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Rebecca Grumet
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Genetics and Genomics of Cucurbitaceae

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Preface

The twenty-first century is a golden age for genetics. Massively increasing DNA sequence information and sophisticated bioinformatic capacity at exponentially decreasing cost allow for a world of knowledge that is no longer limited to model species. Members of the Cucurbitaceae family are among the beneficiaries of these technological gains. The Cucurbitaceae, often referred to as "cucurbits," are probably best known for their large (sometimes extremely large), colorful, and morphologically variable fruits. The most economically important crops are watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), cucumber (*Cucumis sativus*), and various squashes and pumpkins (*Cucurbita pepo, maxima,* and *moschata*), which are produced in widely diverse forms throughout the world. Depending on the specific crop and type, they can be consumed as vegetable or dessert, and in some cases, especially for various squashes and pumpkin, can serve as mainstays of the diet. Additional cultivated crops include bitter, bottle, wax, snake sponge, and ridge gourds, which are primarily cultivated in southern and southeastern Asia. Seeds of cucurbit crops also can be an important source of nutrition. In addition, certain cucurbit species are noted for medicinal properties, and gourd shells have historical uses as containers and musical instruments.

Several genomic initiatives throughout the world are exploring the genetics and genomics of these crops. Genetic features, including diploid genomes and relatively small genome size (~367, 454, 450, and 400 Mbp for cucumber, melon, watermelon, and squash, respectively), facilitate these efforts, and close genetic relationships allow for synergistic approaches. Over the course of the past decade draft genome sequences have been assembled for cucumber, melon, and watermelon; assemblies of *Cucurbita* species are in progress. As would be expected, a major section of *"Genetics and Genomics of the Cucurbitaceae"* is devoted to description of cucurbit genomes and available genomic resources.

Of course genomic information does not exist in a vacuum and must be interpreted within the context of the crop or species and its agronomic, geographic, and evolutionary relationships. The Cucurbitaceae family contains approximately 1000 species, with origins tracing to Southeast Asia prior to subsequent distribution and diversification in Africa and South America. From this great diversity, a small num-

ber were domesticated and carried to current crop status. Over the past century, the most extensively cultivated cucurbits have been greatly improved by plant breeders using conventional plant breeding techniques to increase productivity, yield, fruit size, and quality. This volume provides an overview of use of cucurbits and their evolutionary relationships and explores the genetic resources for the cucurbit crops. Much current effort in each of the crops is devoted to incorporation of resistance to critical diseases for which germplasm collections around the world serve as invaluable, critical resources. Looking forward, molecular breeding approaches facilitated by genomic advances are expected to play an increasing role in facilitating crop improvement, including introgression of novel resistance alleles along with other desirable traits.

Finally, the cucurbits are especially noteworthy for several biological features such as unique phloem structure, highly flexible sex expression patterns, extensively diverse fruit size, shape, colors and patterns, and delicious flavors and aromas. Increasing genomic tools and genetic analyses are making major contributions to our understanding of the fundamental bases for these biological phenomena. Thus, collectively, *"Genetics and Genomics of the Cucurbitaceae"* explores the genetic diversity of cucurbit crops, the current state of knowledge of cucurbit genomics, and evolving applications of genetics and genomics for improvement of cucurbit crops and understanding of cucurbit growth, development, and adaptation to their environments.

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Cultivation and Uses of Cucurbits

James D. McCreight

Abstract Cultivated cucurbits have spread through trade and exploration from their respective Old and New World centers of origin to the six arable continents and are important in local, regional and world trade. Cucumber, melon, pumpkin, squash and gourd, and watermelon comprise the major cucurbits. Bitter gourd, bottle gourd, wax gourd, sponge and ridge gourd, and snake gourd are minor cucurbits from a global perspective that are of import to small shareholder farmers, mostly in Asia. Global production of the major cucurbits increased from 1992 through 2013 in terms of area harvested and yield per hectare, and consequently total production. Production per capita, and presumably consumption, increased in parallel with gains in total production. Cucurbits can play an important role in dietary health. They are low in nutritional value, but can be significant dietary sources of vitamins and minerals. Some cucurbits, such as bitter gourd, have medicinal properties. Cucurbits are generally prized for their delicious fruits, which can be sweet, bitter or aromatic, and may be highly perishable or stored for months with little change in quality. The seeds are good sources of vegetable oil and protein. Gourd shells may be used for storage containers, or as musical instruments. The cultivated cucurbits have been greatly improved by plant breeders using conventional plant breeding techniques for more than 100 years; rapidly advancing molecular technologies are being applied to cucurbits to ensure sustainable production, improve fruit quality and shelf life, and develop novel fruit types.

Keywords Cucumber • Melon • Pumpkin • Squash • Gourd • Cucurbit production • Nutritional value • Genetic resources • Disease resistance • Grafting • Plant breeding

Introduction

Cucurbits encompass a diverse group of annual and perennial species, several of which are of commercial importance worldwide. Cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), pumpkin, squash and gourd (*Cucurbita* spp.), and

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Table 1 Number of countries reported by FAO to produce the four major cucurbit crops, 2013

Crop	Species	No. countries
Cucumber & Gherkin[a]	*Cucumis sativus*	142 (72 %)
Melon	*Cucumis melo*	105 (54 %)
Pumpkin, squash & gourd	*Cucurbita* spp.	123 (63 %)
Watermelon	*Citrullus lanatus*	128 (65 %)

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015), and percentage of total world countries (www.infoplease.com/ipa/A0932875.html Accessed 7 Dec 2015)

[a]Gherkhin likely refers to small cucumbers rather than to the distantly related, sexually incompatible species, *Cucumis anguria* var. *anguria* (Robinson and Decker-Walters 1997; Whitaker and Davis 1962)

watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] comprise the major cucurbits (Table 1). Members of five additional genera are important to smallholder farmers and home gardens in east, south and southeast Asia: bitter gourd (*Momordica charantia* L.), bottle gourd [*Lagenaria siceraria* (Molina.) Standley], wax gourd [*Benincasa hispida* (Tunb.)], sponge and ridge gourd (*Luffa* ssp.), and snake gourd (*Trichosanthes* ssp.) are minor cucurbits from a global perspective that are of import to small shareholder farmers, mostly in Asia. The current state of the genetics and genomics of the major and minor cultivated cucurbits are reviewed in the following chapters.

Cucurbits have, like many crop species, a long history with human culture (Kistler et al. 2015; Robinson and Decker-Walters 1997; Whitaker and Davis 1962). Low in carbohydrates, they have nevertheless been enjoyed in various forms worldwide; indeed, an Indian tribal person in Madhya Pradesh, India stated, in essence, that “A day without a melon is like a day without the sun.”

