosomes. *C. hystrix* ($2n = 24$) was successfully crossed with cucumber (*C. sativus*, $2n = 14$) (Chen et al. 1997). A new species *C.* × *hytivus* Chen and Kirkbride was proposed in 2000 followed by chromosome doubling of the F_1 hybrid (Chen and Kirkbride 2000).

Recently, on the basis of molecular phylogenetic studies, 19 species of five genera including *Cucumella*,

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Table 6.1 Taxonomy of the genus *Cucumis* (Kirkbride 1993)

Cucumis spp.	Chromosome number (*n*)
Subgenus *Melo*	
Section *Aculeatosi*	
Serie *Myriocarpus*	
C. myriocarpus	
subsp. *myriocarpus*	12
subsp. *leptodermis*	12
C. africanus	12
C. quintanilhae	–
C. heptadactylus	24
C. calahariensis	–
Serie *Angurioidei*	
C. anguria	
var. *anguria*	12
var. *longaculeatus*	12
C. sacleuxii	12
C. carolinus	–
C. dipsaceus	12
C. prophetarum	
subsp. *prophetarum*	12
subsp. *dissectus*	12
C. pubituberculatus	–
C. zeyheri	12 (24)
C. prolatior	–
C. insignis	12
C. globosus	12
C. thulinianus	–
C. ficifolius	12 (24)
C. aculeatus	24
C. pustulatus	12, 48, 72
C. meeusei	24
C. jeffreyanus	–
C. hastatus	–
C. rigidus	–
C. baladensis	–
Serie *Metuliferi*	
C. metuliferus	12
C. rostratus	–
Section *Melo*	
Serie *Hirsuti*	
C. hirsutus	12
Serie *Humifructosi*	
C. humifructosi	12
Serie *Melo*	
C. melo	
subsp. *melo*	12
subsp. *agrestis*	12
C. sagittatus	12
Subgenus *Cucumis*	
C. sativus	7
C. hystrix	12

Dicoelospermum, *Mukia*, *Myrmecosicyos*, and *Oreosyce* have been transferred to *Cucumis*, resulting in 14 new combinations, two changes in status, and three new names (*Cucumis indicus*, *C. kirkbrideana*, and *C. oreosyce*). A complete morphological key to all these species now included in *Cucumis* was provided in Schaefer (2007).

6.2.2 Morphology

The genus *Cucumis* includes annual and perennial taxa, and the fruit morphology is the most important character within the genus (Fig. 6.1). Following morphological descriptions are given by combining the data from Kirkbride (1993), Rubatzky and Yamaguchi (1997), and Kristkova et al. (2003).

6.2.2.1 Plants

Herbs, exceptionally semi-shrubs, usually having a trailing or climbing growth habit, are monoecious, or rarely dioecious or andromonoecious; root systems are rarely woody (*C. trigonus*) and extensive, but usually shallow and rarely tuberous (*C. kalahariensis*); stems are angled, sulcate, not aculeate or rarely aculeate (*C. aculeatuc* and *C. ficifolius*), and variously pubescent or rarely glabrous, with non-breakaway hairs or rarely breakaway hairs (*C. sacleuxii*); nodes are geniculate or not geniculate. Each node has a single leaf and a simple tendril (sometimes curling), except that of *C. humifructus,* which has a fasicicle of five to eight tendrils, and that of *C. rigidus,* which lacks them; tendrils of *C. insignis* are either simple or bifid. Tendrils are variously pubescent, rarely glabrous, or rarely aculeate.

6.2.2.2 Leaves

Simple and petiolate. Petioles vary in length (with regard to the length of a leaf blade). They are not aculeate or rarely aculeate and variously pubescent or rarely glabrous, with non-breakaway hairs or rarely breakaway hairs. The majority of species have a uniform type of pubescence on the petioles. *C. sagittatus* and *C. thulinianus* have two pubescence types uniformly mixed over the entire petiole, and *C. myriocarpus* has three types separated into distinct zones

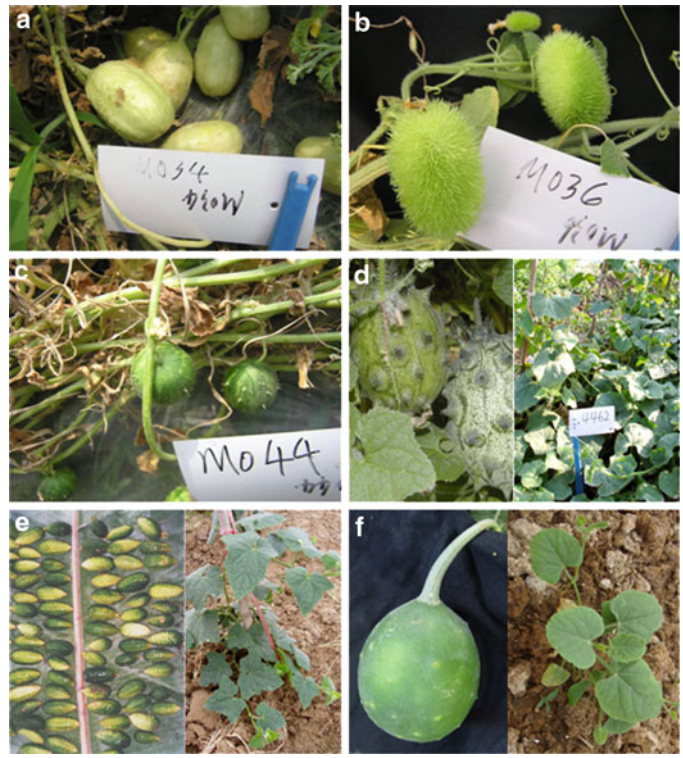

Fig. 6.1 Fruit of some species of the genus *Cucumis*. (**a**) *C. anguria*. (**b**) *C. Dipsaceus*. (**c**) *C. myriocarpus*. (**d**) *C. metuliferus*. (**e**) *C. hystrix*. (**f**) *C. Figarei*

on the petioles, retrose–strigose on the base, hirsute in the middle, and antrorse–strigose at apex; leaf blades are 3- or 5-palmately lobed, trilobite, pentalobate, heptalobate, or entire; Central leaf lobe is symmetrical, entire, or sometimes pinnatifid; lateral leaf lobes are asymmetrical, or sometimes symmetrical, entire, or sometimes pinnatifid.

6.2.2.3 Inflorescences and Flowers

The inflorescence is unisexual and most species are monoecious. *C. humifructus* has only androgynous inflorescences (i.e., inflorescence with both male and female flowers and the female flower below the male ones), and *C. metuliferus* has mainly unisexual inflorescence and a few gynecandrous ones (i.e., inflorescence with both female and male flowers and the female flower above the male ones).

Male inflorescence consists of solitary flowers, or fasciculate, racemose, paniculate, or rarely modified compound dichasial from 1 to 18 flowered, sessile, or rarely pedunculate. Male inflorescences are often multiflowered and rarely branched. When the inflorescences are branched, the male flowers are always pedicellate. Male flowers are 5-merous; pedicel is terete or rarely sulcate in cross section, variously pubescent or rarely glabrous, and without bracteoles or rarely subtended by a bracteole (*C. heptadactylus*). Calyx consists of five or rarely four lobes, linear to oblong, or narrowly to broadly triangular in outline, acute to narrow in the apex, and variously pubescent or rarely glabrous. Corolla is yellow, infundibular, or rarely campanulate and is variously pubescent or rarely glabrous. Corolla is fused into a basal tube. Corolla leaves are elliptic to broad, ovate to shallow, obovate to narrowly, or rarely oblong or broadly triangular in outline, narrowly to broadly acute or obtuse, and sometimes also mucronate at the apex. Three stamens are free, with separation from the free portion of the hypanthium above the ovary. Two of them are 2-thecate and one is 1-thecate. Filaments are terete or radially compressed in cross section and are glabrous or with basal puberculence and glabrous apically. Anther theceae is sigmoid and glabrous with the edges shortly pubescent. Anther connective is extended, obovate, oblong to narrow, transversely broadly

oblong, or ovate, unilobate or rarely bilobate, obtuse or rarely acute in the apex, minutely papillate, sometimes smooth, or rarely glabrous, fimbriate, or crenulate at the apex. Disk is cylindrical or rarely consisting of three papillae and is glabrous.

Female flowers are solitary or rarely in fasciclate inflorescences; sessile flowers arise from leaf axils, very often from secondary branches. They are pedicellate and 5-merous. Pedicel is terete or sulcate in cross section and is variously pubescent, with non-breakaway hairs or rarely with breakaway hairs. Hypanthium is hourglass-shaped. The constricted portion and the lower bulge fused to the ovary. The upper bulge of hypanthium is free from the ovary. Free portion of hypanthium is campanulate. Ovary has three to five placentas with numerous horizontal ovules. Calyx has five, occasionally four or six lobes of the same shape as male flowers. Corolla is yellow and infundibular, with the same shape and types of pubescence as male flower. Corolla tube is present or absent. Three staminodes are present or rarely absent, separating from the free portion of hypanthium above the ovary. Style is terete in cross section, glabrous, subtented by a circular disk, or rarely lacking one. Stigma is copular, lobate, or sometimes entire or sublobate, with one to six or rarely nine finger-like projections on the margin.

6.2.2.4 Fruits and Seeds

Fruits are pendulous; fruit is spherical, oval, oblong, elongated, or blocky in shape and variable in size; fruit surface varies in the number and size of scattered spiny tubercles (warts), or sharp soft hairs. It can be smooth and glabrous, sometimes deeply ridged or covered with a corky (reticulate) netting (e.g., for *C. melo*); skin color varies from pale to very dark green, sometimes with longitudinal indentations or stripes. In maturity, the skin color is white cream to orange brown. Inferior flesh color can be white, green, pink, or orange. The fruit stalk is referred to as a pedicel. The pedicel is sulcate or sometimes terete in cross section and is variously pubescent or rarely glabrous.