A major aspect of cucurbit–human interaction is the movement of cucurbits around the world, far beyond their centers of origin, through exploration and trade. *Cucurbita* ssp. are New World, while the other cucurbits are Old World, either Africa (watermelon) or southern Asia (*Cucumis*, *Momordica charantia, Lagenaria siceraria, Benincasa hispida, Luffa*, and *Trichosanthes*).

Cucurbit Production Worldwide

The four major groups of cucurbits (Table 1) are grown by farmers in 105 (melon) to 142 (cucumber & gherkin) countries across the six hospitable continents (Table 2), among which Asia accounted for 75 % of the area harvested and 83 % of the total production in 2013 (Table 2).

The highest calculated mean yields (tonnes per ha based on FAO statistics for area harvested and total production) were, on average, achieved in Asia (30.7), Australia & New Zealand (23.1), and North America (26.1) (Table 2). Calculated yields varied greatly across the six continents. The range in yields derives from differences in length of growing season, open field *vs.* protected cultivation, and management practices, including irrigation, fertilizer, and pest control.

Table 2 Cucurbit (cucumber, gherkin, gourd, melon, pumpkin, squash, watermelon) cultivation by continent, 2013

Continent	Area harvested (ha)	Production (tonnes)
Africa	856,587 (10.3 %)	11,406,859 (5.0 %)
Asia	6,222,817 (74.8 %)	191,431,365 (83.3 %)
Australia & New Zealand	22,412 (0.3 %)	517,574 (0.2 %)
Europe	746,593 (9.0 %)	16,231,783 (7.1 %)
Northern America	178,478 (2.1 %)	4,651,429 (2.0 %)
South America	294,709 (3.5 %)	5,537,167 (2.4 %)
Total	8,321,596	229,776,177

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015)

Table 3 Asia: Cucurbit (cucumber, gherkin, gourd, melon, pumpkin, squash, watermelon) production by region, 2013

Region	Area harvested (ha)	Production (tonnes)
Eastern Asia	3,932,681 (63.2 %)	152,185,158 (79.5 %)
Central Asia	225,939 (3.6 %)	5,348,178 (2.8 %)
Western Asia	635,452 (10.2 %)	13,058,963 (6.8 %)
South-Eastern Asia	281,688 (4.5 %)	4,396,535 (2.3 %)
Southern Asia	1,147,057 (18.4 %)	16,442,531 (8.6 %)
Total	6,222,817	191,431,365

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015)

Eastern Asia (China, Japan Korea) accounted for 63 % of the Asian cucurbit production (Table 3). Calculated mean yields (tonnes per ha) ranged from 30.7 (Eastern Asia) to 14.3 (Southern Asia). Detailed statistics are not readily available for the minor cucurbits that are common to Asia, but bitter gourd was reportedly harvested from more than 340 K ha (Dhillon et al. 2016), and bitter gourd was harvested in China from more than 200 K ha of plants grafted onto rootstocks of three cucurbit species (Lee et al. 2010). Thus, minor cucurbits contribute substantially to agricultural production.

North Africa produced 81 % of the total cucurbit production of Africa on 42 % of the area harvested (Table 4). Calculated estimates of mean yields (tonnes per ha) ranged across Africa from a world wide low of 1.6 in the middle of the continent to 25.8 in the north, which is above the world mean of 20.6, where water is more abundant and resources are more readily available for export markets.

Eastern Europe accounted for 69 % of the area harvested but only 51 % of the total production in Europe (Table 5). Northern Europe accounted for 0.4 % and 1.4 % of the area harvested and production, respectively, but its calculated mean yield was 75.4 tonnes per ha; 77 % greater than the mean yield of Europe (42.6). Calculated mean yield for the other parts of Europe ranged from 15.8 (Eastern Europe) to 46.3 (Western Europe).

The USA accounted for approximately 50 % of the area harvested and the total production of North America, followed by Mexico at 30 % for these two measures of productivity (Table 6). The highest calculated mean yields (tonnes per ha) in

Table 4 Africa: cucurbit (cucumber, gherkin, gourd, melon, pumpkin, squash, watermelon) production by region, 2013

Region	Area harvested (ha)	Production (tonnes)
Eastern Africa	61,028 (7.1 %)	434,272 (3.8 %)
Middle Africa	342,738 (40.0 %)	539,583 (4.7 %)
Northern Africa	358,347 (41.8 %)	9,235,405 (81.0 %)
Southern Africa	26,145 (3.1 %)	295,307 (2.6 %)
Western Africa	68,329 (8.0 %)	902,292 (7.9 %)
Total	856,587	11,406,859

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015)

Table 5 Europe: cucurbit (cucumber, gherkin, gourd, melon, pumpkin, squash, watermelon) production by region, 2013

Region	Area harvested (ha)	Production (tonnes)
Eastern Europe	519,332 (69.6 %)	8,226,562 (50.7 %)
Northern Europe	2992 (0.4 %)	225,738 (1.4 %)
Southern Europe	194,147 (26.0 %)	6,385,478 (39.3 %)
Western Europe	30,122 (4.0 %)	1,394,005 (8.6 %)
Total	746,593	16,231,783

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015)

Table 6 North America: cucurbit (cucumber, gherkin, gourd, melon, pumpkin, squash, watermelon) production by country, 2013

Country	Area harvested (ha)	Production (tonnes)
Canada	8514 (2.5 %)	349,146 (4.0 %)
Costa Rica	6067 (1.8 %)	192,199 (2.2 %)
Guatemala	30,815 (9.1 %)	697,050 (8.0 %)
Honduras	15,074 (4.4 %)	447,025 (5.1 %)
Mexico	104,443 (30.8 %)	2,697,580 (30.9 %)
Panama	4322 (1.3 %)	46,572 (0.5 %)
United States of America	169,964 (50.1 %)	4,302,282 (49.3 %)
Total	339,199	8,731,854

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015)

North America were achieved by Canada (41.0) and Costa Rica (31.8), which was followed closely by Honduras (29.6). Calculated mean yield for the USA was 25.3 tonnes per ha.

Brazil accounted for approximately 40 % of the area harvested and the total production of South America (Table 7), while Venezuela was a distant second at 17 % and 19 %, respectively, for these two measures of cucurbit productivity. The highest calculated mean yields (tonnes per ha) in South America were achieved by Peru (24.1) Brazil (24.0), Suriname (21.1) and Venezuela (20.5).