Mature seeds have white, cream to yellow color. They are smooth, compressed, ovoid to elliptic, immarginate, with an acute edge, and unwinged or rarely apically winged. *C. humifructus* develops its fruits below ground.

6.2.3 Cytology

Cytologically, the genus *Cucumis*, like all other Cucurbits, is a less studied genus (Ramachandran and Narayan 1985). Most *Cucumis* species are diploid with 12 pairs of chromosomes ($2n = 24$): *C. africanus*, *C. anguria*, *C. dipsaceus*, *C. ficifolius*, *C. hirsutus*, *C. humifructus*, *C. metiluferus*, *C. myriocarpus*, *C. melo*, *C. prophetarum*, *C. pustulatus*, *C. sagittatus*, *C. sacleuxii*, *C. zeyheri,* and *C. hystrix*. Among these species, three have also been reported to be polyploid: *C. ficifolius*, $2n = 48$ (Dane and Tsuchiya 1979; den Nijs and Visser 1985), *C. pustulatus*, $2n = 48$ (Ramachandran 1984; den Nijs and Visser 1985; Ramachandran and Narayan 1985) or $2n = 72$ (Dane and Tsuchiya 1979), and *C. zeyheri*, $2n = 48$ (Dane and Tsuchiya 1976, 1979; Varekamp et al. 1982; Ramachandran 1984; den Nijs and Visser 1985; Ramachandran and Narayan 1985). *C. sativus* is the only species of *Cucumis* reported to have a chromosome count of $2n = 14$ (Fig. 6.2).

There are two base chromosome numbers in *Cucumis*: $x = 7$ and $x = 12$. Two different hypotheses have been put forward to explain the relationship between the two basic chromosome numbers. The fragmentation hypothesis suggests that $x = 12$ has derived from $x = 7$ by fragmentation of particular chromosomes followed by de novo regeneration of centromeres (Bhaduri and Bose 1947; Ayyangar 1967). The fusion hypothesis, on the other hand, says that the basic number $x = 7$ might have arisen from $x = 12$ possibly by unequal translocation or fusion of non-homologous chromosomes (Trivedi and Roy 1970). Comparative genomics between *C. melo* and *C. sativus* may clarify

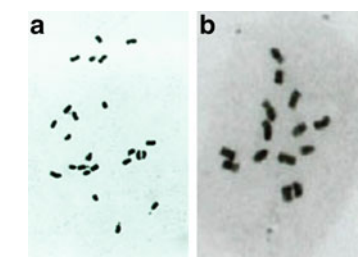

Fig. 6.2 (**a**) Chromosome numbers of *C. hystrix*. (**b**) Chromosome numbers of *C. sativus*

the phylogeny of these species (Danin-Poleg et al. 2001).

As for the karyotype of *Cucumis*, most studies have focused on the cultivated species: cucumber and melon (Figs. 6.3 and 6.4). However, discriminatory information from karyotype analysis for detailing relationships in *Cucumis* has been difficult to access due to the small chromosome size and poor stainability. Ramachandran and Seshadri (1986) used C-banding and pachytene analysis to compare the genomes of cucumber and muskmelon (*C. melo* L.), but their study did not differentiate chromosomes by measurement, and their description of the chromosome morphology and C-banding figures are equivocal.

Chromosomal DNA amounts varied in different species of *Cucumis* (Ramachandran and Narayan 1985). The DNA amounts varied from 1.373 to 2.483 pg in diploids and from 2.846 to 3.886 pg in tetraploids. DNA amount was not correlated with chromosome number and periodicity. Tetraploids were found to have double the quantity of nuclear DNA of diploids.

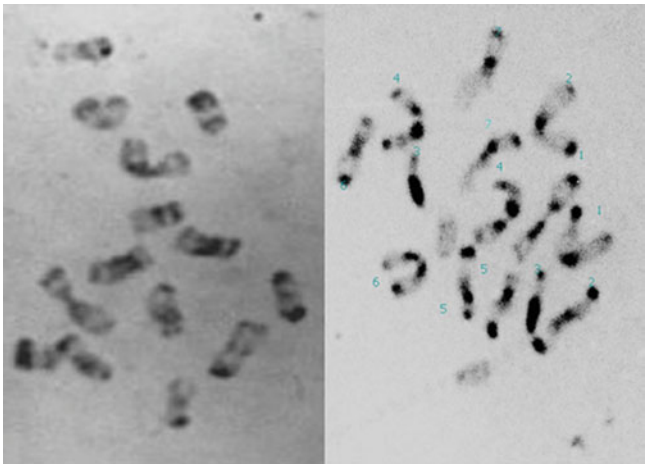

Fig. 6.3 Chromosome C-banding of *Cucumis sativus* L

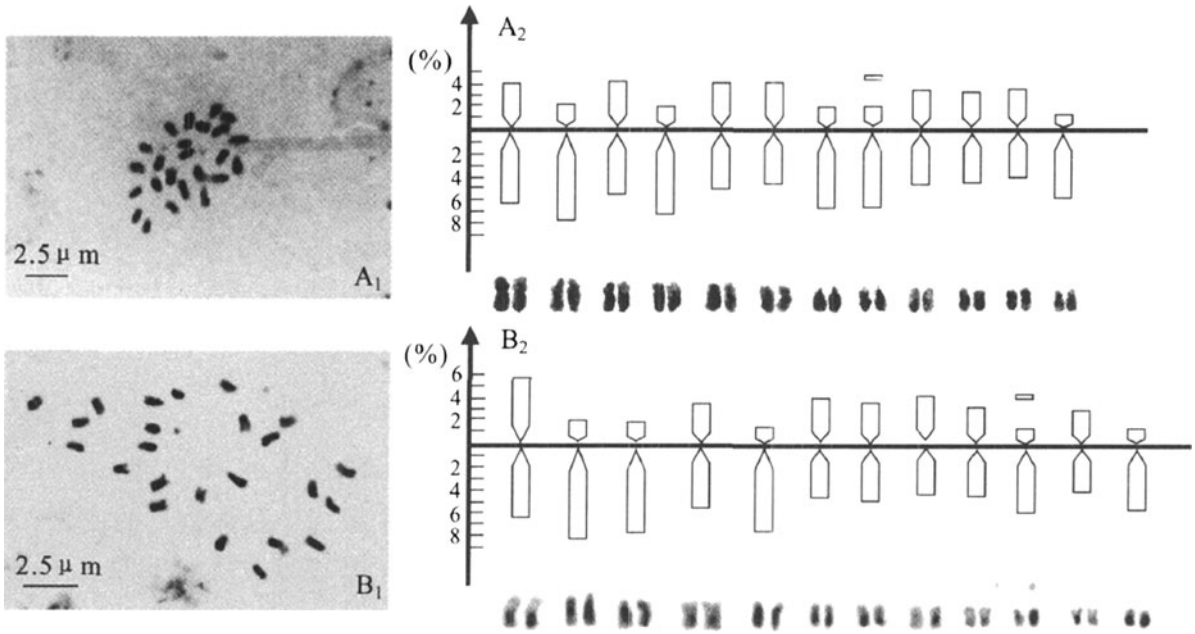

Fig. 6.4 Karyotypes (A1, A2) and their ideograms (B1, B2) of Jiashi and Huangjin melon according to Zhang et al. (2005)

6.2.4 Origin and Distribution

The center of origin for *Cucumis* species is likely Africa for the most wild species with chromosome $2n = 24$, while the Middle East and southern Asia has been considered an important center of diversification for melon and cucumber, respectively (Dane et al. 1980; McCreight et al. 1993; Staub et al. 1999).

Cucumis species occurred in a large scale from 38°N to 37°S of the Old World, with more species in the southern hemisphere than in the northern hemisphere. With latitude rising, *Cucumis* species sharply decreased in the northern hemisphere. There were abundant species near the equator. *Cucumis* species occurred in about 75 countries, but 90.6% of them were from Ethiopia, Kenya, Somalia, South Africa, and Tanzania. Somalia had more rare species in absolute and relative. Thirty-three of 75 countries had only one species (Liu 2007). Table 6.2 presents the details of the distribution of *Cucumis* species.

6.3 Germplasm Conservation

There are some centers, which are engaged in the conservation of *Cucumis* germplasm worldwide. In the United States, plant germplasm is maintained and evaluated by the US National Plant Germplasm System (NPGS). In Europe, the International Plant Genetic Resources Institute (IPGRI) coordinates institutional germplasm holdings. In China, the Crop Germplasm Resources Institute of Chinese Academy of Agricultural Sciences (CAAS) is responsible for the germplasm conservation.

Germplasm information can be found in some good germplasm resources information network web servers, such as Germplasm Resources Information network of United States (http://www.ars-grin.gov), Chinese Crop Germplasm Resources Information System (http://icgr.caas.net.cn), N.I. Vavilov Research Institute of Plant Industry of Russia (http://www.vir.nw.ru), and the European Central Cucurbits Database (http://www.comav.upv.es).

According to the American germplasm resources information network, the regional plant introduction (PI) station of NPGS at Ames, Iowa, houses about 1,486 *C. sativus* accessions of worldwide origin and currently lists 3,074 accessions in its melon inventory. The collection of wild species of *Cucumis* includes 48 *C. africanus* L. f. accessions, 50 *C. anguria* L. accessions, 10 *C. anguria* var. *anguria* accessions, 17 *C. anguria* var. *longaculeatus* J. H. Kirkbr. accessions, one *C. asper* Cogn. accession, one *C. canoxyi* Thulin & Al-Gifri accession, six *C. dipsaceus* Ehrenb. ex Spach accessions, seven *C. ficifolius* A. Rich. accessions, one *C. heptadactylus* Naudin accession, three *C. hirsutus* Sond. accessions, one *C. meeusei* C. Jeffrey accession, 42 *C. metulifer* E. Mey. ex Naudin accessions, 21 *C. myriocarpus* Naudin accessions, two *C. myriocarpus* subsp. *leptodermis* (Schweick.) C. Jeffrey & P. Halliday accessions, three *C. myriocarpus* subsp. *myriocarpus* accessions, three *C. prophetarum* L. accessions, seven *C. pustulatus* Hook. f. accessions, four *C. sagittatus* Peyr. accessions, one *C. subsericeus* Hook. f. accession, eight *C. zambianus* Widrlechner et al. accessions, and nine *C. zeyheri* Sond. accessions. The collection also has 88 accessions labeled *Cucumis* sp., which may include some *C. melo* or *C. metuliferous* accessions (http://www.ars-grin.gov/cgi-bin/npgs/html/genform.pl; Table 6.3).