Cucurbit production increased from 1991 through 2013 in terms of area harvested, yield per hectare, and total production (Fig. 1). Watermelon had the largest absolute increase, 1.3 M ha (60 %), compared with cucumber, 0.9 M ha (78 %), melon, 0.3 M ha

Table 7 South America: cucurbit (cucumber, gherkin, gourd, melon, pumpkin, squash, watermelon) production by country, 2013

Country	Area harvested (ha)	Production (tonnes)
Argentina	31,942 (10.8 %)	503,356 (9.1 %)
Bolivia (Plurinational State of)	4311 (1.5 %)	41,030 (0.7 %)
Brazil	114,042 (38.7 %)	2,729,401 (39.3 %)
Chile	11,474 (3.9 %)	224,952 (4.1 %)
Colombia	21,916 (7.4 %)	3,102,86 (5.6 %)
Ecuador	8775 (3.0 %)	107,218 (%)
French Guiana	93 (0.03 %)	1622 (0.03 %)
Guyana	1730 (0.6 %)	14,957 (0.3 %)
Paraguay	30,757 (10.4 %)	145,140 (2.6 %)
Peru	16,232 (5.5 %)	391,092 (7.1 %)
Suriname	130 (0.04 %)	2746 (0.05 %)
Uruguay	2695 (0.9 %)	28,005 (0.5 %)
Venezuela (Bolivarian Republic of)	50,612 (17.2 %)	1,037,360 (18.7 %)
Total	294,709	1,355,075

FAO. 2015. FAOSTAT http://faostat3.fao.org (Accessed 7 Dec 2015)

(32 %), and *Cucurbita*, 0.8 M ha (89 %). Watermelon increased rapidly from the mid-1990s through 2000 and continued to increase at a slower rate (Fig. 1). Cucumber showed a similar trend from 1991 through 2000, but then leveled off. *Cucurbita* steadily increased at a more or less constant rate. Melon increased more gradually starting in 2000, and exhibited greater seasonal variation than the other cucurbits (Fig. 1).

Yields (Hg per ha) of melon and watermelon increased steadily from 1991 through 2013, 97 % and 59 %, respectively (Fig. 1). Cucumber yields increased slightly through 1999 but thereafter increased steadily through 2013, for an increase of 126 %. *Cucurbita* yield increased slightly (20 %) through 2013.

Total production of cucumber and watermelon increased 302 % and 217 %, respectively, from 1991 through 2013 (Fig. 1). Melon and *Cucurbita* production gains were 111 % and 127 %, respectively. Gains in cucurbit production per capita roughly paralleled gains in total production (Fig. 1). Thus, as global cucurbit production increased through a combination of increased area harvested and yield increases, per capita production of major cucurbits increased 141 % overall, and ranged from 69 (melon) to 218 % (cucumber), with increases of 151 % and 80 % for watermelon and Cucurbita, respectively.

Production Methods

Cucurbits may be harvested from monsoon-fed sand dunes of the Thar Desert area of Rajasthan with no other inputs, or they may be grown with modest, e.g., Turkmenistan, or more precise, e.g., lower desert areas of the southwest USA, control of inputs. They may be cultivated under protection with some control of conditions under plastic, e.g., Spain, or more precise control under glass, e.g., The Netherlands.

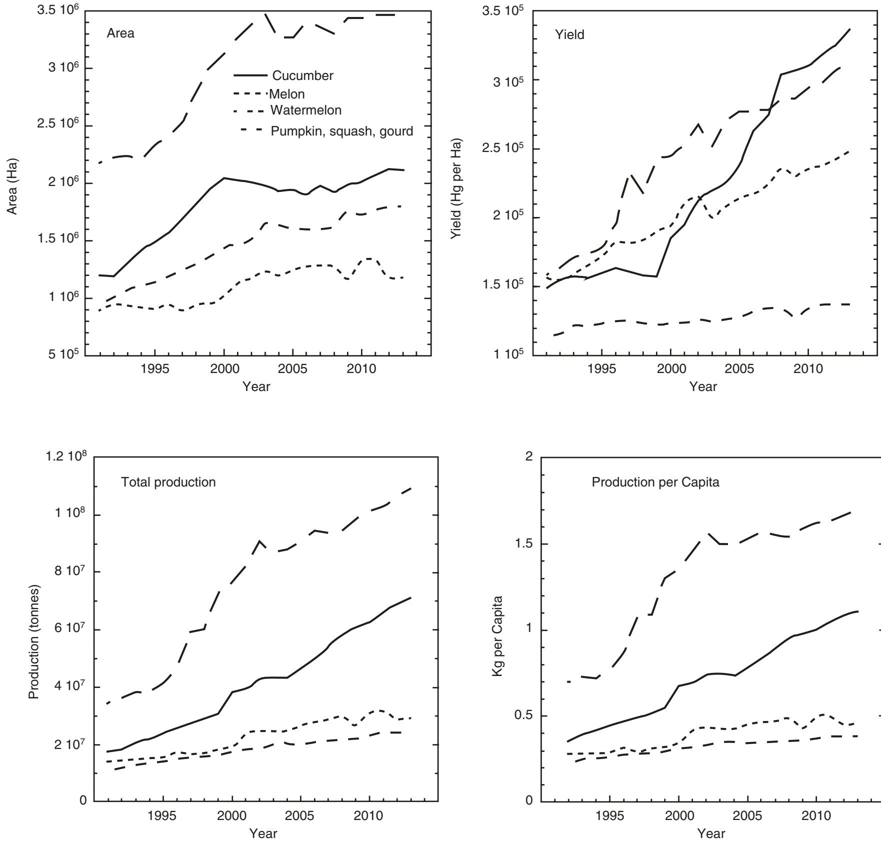

Fig. 1 Trends in area harvested, yield and total production of cucumber, melon, watermelon, and pumpkin, squash and gourd, and their respective production per capita from 1991 through 2013; FAO. 2015. FAOSTAT http://faostat3.fao.org (Area, yield and production-Compare Data link accessed 4 Sept. 2015; Population data for per capita estimate accessed 27 Nov. 2015)

Cucurbits are produced during the dry seasons in areas of abundant rainfall, e.g., south and southeast Asia, and in some of the driest deserts that have external water sources, e.g., Turpan, Xinjiang, China, which is surrounded by the Gobi, Gurbantünggüt and Taklamakan Deserts, or geologic water sources, e.g., Albian Aquifer underlying Algeria, Libya and Tunisia (Foster and Loucks 2006; Mamou et al. 2006; Ouali et al. 2010). Cucurbits are grown in open fields as well under various types of protection, such as wire supported or floating row covers. The plants may be prostrate or trellised. Field cultivation is often done on raised beds for better drainage. Increased soil salinity from surface irrigation in some desert areas must be managed through the use of engineered drainage systems in combination with precision planed (sloped) fields and raised beds, e.g., Imperial Valley, California, USA. Plants in plastic and glass houses may be grown in soil or soilless media, e.g., sand, rockwool, where irrigation and drainage can be precisely managed.

Fig. 2 Irrigated melon in a field claimed from the desert in 2008 by a farmer from the Karakum Desert (in background), Mary, Turkmenistan (Photo courtesy of T.C. Wehner)

The technology used in cucurbit production ranges from low input to maximum input. The native peoples of the Thar Desert area of Rajasthan, India harvest vegetable type melons grown with no other inputs (fertilizer, pesticides) from monsoon-fed sand dunes, as there is no other means of providing water (U. Srivastava, personal communication). In Turkmenistan, farmers are encouraged to expand vegetable production, including watermelon and melon into land newly claimed from the Karakum (Black Sand) Desert that is irrigated with water provided by local irrigation districts and that originated from snowmelt in the mountains of Tajikistan (Fig. 2). Farmers in Ürümqi and Turfan, Xinjiang, China produce watermelon and melons for domestic and export markets using water from the Tian Shan Mountains.