According to the *AD HOC* meeting on Cucurbit genetic resources held in Turkey in 2002, the number of accessions of *Cucumis* species, including landraces, breeding material, and wild relatives, maintained in European collections was 14,333. Among them, there are 33 accessions of *C. anguria*, 31 accessions of *C. dipsaceus*, 11 accessions of *C. ficifolius*, 7,553 accessions of *C. melo*, 11 accessions of *C. metuliferus*, 12 accessions of *C. myriocarpus*, 5,896 accessions of *C. sativus*, 10 accessions of *C. zeyheri*, and 776 accessions of *C.* spp. (Table 6.4).

In China, it is reported that there are 1,506 accessions of *C. sativus* (Sheng et al. 2006) and 1,003 accessions of *C. melo* (Ma et al. 2003); however, the collection of wild species is not clear.

Although *Cucumis* accessions are held by numerous collections around the World, the most wild species are less formal collections for research purposes and through personal exchanges among scientists throughout the world. Those wild species are often not documented or represented in the NPGS base collection or IPGRI collection, so samples acquired through personal contact could be important.

Table 6.2 Distribution of the genus *Cucumis*

Cucumis spp.	Distribution
C. mriopcarpus	Lesotho, Mozambique, South Africa, Zambia, Botswana, Zimbabwe
C. africanus	Namibia, South Africa (Cap Province, Natal Transvaal), Angola, Lesotho, Zimbabwe, Botswana
C. quintanilhae	Botswana (southern most), South Africa (northern most Trasvaal)
C. heptadactylus	South Africa (Cap Province, Transvaal, Orange Free State)
C. kalahariensis	Botswana (central and northwestern), Namibia (northeastern)
C. anguria	Angola, Botswana, Cape Verde Islands, Malawi, Mozambique, Namibia, South Africa, Sierra Leone, Swaziland, Tanzania, Zaire, Zambia, Zimbabwe
C. sacleuxii	Kenya, Madagasar, Tanzania, Uganda, Zaire
C. carolinus	Ethiopia (southeast), Kenya (northeast)
C. dipsaceus	Ethiopia, Kenya, Somalia, Tanzania, Uganda, possibly native to Sudan and southern Egypt
C. prophetarum	Egypt, Mali, Mauritania, Senegal, Somalia, Sudan, Iran, Iraq, Nigeria (northern), Israel, Oman, Saudi Arabia, Socotra, Syria, Unite Arab Emirates, Jordan, India (northeastern), Pakistan. Chad, Ethiopia, Niger, Kenya, Rwanda, Tanzania, Uganda, South Yemen, Yemen
C. pubituberculatus	Central, coastal Somalia
C. zeyheri	Lesotho, Mozambique, South Africa (Cap Province, Natal, and Transvaal), Swaziland, Zambia, Zimbabwe
C. prolatior	Central and south central Kenya
C. insignis	Ethiopia
C. globosus	Tanzania
C. thulinianu	Somalia, only near Erigavo
C. ficifolius	Ethiopia Kenya, Rwanda, Tanzania, Uganda, Zaire (eastern most areas near the Rift Valley)
C. jeffreyanus	Ethiopia, Kenya, Somalia
C. aculeatus	Rarely from southern Ethiopia, Kenya, Rwanda, Tanzania, Uganda, Zaire (eastern)
C. pustulatus	Chad, Ethiopia, Kenya, Nigeria, Sudan, Tanzania, and Uganda. Southwest Asia (Saudi Arabia and Yemen)
C. meeusei	Botswana (northern), South Africa (northern Cape Province), Namibia (northern)
C. hastatus	Somalia (southern)
C. baladensis	Somalia
C. rigidus	Namibia, southern most area along Orange River, South Africa, northwestern Cap Province along the Orange River
C. metuliferu	Botswana, Ethiopia, Mozambique, Kenya, Malawi, Namibia, Senegal, South Africa, Sudan, Swaziland, Tanzania, Uganda, Zaire, Zimbabwe, Cameral African Republic, Liberia, Burkina, South Yemen, Yemen, Angola
C. rostratu	Ivory Coast, Nigeria
C. hirsutus	Botswana, Burundi, Congo, Kenya, Malawi, South Africa, Sudan, Swaziland, Tanzania, Zaire, Zambia, Zimbabwe, Mozambique, Angola
C. humifrucutus	Angola, Ethiopia (very rarely), Kenya, Namibia, South Africa (Transvaal), Zaire, Zambia, Zimbabwe
C. melo	Angola, Benin, Burkina, Cameroon, Cape Verd Islands, Central African Republic, Chad, Egypt, Ethiopia, Gambia, Ghana, Guinea-Bissau, Ivory Coast, Kenya, Madagascar, Malawi, Maldive Islands, Mail, Mauritania, Mozambique, Niger, Nigeria, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Sudan, Tanzania, Uganda, Zaire, Zambia, Zimbabwe, Iran, Iraq, Oman, Saudi Arabia, South Yemen, Yemen, Afghanistan, Bangladesh, Burma, China, India, Japan, Korea, Nepal, Pakistan, Sri Lanka, Thailand, Malaysia, Indonesia, New Guinea, Philippines, Australia, Fiji Islands, Guam, New Britain, Papua New Guinea, Samoa, Solomon Islands, Tonga Islands
C. sagittatus	Namibia, and South Africa (only northwestern Cape province), Angola (southwestern corner next to Namibia)
C. sativus	Burma, China (Yunnan province, GuangXi, GuiZhou), India, Sri Lanka, Thailand
C. hystrix	Burma, China (Yun Nan province), India (Assam), Thailand, Myanmar

Table 6.3 Number of accessions of *Cucumis* species stored in the regional plant introduction station of NPGS at Ames according to the American germplasm resources information net work (http://www.ars-grin.gov/cgi-bin/npgs/html/genform.pl)

Species	Number
C. africanus L.	48
C. anguria L.	50
C. asper Cogn.	1
C. canoxyi Thulin & Al-Gifri	1
C. dipsaceus Ehrenb. ex Spach	6
C. ficifolius A. Rich.	7
C. heptadactylus Naudin	1
C. hirsutus Sond.	3
C. meeusei C. Jeffrey	1
C. metulifer E. Mey. ex Naudin	42
C. myriocarpus Naudin	21
C. myriocarpus subsp. *leptodermis*	2
C. myriocarpus subsp. myriocarpus	3
C. prophetarum L.	3
C. pustulatus Hook. f.	7
C. sagittatus Peyr.	4
C. subsericeus Hook. f.	1
C. zambianus Widrlechner et al.	8
C. zeyheri Sond.	9
Cucumis sp.	88

Table 6.4 Number of accessions of *Cucumis* species stored in the main European genebanks and breeders' collections (from Ad-hoc meeting on Cucurbit genetic resources in Europe held in Turkey, 2002)

Collection curator (Country)	*Cucumis*
Genebanks	
T. Piskunova (Russian Federation)	4,931
A. Börner (Gatersleben, Germany)	975
F. Nuez (Valencia, Spain)	798
E. Křístková (Czech Republic)	967
L. Krasteva (Bulgaria)	1,247
A. Küçük (Turkey)	632
L. Horváth (Hungary)	383
M. Carravedo (BGHZ, Spain)	777
W. Dooijeweert (The Netherlands)	790
T. Kotlińska (Poland)	390
N. Polignano (Italy)	143
R. Farias (BPGV, Portugal)	119
J. Berenji (Yugoslavia)	–
Breeders' collections	
M. Pitrat (France)	605
M. L. Gómez-Guillamón (CSIC, Spain)	561
S. Strajeru (Romania)	280
K. Abak (Turkey)	301
Total	13,899

6.4 Evolution and Phylogenetic Relationships

Knowing the closest relatives and natural composition of the genus *Cucumis* L. is important simply because of the ongoing efforts by plant breeders worldwide to improve melon and cucumber with traits from wild relatives (Renner and Schaefer 2008). Quite a few studies, using morphological, cytology, and molecular characters such as isozymes, random amplified polymorphic DNA (RAPD), chloroplast simple sequence repeat (cpSSR), and internal transcribed spacer (ITS), have been carried out to determine the *Cucumis* phylogeny.

Early studies on karyomorphological investigations of 13 species in the genus *Cucumis* L. indicated that South African annual species are the primitive and identified five distinct groups in taxa with $2n = 24$. They are (1) *C. leptodermis* and *C. africanus*; (2) *C. ficifolius*, *C. hookeri*, and *C. dipsaceus*; (3) *C. myriocarpus*, *C. zeyheri*, *C. prophetarum*, *Cucumis* species CUCU44/74, and *C. anguria*; (4) *C. metuliferus*; and (5) *C. sagittatus* and *C. melo* (Singh and Yadava 1984).

The crossability between species, chromosome pairing, and pollen fertility in F_1 hybrids were also investigated for assessing species relationships and *Cucumis* phylogeny. Deakin et al. (1971) were the first to produce a comprehensive and monographic account on relative cross-compatibility between *Cucumis* species and pollen fertility of their F_1 hybrids. Their studies involved 14 species including cultivated *C. melo* L. On the basis of these data obtained, they grouped *Cucumis* species into four major groups. Singh and Yadava (1984) had investigations on interspecific crossability in eight *Cucumis* species ($2n = 24$, *C. melo, C. dipsaceus, C. anguria* var. *anguria, C. anguria* var. *longipus*, *C. myriocarpus, C. zeyheri*, *C. prophetarum*, and *C. species*). Information on chromosome pairing and pollen fertility of the hybrids from 15 combinations had been utilized for tracing the phylogenetic relationships among these taxa.

Esquinas-Alcazar (1977) studied the alloenzyme variation and relationships in the genus *Cucumis* and divided the genus *Cucumis* into four groups. (1) Ser. *angurioidei*: *C. aculeatus*, *C. africanus*, *C. anguria*, *C. dipsaceus*, *C. ficifolius*, *C. heptactylus*, *C. myriocarpus*,

C. pustulatus, and *C. zeyheri*; (2) Ser. *metuliferi*: *C. aculeatus*, *C. metuliferus*, and *C. sagittatus* (some accessions); (3) Ser *melo*: *C. melo* and *C. sagittatus* (some accessions); and (4) Ser. *sativus: C. sativus*.

From the evolutionary and systematic point of view, Perl-Treves and Galun (1985) compared the phylogenies of *Cucumis* based on cpDNA and nuclear-coded isozymes. The comparison was carried out for 21 *Cucumis* species and the two phylogenies were found to share the main dendrogram features, which also agreed well with most taxonomic data available on *Cucumis*. Accordingly, most of the African *Cucumis* species form a close group ("*Anguria* group," *C. africanus*, *C. anguria*, *C. dipsaceus*, *C. ficifolius*, *C. heptactylus*, *C. meesusei*, *C. myriocarpus*, *C. prophetarum*, *C. pustulatus*, and *C. zeyheri*), which was distant from the melon (*C. melo*), and from a few other distant species (*C. humifructus*, *C. metuliferus*, and *C. sagittatus*), all of which were far apart from each other. The cucumber (*C. sativus*) was the most distant species within the genus.

In 1989, *C. hystrix* Chakr., a wild *Cucumis* species, was rediscovered in Yunnan, China, by Jinfeng Chen (Chen et al. 1994). Subsequent research revealed that *C. hystrix* has $2n = 24$ instead of $2n = 14$ as in the Asian members. This finding challenges the basic chromosome number theory that African *Cucumis* have $n = 12$ and that Asian *Cucumis* have $n = 7$. Isozyme patterns (Chen et al. 1995) suggested that *C. hystrix* has closer genetic affinities with *C. sativus* than with *C. melo*, even though *C. hystrix* and *C. melo* possess the same number of chromosomes.

Chung et al. (2006) used nine chloroplast SSR (cpSSR) markers to investigate the phylogenetic relationships among African *Cucumis* species ($x = 12$) accessions, *C. melo* accessions, *C. sativus* accessions, and *C. hystrix* accessions. Sequence variation analysis identified a group of African *Cucumis* species and a group composed of *C. melo*, *C. sativus*, and *C. hystrix* species leading to the conclusion that *C. hystrix* is the progenitor species of *C. sativus*, or that they at least share a common ancestral lineage.

Zhuang et al. (2006) investigated the phylogenetic relationships in *Cucumis* species using RAPD. Their focus was mainly on the analysis of genetic relationship among *C. hystrix*, *C. sativus,* and *C. melon* and *C.* × *hytivus,* a new synthetic species. On the basis of results, a modified taxonomic system was proposed that *C. hystrix* should remain in subgen. *Cucumis*, although it had a chromosome number different from that of *C. sativus*. With the interspecific hybrids *C.* × *hytivus* as the third species, subgen. *Cucumis* was thus made up by three species. Although the basic chromosome number and geographic location theorized were challenged by the proposed system, the use of it will likely assist in the exploitation of the wild *Cucumis* species in Asia.

Using sequences of the internal transcribed spacer (ITS) 1 and 2 regions of the nuclear ribosomal RNA genes, Jobst et al. (1998) evaluated the phylogenetic relationships among different members of the family Cucurbitaceae. Six *Cucumis* species along with *C. melo* and *C. sativus* were analyzed in the study and the results obtained by ITS sequence data were highly congruent with isoenzyme data of Puchalski and Robinson (1990). Garcia-Mas et al. (2004) defined phylogenetic relationships among *Cucumis* species also using the nuclear ribosomal DNA ITS region and microsatellite markers. In their study, the genus *Cucumis* was splited into five groups: cucumbers, melons, *C. metuliferus*, a group containing 12 wild species of *Cucumis*, and Oreosyce Africana, and a fifth group comprising *C. sagittatus* and *C. globosus*.

Kocyan et al. (2007) presented a multilocus chloroplast phylogeny for the Cucurbitaceae that included all putative close relatives of *Cucumis*. Their results support a paraphyletic *Cucumis*, with *Cucumela*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce* nested among species of this genus. Although evolutionary relationships are not completely resolved, following the discovery by Koeyan and coworkers that *Cucumis* as traditionally circumscribed (Kirkbride 1993) was highly unnatural, two molecular phylogenetic studies reinvestigated species relationships in a much more broadly circumscribed *Cucumis* (Ghebretinsae et al. 2007; Renner et al. 2007).

Ghebretinsae et al. (2007) presented a comprehensive molecular phylogeny of *Cucumis* and the traditionally related genera based on sequences from both nuclear and chloroplast genomes. Their study used a much more complete sampling of species within *Cucumis* than did previous studies and includes representatives of *Cucumella*, *Dicaelospermum*, *Mukia*, *Myrmecosicyos*, and *Oreosyce*. Their combined phylogenetic analyses did not support Kirkbride's (1993) subdivision of *Cucumis* into two subgenera based principally upon chromosome number and geographical distribution. *Cucumis* sensu Kirkbride (1993) is

paraphyletic and the Asian *Cucumis* (subg. *Cucumis* of Kirkbride) forms a well-supported clade nested within African *Cucumis* s.s. They identified six clades within the *Cucumis* complex, designated as Clades I, II, III, IV, V, and VI. Clade I comprised all non-domesticated African *Cucumis* s.s. after the exclusion of *C. hirsutus*, *C. humifructus*, *C. metuliferus*, *C. rostratus*, and *C. sagittatus*; *C. hystrix* and *C. sativus* were included in Clade II. Clade III comprised *C. melo* and *C. sagittatus*. Clade IV consisted of *C. metuliferus* and *C. rostratus*. Clade V comprised four species of *Cucumella* and *Oreosyce*. Clade VI consisted of *C. hirsutus* and *C. humifructus*.

Based on these two recent molecular phylogenetic studies of *Cucumis*, Renner and Schaefer (2008) summarize what is now known about phylogenetic relationships in *Cucumis*. The phylogeny of *Cucumis* resulting from combined nuclear and chloroplast data (Fig. 6.5) implied that the deepest divergence lies between the common ancestor of *C. hirsutus*/*C. humifructus* and the stem lineage of the remainder of the genus. The area of origin of *Cucumis* cannot be inferred because its sister genus, *Muellerargia*, has one species in *Madagascar* and the other one in tropical Australia and Indonesia. The next closest relatives are in African/Asian clades including the genera *Coccinia*, *Zehneria*, *Neoachmandra*, and *Peponium*, but their exact position is still unresolved. The earliest divergence events in *Cucumis* likely took place in Africa. However, contrary to the traditional classification (Kirkbride 1993), which grouped *C. melo* with the African *C. hirsutus*, *C. humifructus*, and *C. sagittatus*, melon instead is closest to an Australian/Asian clade.

6.5 Interspecific Hybridization Among Cucumis Species

6.5.1 Progress of Interspecific Hybridization

Interspecific hybridization is used to improve crops by transferring specific traits, such as pest and stress resistance, from their wild relatives (Bowley and Taylor 1987). Interspecific hybrids in the Cucurbitaceae have been produced in several genera, including *Cucumis* (Deakin et al. 1971), *Citrullus* (Valvilov 1925), *Luffa* (Singh 1991), and *Cucurbita* (Weeden and Robinson 1986). In the genus *Cucumis*, an amphidiploid was reported from the cross of *C. anguria* L. and *C. dipsaceus* E. ex S. (Yadava et al. 1986). However, in the Cucurbitaceae only in *Cucurbita* interspecific hybridization has been successfully utilized for crop improvement (Robinson and Decker-Walters 1997).

Cucumis contains two species of economic importance: melon (*C. melo* L., $2n = 24$) and cucumber (*C. sativus* L., $2n = 14$). The importance of wild *Cucumis* species has long been recognized because they possess resistance to pathogens, such as powdery mildew, downy mildew, anthracnose, and Fusarium wilt (Leppick 1966; Lower and Edwards 1986; Kirkbride 1993). Genetic variation is relatively limited in cucumber; thus, efforts to create interspecific hybrids have become more critical and meaningful.

The first recorded attempt to make crosses between cucurbits by removing the male flowers and transferring pollen by hand was made by Naudin. He was unsuccessful in obtaining a cross between *C. melo* and *C. myriocarpus* (Naudin 1859). Many more studies have also been attempted to make interspecific crosses in *Cucumis* (Betra 1953; Andrus and Fassuliotis 1965; Deakin et al. 1971; Chelliah and Sambandam 1972; Fassuliotis 1977; Dane et al. 1980; Kho et al. 1980; Visser and den Nijs 1983; Singh and Yadava 1984; den Nijs and Visser 1985; den Nijs and Custers 1990; Chatterjee and More 1991, etc.).

The first comprehensive crossability analysis of the genus was published by Deakin et al. (1971), who observed that crosses among wild species were frequently possible, but that all attempts to cross any of these with the two cultivated species, *C. sativus* and *C. melo*, failed. Raamsdonk et al. (1989) summarized the data in two crossing polygons. The species *C. heptadactylus*, *C. humifructus*, *C. melo*, and *C. sativus* have never been successfully crossed with any other species of *Cucumis* to produce a fertile F_1 generation. The following species can be crossed to a limited extent among themselves: *C. africanus*, *C. anguria*, *C. dipsaceus*, *C. ficifolius*, *C. metuliferus*, *C. myriocarpus*, *C. prophetarum*, *C. pustulatus*, and *C. zeyheri* (Kirkbride 1993). Some wide-cross attempts between cultivated and wild *Cucumis* species are presented in Table 6.5.