Cucurbits are direct seeded in many areas of the world, but transplants are commonly used for greenhouse production. Grafting is common for stand establishment in Japan, Korea and China, and is expanding to other countries for control of soil-borne diseases, improved vigor, and tolerances to cold temperatures, salinity, drought and flooding (Davis et al. 2008; Lee et al. 2010). Several cucurbit species are suitable as rootstocks.

Cucurbits are subject to many diseases and insect pests. Powdery mildew incited by the two obligate erysiphaceous ectoparasites (*Golovinomyces orontii s.l.*, and *Podosphaera xanthii*) is nearly ubiquitous (Jahn et al. 2002). Many other diseases are also important in one or more regions of the world (Zitter et al. 1996). Their control is not always successful and is done using cultural practices, including chemical protectants or eradicants, and host plant resistance. The succeeding chapters in this review will address host plant resistance, as appropriate.

Consumption and Use of Cucurbits

Increased production per capita likely means increased consumption per capita, assuming other factors constant, e.g., losses due to spoilage, and is likely due to the interplay of factors beyond the scope of this introduction. One factor of note is the

educational emphasis by western governments, e.g., 5 A Day for Better Health Program (http://www.fns.usda.gov/5-day), and non-governmental organizations, health care providers and individuals to reverse the trend of increasing obesity and other diseases, e.g., type 2 diabetes, associated with western diets (Taubes 2011).

Cucurbits can play an important role in improved dietary health. They are low in nutritional value, compared with other vegetables, but they provide ranges of sweetness (from subtle, e.g., freshly sliced cucumber, to bold, e.g., watermelon), texture (from crunchy, e.g., Piel de Sapo melons, to stringy, e.g., spaghetti squash, *Cucurbita pepo*), color, and low calorie bulk as fresh alternatives to the proliferating array of readily available, carbohydrate rich, processed foods.

Fruit water content ranges from 86 (winter squash) to 95 % (cucumber), and caloric content per 100 g fresh material ranges from 15 kcal for cucumber to 46 kcal, on average, for winter squash (Ensminger et al. 1983). The minor cucurbits addressed in this volume are comparable to the major cucurbits for water content and caloric value, e.g., bottle gourd (*Lagenaria siceraria* (Molina.) Standley) 92 % water and 26 kcal/100 g fresh fruit wt., and wax gourd (*Benincasa hispida* (Thunb.) Cogn.) 96 % water and 13 kcal/100 g fresh fruit wt (Ensminger et al. 1983).

Sweet or bland, bitter or aromatic, highly perishable or storable for months with little change in quality, cucurbits are, with few exceptions, prized for their delicious fruits that may be consumed immature or mature, and the seeds may be used for vegetable oil and protein (Jacks et al. 1972) (Fig. 3). Cucumbers are enjoyed either fresh or pickled. Watermelon is prized for its sweet crunchy flesh that is typically eaten fresh but which can be prepared into a jam. Leaves may be consumed for food or medicinal purposes: melon in Tanzania (B.D. Jensen, personal communication), *Citrullus* in China (Yang and Walters 1992), and *Cucurbita maxima/moschata* in Zimbabwe (Ndoro et al. 2007), and *Cucumeropsis mannii* Naudinin Benin (Achigan-Dako et al. 2008). Both male and female *Cucurbita pepo* flowers are commonly consumed in Mexico and Italy in soups and other foods (L. Wessel-Beaver, personal communication). Pickled watermelon rinds are commercially available in many countries, and recipes are easily found online. Mature seeds are consumed in numerous countries, e.g., China. Pumpkin seed oil from the Styria region of Austria is a European Union Protected Designation of Origin (PDO) product. “Roaster mixes” of several cucurbit species are common in Asian countries; indeed there were cucumber and melon seeds in one such mix (cucumber PI 175111) purchased at a market in Mussoorie, India. The melon fraction was re-numbered as PI 371795, which after selection for uniform reaction to melon aphid, *Aphis gossypii* gave rise to PI 414723 (Fig. 4), a rich source of genes for host plant resistance to several diseases (Dhillon et al. 2012; McCreight et al. 1992). Fruit of “wild” melons that are small, non-sweet, thin-fleshed and mostly seed are used in Madhya Pradesh, India in cooked dishes and may be dried for use at a later time (Fig. 5).

Winter squashes may be stored for months (Robinson and Decker-Walters 1997). Long season melons grown in the central Asian countries of Uzbekistan and Turkmenistan can be stored for up to 6 months with no loss of quality (14–18 % soluble solids), range in weight from 8 to 35 kg per fruit (Anon 2008; Mavlyanova et al. 2005a), and were prized by European monarchy (Anon 2008).

Fig. 3 Four melon products at a roadside melon market, Tejen, Turkmenistan; background, long season and long shelf-life Vaharman-type fruit with 14–18 % soluble solids; melon seeds in sack, bottled melon seed oil, and cellophane-wrapped "gavun kak," which consists of dried and twisted slices of Vaharman-type fruit (Anon 2008; McCreight et al. 2013. Photo courtesy of J.D. McCreight)

Fig. 4 Sample of fruit diversity of melon; from left to right, Iran H, 'Top Mark', PI 414723, PI 124111, PI 124112, and PI 313970; Imperial Valley, California (Photo courtesy of J.D. McCreight)

Dried gourd shells may be used as ornaments, storage containers, or as musical instruments. Some cucurbits, such as bitter gourd, have medicinal properties (Dhillon et al. 2016). Cucurbits can be used for skin care, e.g., *Luffa cylindrical*

Fig. 5 "Wild" melon fruits split open and drying for later use in soups or stews, Madhya Pradesh, India (Photo courtesy of J.D. McCreight)

used as a sponge, and as extracts in cosmetics (Athar and Nasir 2005). Winter squashes are used in some areas as cattle fodder (Grisales 2015).

See Robinson and Decker-Walters (1997); Whitaker and Davis (1962) for a more thorough overview of cucurbit uses, including many minor species not addressed in this review of major and major-minor cucurbits.