A successful interspecific hybridization between *C. hystrix* and *C. sativus* was reported (Chen et al. 1997). It was the first reproducible cross between a

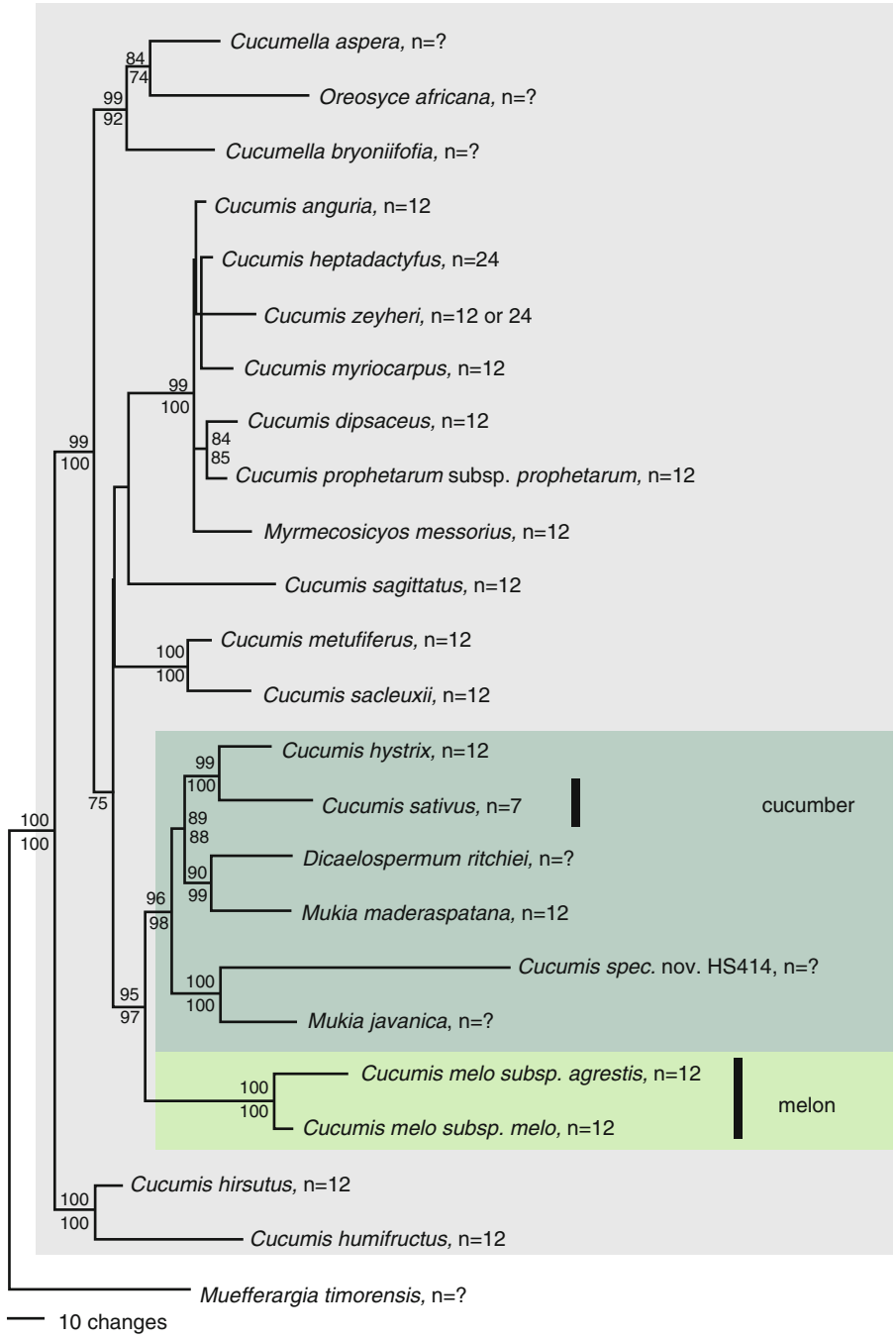

Fig. 6.5 Parsimony tree for *Cucumis* based on combined chloroplast and nuclear DNA sequences according to Renner et al. (2007)

cultivated *Cucumis* species and a wild relative, and it represented a breakthough in interspecific hybridization in *Cucumis*. The success of this cross was even more surprising because the parental species have different chromosome numbers. The original F_1 hybrid ($2n = 19$), obtained by embryo rescue following pollination of *C. sativus* by *C. hystrix* (Fig. 6.6), has 7 chromosomes from *C. sativus* and 12 from *C. hystrix* and was both male- and female-sterile. To restore fertility, reciprocal crosses were made and the

Table 6.5 Wide-cross attempts between cultivated and wild *Cucumis* species

Cross	Result	Source
C. sagittatus × *C. melo*	Embryos only	Deakin et al. (1971)
C. metuliferus × *C. melo*	Embryos only	Fassuliotis (1977)
C. sativus × *C. melo*	Globular stage embryos only	Niemirowicz-Szczytt and Kubicki (1979)
C. metuliferus × *C. melo*	Fertile F_1	Norton and Granberry (1980)
C. prophetarum × C. melo	Fruit with non-viable seeds	Singh and Yadava (1984)
C. zeyheri × *C. sativus*	Fruit with non-viable seeds	Custers and Den Nijs (1986)
C. sativus × *C. metuliferus*	Embryos only	Franken et al. (1988)
C. melo × *C. metuliferus*	Embryos only	Soria et al. (1990)
C. sativus × *C. hystrix*	Sterile plant ($2n$ and $4n$)	Chen et al. (1997)
C. hystrix × *C. sativus*	Fertile plants ($4n$)	Chen et al. (1998)

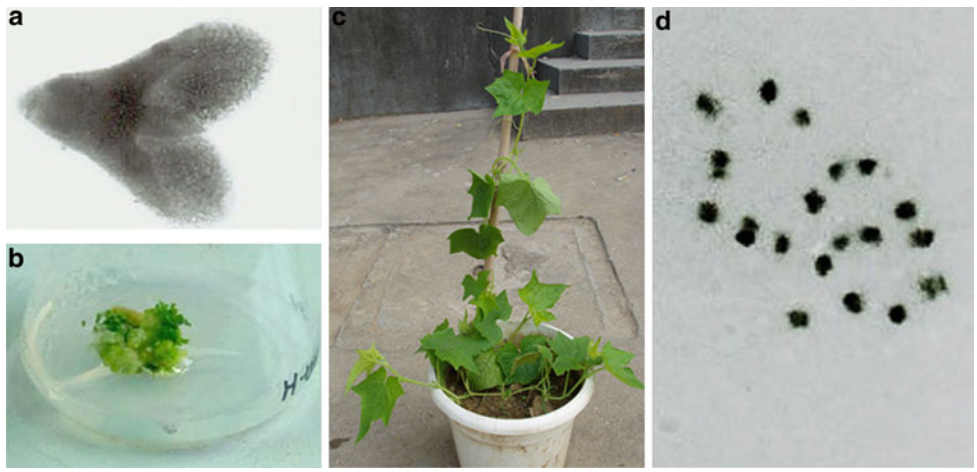

Fig. 6.6 Production and chromosome counting of interspecific hybrids F_1 between *C. hystrix* and *C. sativus*. (**a**) Embryo of hybrid. (**b**) Regeneration from the young embryo. (**c**) Acclimatized plants. (**d**) Metaphase chromosomes of interspecific hybrid F_1 ($2n = 19$)

chromosome numbers of the progeny were successfully doubled (Chen et al. 1998). Pollen grains were produced by these progeny when *C. hystrix* was used as the seed parent; the plants produced fertile flowers and set fruit with viable seeds, indicating that fertility was restored. This restoration of fertility marked the creation of a new synthetic species, which has close phylogenetic relationships with its parental species, but is distinctively different from each parent. The new species (*Cucumis* × *hytivus* Chen and Kirkbride; Fig. 6.7) has genome HHCC, where H represents the genome of *C. hystrix* and C represents the genome of *C. sativus* and chromosome number $2n = 4x = 38$ (Chen and Kirkbride 2000). This synthetic species might be useful as a new *Cucumis* crop. In addition, as a *C. hystrix* × *C. sativus* hybrid, it might be useful as a bridging species for transfer of useful traits to cucumber. Figure 6.8 shows the polygon of crossability in *Cucumis* species.

6.5.2 Major Problems in Interspecific Hybridization

6.5.2.1 Hybridization Barriers

Many experiments have indicated the presence of a strong barrier to interspecific hybridization in *Cucumis*. The nature of cross-incompatibility between cultivated *Cucumis* species and their wild relatives is not well understood. Incompatibility is characterized by delayed growth of pollen, or arrested pollen tube growth to reach the ovules (Kishi and Fujishita 1969), as well as lack of cell division of the zygote, and abortion of the endosperm (Kishi and Fujishita 1970).

Several traditional approaches in interspecific hybridization have been used to overcome the hybridization barriers in *Cucumis*. These include growth

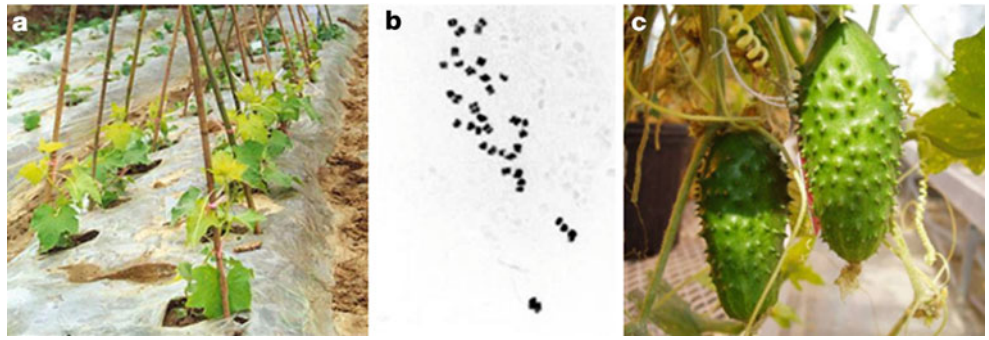

Fig. 6.7 (**a**) *Cucumis* × *hystivus* plant in the field. (**b**) Chromosome numbers of *Cucumis* × *hytivus*. (**c**) Fruit of *Cucumis* × *hystivus*

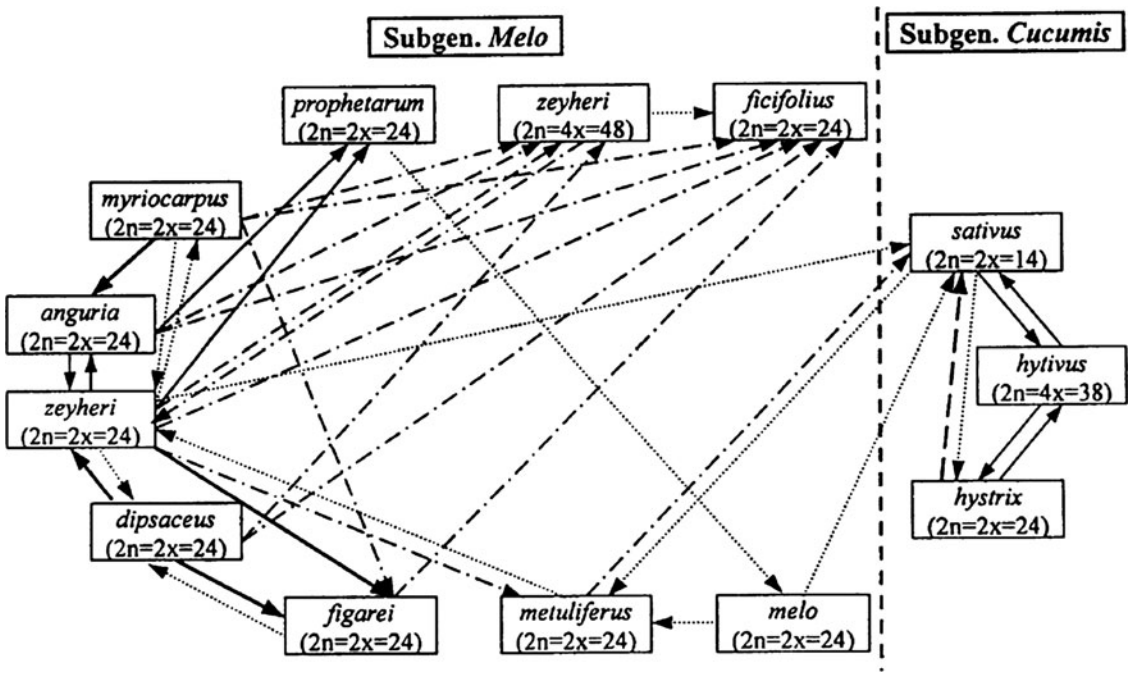

Fig. 6.8 Polygon of crossability in *Cucumis* species according to Zhuang (2003). *Arrows* point to the female parent; moderately to strongly self-fertile and cross-fertile hybrids (*thick solid line*); sparingly self-fertile and moderately cross-fertile hybrids (*thin solid line*); self-fertile, usually not cross-fertile hybrids (*dashed* and *dotted line*); inviable seeds or seedlings (*dashed line*); self-sterile and cross-sterile hybrids (*thick dashed line*); self-sterile and cross-fertile hybrids (*long dashed line*); *absence of a line* indicates that seed fruits were not obtained

regulator application (Custers and Den Nijs 1986), pollen irradiation (Beharav and Cohen 1994), use of mentor pollen (Kho et al. 1980), and bud pollination (Chatterjee and More 1991). Biotechnological techniques, such as somatic hybridization, have also been applied as possible tools for overcoming these barriers in *Cucumis* (Tang and Punja 1989; Chatterjee and More 1991). Likewise, fusion of *C. sativus* and *C. melo* protoplasts has been attempted, but the results indicated that successful hybridization is still unpredictable (Fellner et al. 1996).

The interspecific hybrid between *C. sativus* and *C. hystrix* represents an important step in interspecific hybridization in *Cucumis*. If *C. hystrix* and *C. melo* are cross-compatible and if the F_1 derived from either interspecific hybridization can be made fertile through crossing and/or chromosome doubling, then *C. hystrix* could act as bridge species between *C. melo* and *C. sativus*.

6.5.2.2 Post-fertilization Abortion and Embryo Rescue

In higher plants, post-zygotic failure of hybrid embryos is not often due to incompatibility between the parental chromosomes, but incompatibility problems in the endosperm. In such cases, embryos from interspecific hybridization have to be rescued; otherwise, they will fail due to embryo abortion and/or endosperm degeneration. Successful embryo rescue in tissue culture allows further advances in interspecific hybridization.

Embryos can sometimes be rescued, even if they are immature or lack endosperm (Laibach 1925). In *Cucumis*, fruits with non-viable seeds were obtained in the cross between *C. prophetarum* L. and *C. melo* (Singh and Yadava 1984). The barriers between these two species may be post-zygotic. If the embryo rescue technique had been employed, the experiment might have been successful.

6.5.2.3 Sterility in F_1 Hybrids

A common problem on utilizing germplasm of wild species for crop improvement was sterility in F_1 hybrids. In many cases, this sterility was associated with meiotic abnormalities and was a large obstacle that followed hybridization and hindered utilization.

The ability to cross *C. sativus* and *C. hystrix* offered the promise of introgressing desirable characters from *C. hystrix* to *C. sativus*. However, self-pollination and backcrossing of the F_1 plants to either parent was unsuccessful because the original hybrid was both male- and female-sterile, probably because of the non-functional gametes containing odd chromosome numbers. When chromosomes were doubled, each chromosome had a homologous partner for pairing during meiosis; if there were no cytoplasmic incompatibility, the chromosome-doubled F_1 hybrid might have produced viable gametes, and fertility restoration was anticipated.

External application of chemical agents is the usual way to double chromosome number. Among various agents, colchicine was one of the antimitotic substances most frequently used for this purpose (Chen and Staub 1997). Colchicine at an aqueous solution of 0.05–0.5% (w/v) is believed to be most effective for many plant species. Since colchicine is poisonous to plants, germinating seeds or young seedlings are often preferred for treatment because they grow rapidly and recover more readily than more mature plants do.

When the experimental material does not respond well to chemical treatment, in vitro chromosome doubling (spontaneous polyploidy as a consequence of tissue culture) could be an alternative (D'Amato 1977). When and how the polyoloidization happened in tissue culture was not entirely clear, but it occurred at a low rate during plant formation from axillary buds (Adelberg et al. 1994), callus (Osifo et al. 1989) and culture of protoplasts (Tabei et al. 1992). Polyploidization can be generalized as a universal phenomenon in melon tissue culture (Ezura et al. 1992), although genotype is an important factor in determining the rate of chromosome doubling (Adelberg and Chen 1998).

6.6 Utilization of Wild Species

Although there are many wild *Cucumis* species, not all of them have been fully and properly characterized and documented. Still a few species have been actually used in breeding programs, despite the fact that several of them have been reported to have one or more excellent features to be used as donors (Fassuliotis 1967; Norton and Granberry 1980). Intergroup incompatibility has been assigned as the main reason for this (Deakin et al. 1971; Fassuliotis and Nelson 1988). However, the research work on transfer of useful genes from wild relatives was strengthened with the successful interspecific hybridization between *C. hystrix* and *C. sativus*.

6.6.1 *Resistance in* C. hystrix

C. hystrix is a wild species of *Cucumis* subgen. *Cucumis*, which originated in Asia (Kirkbride 1993). It has a sour taste that is not really like cucumber. As it has been described before that although it bears morphological similarity with cucumber, its diploid chromosome number is 24, the same number as in melon (Chen et al. 1997). Since the first interspecific hybridization successfully made in 1997 and development of the synthetic allotetraploid species (*C.* × *hytivus* Chen and Kirkbride, $2n = 4x = 38$) in 2000, several disease screens were undertaken to characterize the response of *C. hystrix* and its progenies derived from the interspecific hybridization to common cucurbit diseases (Chen and Lewis 2000; Chen et al. 2004b).

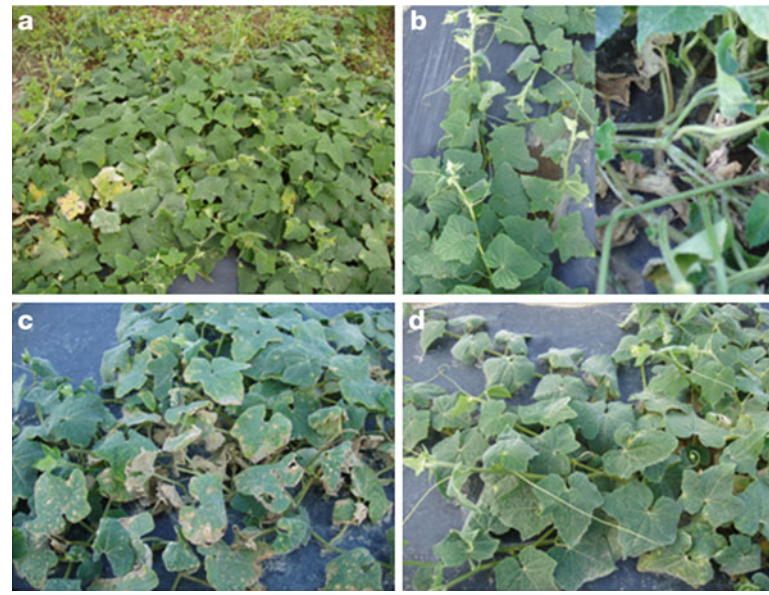

Fig. 6.9 Gummy stem blight resistance shown in *C. hystrix* (**a**, **b**) in the field. *C. hystrix* showing susceptibility to WMV-2 (**c**) and resistance to PRV, CMV-C, and ZYMV (**d**)

6.6.1.1 Gummy Stem Blight

Resistance evaluations were made in a field at Cornell University using the highly virulent *Didymella bryoniae* isolate NY1. Resistance was found in *C. hystrix* plants in the field (Fig. 6.9a, b). Few symptoms were found on stem and slightly more on the leaves. There was no segregation observed among the plants.