Improvement of Cucurbit Germplasm

The cultivated cucurbits have been improved greatly by plant breeders using conventional plant breeding techniques for more than 100 years. Cucurbit germplasm resources have and will continue to serve as valuable resources for new genes and alleles for important production, e.g., disease resistance, and market traits, including new market types. The USDA, ARS germplasm repositories and associated Germplasm Information Network (GRIN) database (http://ars-grin.gov) serve genetic improvement of cucurbit crops worldwide (Clark et al. 1991; Dhillon et al. 2012). In addition, there are many other cucurbit germplasm repositories/collections worldwide (Esquinas-Alcazar and Gulick 1983), e.g., AVRDC – The World Vegetable Center and Kasetsart University (http://avrdc.org/seed/improved-lines/[accessed 9 Mar 2016]), Centre for Genetic Resources, the Netherlands (http://www.wageningenur.nl/en/[accessed 9 Mar 2016]), Uzbek Research Institute of Plant Industry, Uzbekistan (Mavlyanova et al. 2005a, b).

As we look to the future, rapidly advancing molecular technologies and genomic approaches are being applied to cucurbits to ensure sustainable production, improve fruit quality and shelf life, and develop novel fruit types. The subsequent chapters in this book will describe genetic and genomic resources for the major and minor cucurbit crops and application of those resources to crop improvement and understanding of cucurbit crop biology.

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Phylogeny and Evolution of the Cucurbitaceae

Susanne S. Renner and Hanno Schaefer

Abstract The Cucurbitaceae family contains about 1000 species in 96 genera. Representatives of all genera (except the extinct *Khmeriosicyos*) and a large percentage of the species have been sequenced for the ribosomal RNA transcribed spacer regions and variable regions of the plastid and mitochondrial genome. These data have allowed to infer evolutionary relationships in the family. The major phylogenetic structure of the family is now clear, and this chapter includes an up-to-date phylogenetic scheme with the placement of all genera. The Cucurbitaceae clade originated in mainland Southeast Asia sometime in the Late Cretaceous, and the five deepest evolutionary divergences in the family all date to the Late Cretaceous, 70–80 Ma. Two of these ancient clades, the Gomphogyneae and *Actinostemma*, are now almost restricted to Asia. A third ancient group, the Triceratieae, is mainly Neotropical, except one African genus; other clades and tribes are more widespread. The economically most important genera are concentrated in the Cucurbiteae and Benincaseae, and species of *Cucumis* and *Citrullus*, with well-annotated genomes, therefore have largely comparable (homologous) linkage groups. In contrast to the relatively good data on the family's phylogeny, data on its ecology, physiology and morphological evolution are scarce and collection and study of wild species, many of them in threatened habitats is much needed.

Keywords Collections • Molecular phylogenetics • Molecular clock • Publicly available herbarium specimens • Sister groups • Crop wild relatives

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Introduction

The Cucurbitaceae are a tropical and subtropical family that is related to the Begoniaceae, Datiscaceae, and Tetramelaceae, with which it shares inferior ovaries and parietal placentation (Zhang et al. 2006). The precise relationships among these four families remain unresolved. Their tendrils readily distinguish Cucurbitaceae from their closest relatives, and the family's monophyly is well supported by molecular analyses that have included a dense sampling of both outgroups (potential relatives) and Cucurbitaceae themselves (Kocyan et al. 2007; Schaefer et al. 2009; Schaefer and Renner 2011a, b). Indeed, species from all genera of Cucurbitaceae except *Khmeriosicyos*, a genus only known from the type, a specimen collected in Cambodia in 1873 and now in the Paris herbarium, have been sequenced for at least one nuclear DNA region and one or more plastid regions. Maximum likelihood and Bayesian phylogenies inferred from these large data matrices reveal five statistically well-supported clades. This chapter summarizes the phylogenetic placement of all genera as well as the ages of the family's major clades based on fossils and molecular clock approaches. We conclude with a brief review of morphological trends and historical geographic expansion of the family.

The Main Clades (and Taxonomic Tribes) of the Cucurbitaceae

The most comprehensive molecular phylogenetic analysis of the Cucurbitaceae is that of Schaefer and Renner (2011b) who included ribosomal RNA transcribed spacer regions, two mitochondrial regions, and nine regions of the plastid genome for 664 species of Cucurbitales (most of them Cucurbitaceae, which were represented with 95 genera). Figure 1 shows the placement of all currently recognized genera and is up-dated from the most recent taxonomic classifications of the Cucurbitaceae (Schaefer and Renner 2011a, b). The deepest phylogenetic divergences in the family can be 'captured' in five major groups of genera, namely, (I) a group that includes *Alsomitra*, *Bayabusua*, and *Neoalsomitra*, which corresponds to tribe Gomphogyneae of Bentham & Hooker; (II) a group of one African genus and five Neotropical genera, including *Fevillea* and *Sicydium*, which corresponds to tribe Triceratieae of A. Rich.; (III) a group of four or five genera from Madagascar, continental Africa, Asia, and South America, corresponding to tribe Zanonieae of Bentham and Hooker; (IV) a clade consisting of the Asian *Actinostemma*; and (5) a group of c. 80 genera that has traditionally been ranked as subfamily Cucurbitoideae of Kosteletzky. Before molecular data, the groups (1) to (4) (above) were placed together in a subfamily called Zanonioideae (Benth. & Hook.f.) Luerss. or Nhandiroboideae (Kosteletzky 1833; Jeffrey 1980, 1990, 2005), however, Nhandiroboideae is an illegitimate name, and Zanonioideae is a taxonomic synonym of Fevilleoideae Burnett (Burnett 1835). Neither morphological data nor molecular phylogenetic results support the division of the family Cucurbitaceae into

Fig. 1 Cladogram showing the tribal classification of the Cucurbitaceae based on 14 DNA regions from chloroplast, nuclear and mitochondrial DNA sequences. Up-dated from Schaefer and Renner (2011a, b, the latter paper showing the statistical support for this tree). Numbers in parentheses refer to species numbers (Photos by H. Schaefer)

two equal-aged or otherwise equivalent groups, and the morphological characters thought to distinguish Zanonioideae from Cucurbitoideae, namely, striate pollen, winged seeds, and "zanonioid" tendrils (tendrils in which the lower section is capable of curving), all occur also in Cucurbitoideae. We therefore recommend not using

any subfamily division in this relatively small family. Brief descriptions of the 15 tribes (Fig. 1), including comments on geographic occurrence, and chromosome numbers are provided in Schaefer and Renner (2011b).