6.6.1.2 Downy Mildew

Resistance tests were conducted in Nanjing Agricultural University, China. The disease index in *C. hystrix* was 5.3, indicating that it is highly resistant to downy mildew. This resistance was partially transmitted to the *C. hytivus*, and the progenies from backcross. Compared to the susceptible cucumber cultivar "Jinlu," all the materials derived from this interspecific hybridization possess at least moderate resistance.

6.6.1.3 Viruses

C. hystrix was evaluated for resistance to four viruses: CMV-C, WMV-2, PRV, and ZYMV in the field at Cornell University. *C. hystrix* plants generally suffered from the WMV-2 inoculation (Fig. 6.9c), but showed stronger resistance to PRV and moderate resistance to CMV-C and ZYMV (Fig. 6.9d).

6.6.1.4 Nematodes

Nematode resistance tests were carried out. The three groups (*C. hystrix*, *C. sativus*, and reciprocal interspecific hybrids) varied greatly in their response to *M. incognita*. *C. hystrix* had a high level of resistance to *M. incognita* with mean gall index of 1.8 (Fig. 6.10). In contrast, cucumbers were confirmed as being highly susceptible possessing a mean gall index of 4.8–5.0. The interspecific F_1 hybrid was intermediate in resistance to the two parents, with a mean gall index 3.4. The transmission of resistance was observed in backcross progeny of the chromosome-doubled F_1 to cucumber.

6.6.2 Synthesis, Characterization, and Utilization of Novel Germplasm

6.6.2.1 Allotetraploid

The first repeatable interspecific hybridization between cucumber (*C. sativus* L., $2n = 14$) and *C. hystrix* ($2n = 24$) was successfully made through embryo rescue (Chen et al. 1997). Hybrid plants ($2n = 19$; 12 from *C. hystrix* and 7 from cucumber) were sterile, but morphologically uniform. The multiple-branching habit, densely brown hairs (on corolla and pistil), orange–yellow corolla, and ovate fruit of

Fig. 6.10 *C. hystrix* showing resistance to root-knot nematode *M. incognita* (*right*), susceptible control "Beijingjietou" (*left*), and resistant control *C. metuliferus* (*middle*)

F_1 hybrid plants were similar to that of the *C. hystrix* parent, and the appearance of the first pistillate flower was more similar to that of *C. sativus* parent. The diameter and internode length of the stem and the shape and size of leaves and flowers were intermediate when compared to the parents.

To restore fertility, chromosome doubling of the F_1 hybrid plants was carried out (Chen and Staub 1997). Sixty-two chromosome-doubled plants were obtained. The chromosome-doubled F_1 plants were morphologically distinct from the parents and other progeny in traits such as a curve on leaf margins and shorter and stronger internodes. The fruits at two ploidy levels vary in morphology (Fig. 6.11). While diploid fruit ($2n = 19$, seedless) was longer and spindle-like in shape, the tetraploid fruit was shorter and column-shaped. After fertility selection, two primary allotetraploid produced fertile flowers and set fruit with viable seeds (Chen et al. 1998). The restoration of fertility in the chromosome-doubled F_1 hybrid marks the creation of a new combination of genomes and a new synthetic species that did not exist previously.

Several photosynthetic characters of the hybrid species *Cucumis* × *hytivus* Chen and Kirkbride under weak light condition were studied (Qian et al. 2002). The light compensation point of allotetraploid was 11.25 μE m^{-2} s^{-1}. After treatment with low intensity light for 2 weeks, the leaf contents of chlorophyll a and b increased, while the value of chlorophyll a/b decreased, indicating that the allotetraploid has good tolerance to low irradiance.

Zhuang et al. (2002) investigated the responses of seedlings of the new species *Cucumis* × *hytivus* and its progenies from backcross with cucumber to chilling injury. The abnormal metabolism was observed in *C. hytivus* as it was subjected to low temperature treatment; however, the progenies from backcrossing the new species to cucumber showed high tolerance to chilling injury.

Chen et al. (2007) studied the genomic events in the early generations of the synthesized allotetraploid. Extensive genomic changes were detected by amplified fragment length polymorphism (AFLP) analysis. The changes mainly involved loss of parental restriction fragments and gain of novel fragments. The total detectable changes were from 11.1 to 32.1%, and the frequency of losing parental fragments was much higher than that of gaining novel fragments. Although no significant differences were detected in the reciprocal crosses, the data showed that the frequency of sequence loss in *C. sativus* was two times higher than that in *C. hystrix*. The results demonstrated that the sequence elimination was the major event of genomic changes, and it might provide the physical basis

Fig. 6.11 Morphological comparison between the interspecific hybrid and synthetic allotetroploid *C.* × *hytivus* (**a** and **b**). The interspecific hybrid F_1 diploid, sterile, hybrid plant from embryo rescue (*left*) and its chromosome-doubled tetraploid, fertile plant (*right*). (**c**) Female flowers of F_1 hybrid (*left*) and allotetraploid (*right*). (**d**) Fruit of F_1 hybrid (*left*) and allotetraploid (*right*). (**e**) Seeds harvested from the allotetraploid and its diploid progenitors

for the diploid-like meiotic behavior in the diploidization of the newly formed allopolyploids.

In order to explore the molecular involvement of epigenetic phenomena, cytosine methylation was investigated in *C.* × *hytivus* by using methylation-sensitive amplified polymorphism (MSAP) (Chen and Chen 2008). Twofold difference in the level of cytosine methylation in the reciprocal F_1 hybrids and in the allotetraploid was observed. Pattern analysis found that 2.0–6.4% of total sites changed in both the F_1 hybrids and the allotetraploid compared to their corresponding parents. 68.2–80.0% of the changed sites showed an increase in cytosine methylation, and most of the methylated sites were from the maternal parent. The extent of cytosine methylation pattern changes was greatly decreased during selfing process, suggesting stability in advanced generations.

Changes of gene expression played an important role in the evolution of plant allopolyploids. Characters of the changes of gene expression between the allotetraploid *C.* × *hytivus* and its diploid parental species were analyzed by using cDNA-AFLP technique. The results indicated that most genes from parents could be stably expressed in the allotetraploid, while some genes expressed differentially. A total of 36 (3.37%) differentially expressed transcripts were detected and classified into three types: no expression of genes from parents, expression of genes from one parent, and novel expression of new genes. The majority was the expression of genes from one parent. The data also showed that the genes from female parent were easier to be changed. Those results indicated a rapid change in gene expression in early generations of *C.* × *hytivus*.

In Cucurbitaceae, amphidiploidy was reported from *C. maxima* × *C. moschata* (Pearson et al. 1951) and *C. anguria* × *C. dipsaceus* (Yadava et al. 1986). There were no successful efforts on the two most commercially important *Cucumis* spp.: cucumber and melon. *C.* × *hytivus* as a synthesized allopolyploid can be a useful model system to study polyploidization and may also serve as a genetic bridge in *Cucumis* and thus is a source for broadening the genetic base of *C. sativus*.

6.6.2.2 Allotriploid

A primary allotriploid cucumber ($2n = 3x = 26$; HCC) was obtained through backcrossing the synthetic allotetraploid (HHCC, maternal parent) to cultivated cucumber (CC, paternal parent) (Chen

et al. 2003). The allotriploid showed many novel characters, such as strong heterosis, sequential fruit set, tolerance to low temperature and low light, and high nutritional value of the fruit. In morphology, fruit weight, number of branches, ovary length, fruit length, and spine and mature fruit of progeny more closely resembled their maternal *C.* × *hytivus* parent (Fig. 6.12); developmental vigor (i.e., relative growth rate) and leaf color (i.e., dark green vs. reseda of *C.* × *hytivus*) were more similar to and characteristic of the paternal, *C. sativus*, parent. Progeny were more parthenocarpic than either parent bearing abundant seedless fruit (i.e., 84.8% developed fruit under greenhouse conditions). While their parents, *C.* × *hytivus* and *C. sativus,* were lower (26.7 and 45.2%, respectively) (Table 6.6).

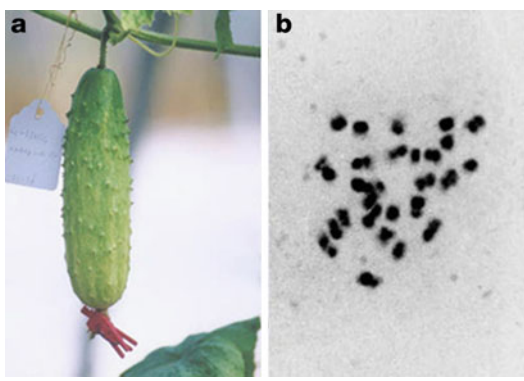

Fig. 6.12 (**a**) Fruit of *Cucumis* allotriploid plants derived from a cross between *C.* × *hytivus* and *C. sativus*. (**b**) Chromosome number of allotriploid plants

Recently, 19 cucumber cultivars were used to make crosses with *C.* × *hytivus* to identify crosses producing allotriploid fruits with viable seeds. Only three crosses had fruits with viable seeds. However, this marked significant progress toward commercial use of this seedless novel cucumber. The putative allotriploid plants were confirmed by molecular and cytological analysis.

6.6.2.3 Monosomic Alien Addition Lines

Two monosomic alien addition lines (MAALs) (14 CC + 1 H, $2n = 15$) were recovered among 252 regenerated plants, when the allotriploid was treated with colchicine to induce polyploidy (Chen et al. 2004a). Both the putative MAALs, plant numbers 87 and 517, grew more slowly and were easily differentiated morphologically from the allotriploids, *C.* × *hytivus*, and *C. sativus*. The leaf shape of these plants was palmate and hastate, respectively, in contrast to the pentagon shape observed in allotriploid and the wavy leaf edges observed in the allotetraploid *C.* × *hytivus*. Fruit length of plant numbers 87 and 517 was 23.6 ± 0.9 and 25.4 ± 1.5 cm, respectively, much longer than the allotriploids (12.1 ± 2.1 cm) (Fig. 6.13). Both plants showed white spines, characteristic of *C. sativus*, in contrast to black spines observed in progenies of the interspecific hybrids from the female parent, *C. hystrix*, indicating that the gene for black spines is not located on the alien chromosome in either plant number 87 or 517; both

Table 6.6 Morphological characteristics of *Cucumis hytivus*, *C. sativus* L. cv. Beijing jietou, and allotriploid plants derived from a *C. hytivus* × *C. sativus* mating

Traits[a]	*Cucumis hytivus*	Allotriploid plantlets	*Cucumis sativus*
Average number of branches	4.6 ± 1.9[b]	4.9 ± 1.2	1.3 ± 0.6
Ovary length (cm)	1.3 ± 0.2	1.9 ± 0.2	5.4 ± 0.7
Average fruit length (cm per fruit)	7.5 ± 1.4	12.1 ± 2.0	51.7 ± 3.5
Average fruit weight (gm per fruit)	35.4 ± 4.3	123.2 ± 5.8	322.4 ± 14.4
Mean pollen grain stainability (%)	21.9 ± 2.6	9.8 ± 2.5	94.4 ± 4.28
Fruit length: diameter ratio	2.2 ± 0.3	3.4 ± 0.2	12.5 ± 0.9
Parthenocarpic rate (%)	26.7	84.8	45.2
Fruit spine color	Black	Black	White
Leaf color	Reseda	Dark green	Dark green
Chromosome number	38 (4*n*)	26 (3*n*)	14 (2*n*)

[a]Primary branch number was recorded before season-end vegetative decline; ovary length and pollen grain stainability were recored at anthesis; five mature fruits were used for the determination of fruit weight, length and diameter fruit index, and spin color and leaf color was observed during the vegetative growth period.
[b]Data are shown with mean ±SE.

MAALs also showed a multibranching character reminiscent of *C. hystrix*.

Alien chromosome addition lines harboring one single chromosome from the wild species *C. hystrix* might be used as a bridge to transfer genes of interest originating in *C. hystrix* to individual chromosomes of *C. sativus* via recombination or translocation events.

6.6.2.4 Introgression Lines

C. sativus–hystrix introgression lines were obtained among progenies of interspecific hybrids after the allotetraploid was backcrossed to cultivated cucumber.

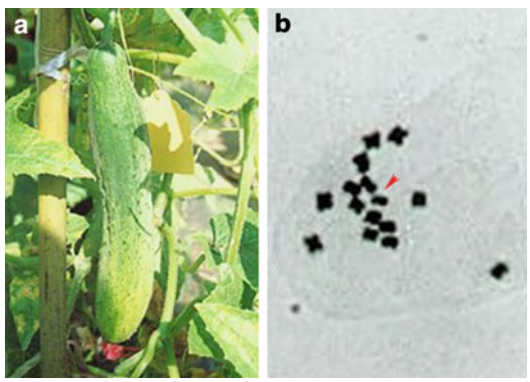

Fig. 6.13 (**a**) Fruit of the monosomic alien addition lines (MAAL). (**b**) Chromosome number of MAAL ($2n = 15$)

These introgression lines were genetically stable and differed from each other as well as their two parents in many morphological traits (Fig. 6.14). Substantial genomic changes were detected in introgression lines, including *C. hystrix*-specific fragments, deletion of fragments originally observed in *C. sativus*, and novel bands not from both parents (Zhou et al. 2009).

Zhou et al. (2009) used SSR markers to detect the introgression from *C. hystrix* to *C. sativus*, and one locus at 210 bp was revealed and assigned to introgressive fragment of *C. hystrix* genome in introgression line 56. This line was characterized to have small-sized leaf, short fruit, and multibranching habit, which were closer to the wild parent (*C. hystrix*), and had fertility as high as that of cultivated cucumber. Moreover, this line was found to have a desirable response to downy mildew and Fusarium wilt. Several molecular markers linked to these introgressed traits are now being developed. The introgression lines could be used as vehicle for transferring desirable characters from *C. hystrix* and valuable for the improvement of cucumber cultivars.

6.6.2.5 New Pickling Cucumber F_1 Hybrid

A new pickling cucumber line 7012A was developed through subsequent backcrossing of the hybrid to cucumber and selection from the selfed progenies

Fig. 6.14 Variation in fruit shape of introgression lines

Fig. 6.15 Fruit of Ningjia #1

(Chen et al. 2005). This line was used to cross with an elite American pickling cucumber line (7011A) from the University of Wisconsin to produce F_1 (Fig. 6.15). The results indicated that the F_1 has significant heterosis over its parents in yield and growth vigor. The plants set uniform fruits with good quality. Fruits could be set on both the main and lateral branches. It has highest late yield and its total yield was higher than all the other cultigens tested and this F_1 was subsequently designated as Ningjia #1.

6.7 Genomic Resources

The development of genomic tools in *Cucumis* species has been very limited in spite of the importance of these species. In recent years, there has been an effort toward the development of genomic resources mainly in melon and cucumber, the cultivated species of *Cucumis*.

The Cucurbit genomics database of the International Cucurbit Genomics Initiative offers comprehensive information on express sequence tags (ESTs) and maps developed in the cucurbits. The version 2 of melon EST collection contains 34,451 ESTs and published genes, representing 16,128 unigenes. In cucumber, 4,331 ESTs are also available from leaf, fruit, and flower tissues representing around 3,000 unigenes. Several genetic maps are available for melon and cucumber. They have been constructed using different types of molecular markers and populations; however, these maps are still far from saturation (Garcia-Mas 2008).

Several bacterial artificial chromosome (BAC) libraries have been constructed in melon (Luo et al. 2001; van Leeuwen et al. 2003) and cucumber (Nam et al. 2005). A fosmid library of cucumber was also constructed (Havey et al. 2008). These libraries can be accessed from the website of the Clemson University Genomics Institute (http://www.cugi.org). Construction of a BAC library of *C. hystrix* is now undergoing in China.

The Cucumber Genome Initiative has reported the progress of the sequencing of the cucumber genome. The sequencing of the cucumber genome has been completed and about 30,000 genes were annotated. A genetic map was constructed, consisting of 1,200 diversity array technology (Dart) markers and 1,000 SSR (microsatellite) markers, distributed over seven linkage groups. By fluorescent in situ hybridization (FISH) mapping of 60 SSR-anchored fosimids, an integrated genetic and cytogenetic map was constructed and chromosomal rearrangements between subspecies of cucumber were discovered (Huang et al., pers comm). The cucumber genome sequence will provide a rich resource for investigating the pathways of gene and genome evolution.

6.8 Recommendations for Future Actions and Future Prospects

Wild species are an important reservoir of useful genes and offer great potential to incorporate such genes into commercial cultivars for resistance to major diseases, insects, and tolerance to various abiotic stresses. Moreover, many of the useful alien genes are different from those of the cultivated species and are thus useful in broadening the sources of resistance/tolerance to various stresses. However, despite the importance of wild species of *Cucumis*, very little information is available on the studies of wild species. Morever, use of exotic germplasm for the development of lines and populations with unique traits has been limited. The application of genetic markers to germplasm management or for marker-assisted selection has not been clearly defined in *Cucumis*. Therefore, we hope that future attention should be paid to research on the wild species.

As a first step, germplasm acquisition of all the *Cucumis* species available from every country should be initiated. Our objective should not be limited to the maintenance of germplasm. Instead, what is required is a well-organized research program on germplasm characterization, utilization, and enhancement.

An important long-term objective for *Cucumis* breeders is the introduction of genes from wild relatives. Some wild relatives, such as *C. metuliferus* E. Meyer ex Naudin (nematode resistance) and *C. figarei* Naudin (virus resistance), have long been attractive to scientists. However, the limits to utilization of wild relatives depend on the breeders' ability to produce interspecific hybrids, but hybrids may be sterile. Future research should focus on producing fertile interspecific hybrids through biotechnological or other approaches.

The rapid advancement in the molecular genetics has now opened a new era of technologies and the application of MAS in crop breeding has become more and more important. There is thus a need to develop molecular markers tightly linked to the useful traits of wild species. Generation and utilization of novel genetic stocks such as introgression lines, substitution lines, and deletion lines will further facilitate the genetic analysis of complex agronomic traits and the introgression of desirable genes into commercial cultivars. Construction of the bacterial artificial chromosome (BAC) library of wild species of *Cucumis*, along with the tightly linked markers of desirable traits, may greatly promote the identification and isolation of useful genes for cucumber and melon improvement.

Wild relatives are useful sources of characters desired to improve cultivated *Cucumis* species. Although many of these wild species are resistant to pests and diseases or adapted to adverse environments, the utilization of wild relatives is limited due to the cross-incompatiability problems. However, the successful development of the synthetic allotetraploid species *C.* × *hytivus* Chen and Kirkbride via chromosome doubling of an interspecific hybrid between cucumber and *C. hystrix* Chakr. provides a step toward transferring desirable genes from the wild species *C. hystrix*. Future works emphasized on map-based cloning of the downy mildew and root-knot nematode resistance genes from *C. hystrix* will be of great value for the improvement of cucumber and melon.

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