Morphological Evolutionary Trends in the Cucurbitaceae

Sexual Systems

Cucurbitaceae are usually hairy climbers with simple or branched, lateral tendrils (very rarely, the tendrils are lost, e.g. in the cucumber tree, *Dendrosicyos socotranus*), yellow or whitish unisexual flowers, an inferior ovary with parietal placentation and numerous, relatively large seeds. About 50% of their species are monoecious and 50% dioecious; no wild Cucurbitaceae have only bisexual flowers although a few have individuals with bisexual flowers and others with staminate flowers (individuals with bisexual flowers regularly occur in *Schizopepon bryoniifolius* and *Zehneria hermaphrodita*; Schaefer and Renner 2011a). Dioecy appears to be the ancestral condition in the family, with much back and forth between dioecy and monoecy (Zhang et al. 2006; Volz and Renner 2009; Schaefer and Renner 2010), and a dioecious mating system may even go back to the common ancestor of Begoniaceae, Cucurbitaceae, Datiscaceae, and Tetramelaceae, all of which have normally unisexual flowers and are entirely or mostly dioecious (Zhang et al. 2006). Spontaneous mutations that modify flower sex phenotype are common and were favored by breeding targeted towards femaleness (see also The Genomics of Wood Formation in Angiosperm Trees). In *Cucumis*, a genus of 66 species, most of them monoecious but a few dioecious, e.g. *Cucumis hirsutus*, (Sebastian et al. 2010), breeders have selected gynoecious or 'all-female' cultivars of *C. sativus* because of their high fruit yield. Gynoecy, coupled with parthenocarpy, is valued for greenhouse production. Field production of gynoecious varieties requires a few androecious plants (with only male flowers) or monoecious plants with both types of flowers to ensure fertilization. Their sexual lability and economic importance have made cucurbits an important system for the developmental genetics of sex determination (The Genomics of Wood Formation in Angiosperm Trees). The ancient presence of unisexual flowers and dioecious populations in Cucurbitaceae may be the evolutionary 'reason' for the absence of genetic self-incompatibility in this family. All species that have been investigated in this regard are self-compatible. However, the sexual systems of most species are only inferred from a few herbarium specimens rather than field observations of wild populations.

Flower Morphology and Its Evolutionary Trends

Floral symmetry is mostly actinomorphic (radially symmetric), although zygomorphy (mirror symmetry) has evolved in a few species, for example, in *Gerrardanthus* (Fig. 2a), *Xerosicyos,* and a few species of *Momordica.* Male and

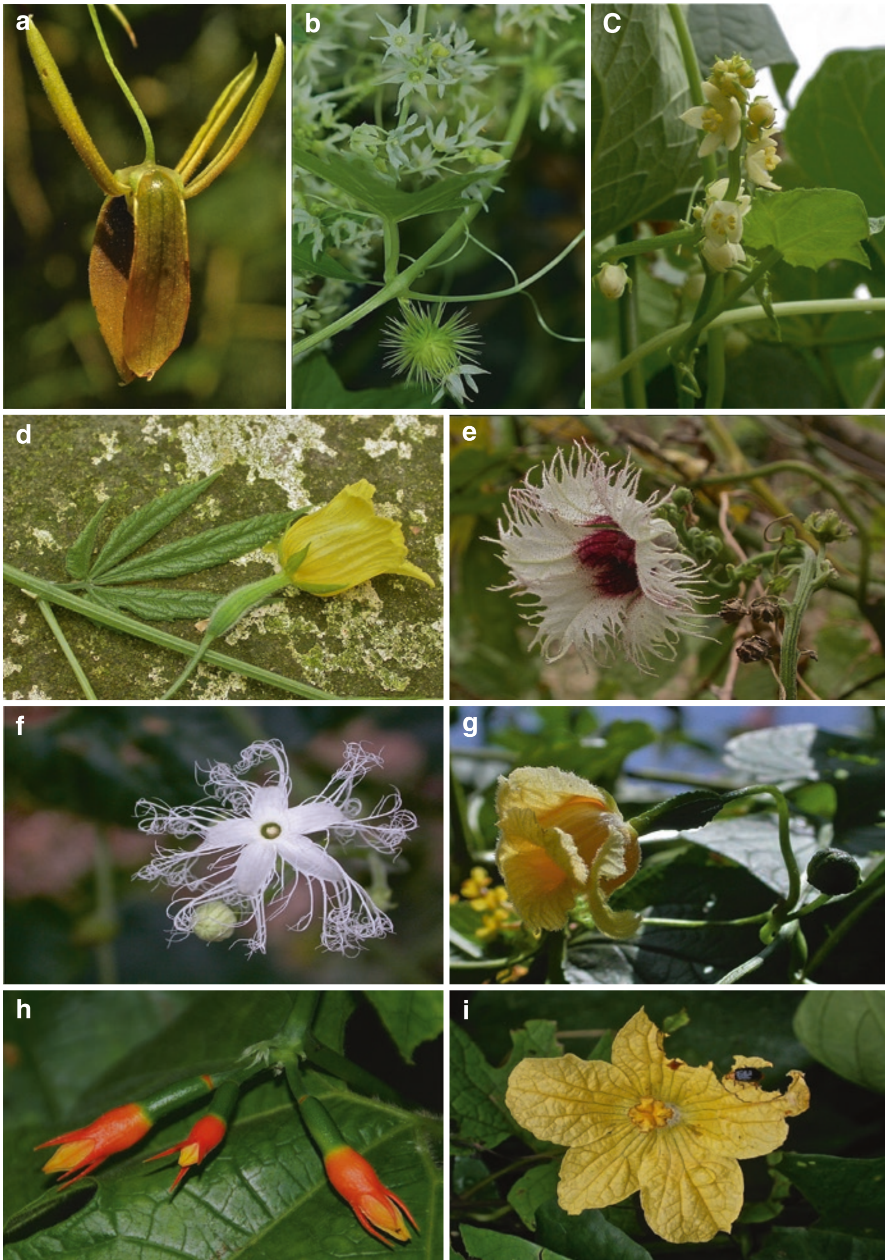

Fig. 2 Diversity of flower morphology in Cucurbitaceae: (**a**) *Gerrardanthus grandiflorus*, male flower (Tanzania); (**b**) *Echinocystis lobata*, male and female flowers (USA); (**c**) *Sicyos edulis* (syn. *Sechium edule*), male flowers (Brazil); (**d**) *Thladiantha hookeri*, female flower (China); (**e**) *Telfairia occidentalis*, male flower (Nigeria); (**f**) *Trichosanthes cucumerina*, male flower (China); (**g**) *Momordica leiocarpa*, female flower (Tanzania); (**h**) *Gurania makoyana*, female flower (Peru); (**i**) *Ruthalicia eglandulosa*, male flower (Sierra Leone); all photographs Hanno Schaefer

female perianths are usually similar, probably to make pollinators visit the often rewardless female flowers; occasionally, they differ in size and exceptionally also in shape. At about day six of floral development (at least in *Cucumis*), either the stamen primorida or the carpel primordia begin to expand rapidly, while the primorida of the other sex are arrested (Kater et al. 2001). In female flowers, the aborted stamens are visible as staminodes. The calyx and corolla are usually pentamerous, and the corolla consists of distinct or more or less fused petals that are highly variable in size and shape, but rather uniformly white, yellow, or orange, rarely red or pink (Fig. 2). Especially striking are the long-fringed petals of the nocturnally flowering species of *Trichosanthes* (Fig. 2f), *Linnaeosicyos*, *Hodgsonia*, and *Ampelosicyos*, which are pollinated by long-tongued hawkmoths (De Boer et al. 2012; Mitchell et al. 2015).

The hypanthium derives from the expansion of sepal and petal bases, and their receptacular insertion area (cf. Schaefer and Renner 2011a for references to morphological studies). In male flowers, stamens arise at the bottom of the hypanthium or are inserted at different levels on the hypanthium wall, with the stamen bases then contributing to the hypanthium. These “appendicular” hypanthia may take a wide range of shapes: from flat and patelliform (*Cyclanthera*) to long and tubular (*Ceratosanthes*). The hypanthium floor typically bears a nectary, which can be mesenchymal (most genera) or trichomatous (Sicyoeae). In female flowers, the hypanthium includes the ovary wall, and the gynoecia consist of 1–5 carpels, the 3-carpellate condition being the most common.

A taxonomically useful character is the number of stylodia: Gomphogyneae, Fevilleeae, and Zanonieae usually have three (sometimes two or five) distinct stylodia. The more derived clades (Fig. 1) have a single style with 2–3(−5) stigmas that can be enlarged to mimic an androecium, probably to attract pollen-seeking bees. Stigma shape is diverse and likewise taxonomically useful.

Ovules are anatropous and bitegmic, their number ranging between 1 to several hundreds (Matthews and Endress 2004). In the more derived clades, the ovules are embedded within individual chambers that at maturity can be liquid filled (e.g, *Cionosicyos macranthus*). Ovule orientation in the *Actinostemma* clade, Fevilleeae, Gomphogyneae, and Zanonieae (as well as in the derived clade Sicyoeae) is mostly pendent. Ovule orientation in the remaining clades is typically horizontal or entirely erect (*Cayaponia*). However, ovule orientation is not known for many groups.

A family-wide evolutionary trend is an increasing fusion of neighboring stamens, and an enlargement of the pollen-producing space through sigmoid coiling of the thecae. Five distinct, bithecal stamens probably is the ancestral state, and as far as is known, all cucurbit flowers initiate five distinct stamen primordia, even those with highly connate stamens (Matthews and Endress 2004). The condition survives in a few unrelated groups (*Anisosperma* and some *Telfairia*; see Fig. 1 for their placement). Five distinct monothecal stamens occur only rarely, while androecia with three stamens, four stamens (via the loss of one), or two stamens evolved repeatedly and are common. Another type of fusion involves the filaments, which may form a central column, a condition that has evolved repeatedly.

Fossils, Biogeography, and Divergence Times of Major Groups of Cucurbitaceae

The oldest fossils of Cucurbitaceae are seeds from the Uppermost Paleocene and Lower Eocene London Clay (65 Ma) that, based on their shape and testa morphology, represent Cucurbitaceae (Collinson et al. 1993). The earliest pollen of Cucurbitaceae is *Hexacolpites echinatus* from the Oligocene of Cameroon (Salard-Cheboldaeff 1978); these grains under the light microscope are hexacolpate or stephanocolpate, and resemble polycolpate pollen of New World Sicyoeae (Schaefer et al. 2008a). Seeds of various species of *Cucurbitospermum* have been described from the Early Miocene (17.8 Ma) sites of Rusinga Island in Lake Victoria, Kenya (Collinson et al. 2009). *Bryonia*-like seeds from fossil beds at Tambov, Western Siberia (Dorofeev 1963, 1988) date to the Lower Sarmat, 15–13 Ma ago. A relatively high geologic age for the clade comprising *Austrobryonia, Bryonia*, and *Ecballium* (Fig. 1) matches its geographically distant and biogeographically unusual range in Eurasia and Australia (Schafer et al. 2008b; Volz and Renner 2009).

Subfossil records of *Cucurbita pepo* have been dated to 8000–7000 B.P. at Guilá Naquitz, and to about 7000–6500 B.P. at Ocampo Cave, Tamaulipas (Smith 1997), those of *C. moschata* in the northern Peruvian Andes to up to 9200 B.P. (Dillehay et al. 2007; Paris, The Cucumber Genome). *Lagenaria siceraria* rind fragments from Mesoamerican archaeological deposits have been radiocarbon-dated to 10,000 B.P, indicating that the bottle gourd was present in the Americas as a domesticated plant by that time.

Cucurbitaceae apparently originated in Asia sometime in the Late Cretaceous (Schaefer et al. 2009). The five deepest evolutionary divergences in the family all date to the Late Cretaceous, 70–80 Ma. Two of these ancient clades [the Gomphogyneae (I) and *Actinostemma* (IV) clade; Fig. 1] are now almost restricted to Asia. A third, the Triceratieae (II), is mainly Neotropical, except for a small African genus, *Cyclantheropsis*. The ancestors of another early-diverging clade (Fig. 1), the Zanonieae (III), apparently reached the African continent early, and from there dispersed to Madagascar (the early Eocene *Xerosicyos* lineage). Later, in the Oligocene, at least two long-distance dispersal events brought two members of this clade, the *Siolmatra* lineage, to America, and the *Zanonia* lineage, back to tropical Asia. The younger tribes towards the top of the tree (Coniandreae, Benincaseae, Cucurbiteae) in Fig. 1 all have relatively large geographic ranges that they often extended by transoceanic dispersal (Schaefer et al. 2009). Striking examples of such transoceanic dispersal are found in the sponge gourd genus, *Luffa*, three of whose eight species occur in the New World, four in tropical and subtropical Asia, and one in northern Australia (Telford et al. 2011a, b; Filipowicz et al. 2014), and in *Sicyos*, which has 14 species, all descending from a single ancestor that arrived c. three million years ago, two species on Galapagos that arrived independently, and three species in Australia and New Zealand (Sebastian et al. 2012; Telford et al. 2012). Finally, the bottle gourds, *Lagenaria*, are of African origin but very likely arrived in Central America with sea currents and were domesticated there some 10.000 years ago (Clarke et al. 2006; Kistler et al. 2014).

The native European cucurbit flora belongs to a single clade, *Bryonia*, with 10 species, and its monotypic sister *Ecballium*, the squirting cucumber, which diverged from a sister group that reached Australia (*Austrobryonia*) c. 36 (50–24) million years ago (Schaefer et al. 2008b). The remaining cucurbit species occurring in Europe are the result of recent introductions (*Echinocystis lobata*, *Sicyos angulatus*, *Thladiantha dubia*), or casual escapes from cultivation (*Citrullus lanatus*, *Cucumis melo*, *C. sativus*, *Cucurbita pepo*).

African Cucurbitoideae, classified in some 25 genera, evolved from five successful dispersals from Asia to Africa, and two from America to Africa (in *Melothria* and *Cayaponia*; Schaefer and Renner 2010a; Duchen and Renner, 2010). The famous cucumber tree, *Dendrosicyos socotranus*, endemic on Socotra some 350 km from the Arabian peninsula, was thought to have diverged from its closest relative 34 (47–22) Ma, while the Socotra archipelago is only some 10 million years old (Schaefer et al. 2009) but more comprehensive sampling revealed previously overlooked close relatives in the genus *Kedrostis*, which led to a much reduced age estimate of *Dendrosicyos* of 14 (8–19) Ma in accordance with the age of Socotra (Schaefer et al. in prep.). Madagascar has 50 native species of Cucurbitaceae that are currently classified in 16 genera. Based on molecular sequence data, it appears that this diversity evolved from 13 ancestral lines that reached Madagascar from the African mainland (Schaefer et al. 2009).

South America has about 360 species of Cucurbitaceae that descend from just a few transoceanic dispersal events, mostly from Africa to South America. These events involved the ancestors of the Cucurbiteae, lineages of the Sicyoeae, part of the Coniandreae, and *Melothria*, *Lagenaria*, and *Luffa* (see under these genera). For *Melothria*, it appears that its ancestors came across the Pacific, since the sister group of *Melothria*, *Indomelothria*, is endemic in Southeast Asia. North American cucurbits descend from seven expansions of Central and South American lineages that occurred at widely different times (Schaefer et al. 2009).

The indigenous Australian Cucurbitaceae flora, finally, consists of 30 species in 12 genera of which two are endemic, *Nothoalsomitra*, a single liana species of Queensland's humid rainforests, and *Austrobryonia*, four species of trailers or creepers in the dry regions of (mostly) Central Australia (Schaefer et al. 2008b).

A 'time tree' or 'chronogram' for the Cucurbitaceae, which provides divergence times in million years, along with confidence intervals, is shown in Schaefer et al. (2009), and more detailed chronograms, all calculated using Bayesian molecular clock approaches, are available for the c. 66 species of *Cucumis* (Renner et al. 2007, Sebastian et al. 2010), all 47 species of *Momordica* (Schaefer and Renner 2010a, b), all 27 species of *Coccinia* (Holstein and Renner 2011), 52 of the 90–100 species of *Trichosanthes* (De Boer et al. 2012), all seven species of *Citrullus* (Chomicki and Renner 2015). Additional studies will be published in the coming months for the three species of *Dactyliandra* (Lindner et al. 2017), the six species of *Siraitia* (Schaefer, unpublished), and all species of *Kedrostis* and *Corallocarpus* (Schaefer, unpublished).

The Need for Fieldwork and Sequencing of Publicly Available Herbarium Vouchers

Even though the phylogeny and geographic history of the cucurbit family is now reasonably well understood (especially when compared to other tropical clades of similar species richness), every sequencing project so far has resulted in the discovery of overlooked species, including completely unexpected wild relatives of crop species such as cucumber, melon, and watermelon (Sebastian et al. 2010; Chomicki and Renner 2015). There is an urgent need to collect (and DNA-sequence) wild material, but also to sequence the type specimens (housed in herbaria) of the many overlooked existing species names. This would help breeders and genomics researchers to become aware of the correct names of the entities they are studying. Knowing the precise phylogenetic position of a taxon allows better choices for crossing experiments, facilitates genome annotation, and permits inferences about area of geographic origin or likely native climate niche. One example of this need is an entity often discussed as *Cucumis sativus* L. 'var. *xishuangbannanesis*' (e.g, most recently by Bo et al. 2015). This name has never been validly published, meaning it does not exist under the *Codes of Nomenclature*. To validate any scientific name requires a diagnosis, which need not comprise a lengthy text, but instead can consist of clearly stated and publicly verifiable DNA differences (characters) linked to one or more specimens deposited in a permanent and public collection (a herbarium, not a germplasm collection, which is not permanent) (Renner 2016). A similar case, now solved, was the watermelon, where the fully sequenced genome (Guo et al. 2013) was in fact not the species represented by the type specimen of *Citrullus lanatus* collected by one of Linnaeus's students (Chomicki and Renner 2015). The watermelon name problem can be rectified by selecting a new type for this name that agrees with our current usage as proposed to the International Nomenclature Commission (Renner et al. 2014). Lastly, and most importantly, more fieldwork, especially in the diversity centers of the family, the Himalayan foothills of India, the Australasian region, West Africa, and Madagascar, is needed to better understand the evolution of the family.

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Melon Genetic Resources: Phenotypic Diversity and Horticultural Taxonomy

Michel Pitrat

Abstract Melon (*Cucumis melo* L.) presents extensive diversity, particularly at the fruit level. The combination of independent phenotypic traits such as sex expression, fruit shape and size, skin colour, flesh colour, presence of gelatinous sheath around the seeds, and seed size can be used to define horticultural Groups and Subgroups. This chapter presents a brief description of 19 Groups of wild, feral and domesticated melons, and for some of them of Sub-groups. Two new Groups are proposed: “kachri” for accessions intermediate between wild and cultivated, and “indicus” for certain accessions cultivated mainly in central India. It is proposed to merge the “cantalupensis” and the “reticulatus” Groups, and to split the large “inodorus” Group into three Groups, namely “cassaba”, “ibericus” and a smaller “inodorus”. A key for the determination of the Groups is proposed.

Keywords *Cucumis melo* • Cultigroup • Fruit trait • Sex expression • Infraspecific classification • Taxonomy

Introduction

Although melon (*Cucumis melo* L.) has been cultivated for at least 4000 years, it is not known when and where it was first domesticated, and whether there were one or several independent domestication events, as there are very few archaeological remains. In the old texts, there is often confusion of non-sweet forms of melon with cucumber (*Cucumis sativus* L.), or between sweet forms of melon and watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai]. It has been proposed that melon was first cultivated for its seeds, like *Cucurbita* and *Citrullus*. Indeed, the seeds are a good source of proteins and lipids. The fruit flesh of wild melons is very thin (1 or 2 mm thick) and may be bitter due to the presence of cucurbitacins. Selection in different countries from the Mediterranean area to eastern Asia has resulted in a large phenotypic diversity. Classification of accessions can be made using different types of data: geographical origin, morphological and horticultural traits, and

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genetic data such as neutral molecular markers or sequences. The aims of this review are to make a synthesis of the phenotypic variation in melon and to summarize and illustrate the different groups at the infraspecific level.

Cucumis melo and Its Relationship with Other *Cucumis species*

Despite attempts to separate melons in the genus *Melo* (Ashurmetov 1995; Miller 1724; Pangalo 1950), botanists largely agree that melon belongs to the genus *Cucumis*. Recent publications have redefined this genus, including the former genera *Mukia*, *Dicaelospermum*, *Myrmecosicyos*, and *Oreosyce*. More than 50 species are now considered as belonging to this genus (Ghebretinsae et al. 2007; Renner et al. 2007). The basic chromosome number (when it is known) is usually x = 12 except for *C. sativus* (cucumber) with x = 7. The genus *Cucumis* includes two important crops, cucumber and melon; *Cucumis metuliferus* Meyer ex Naudin and *Cucumis anguria* L. are locally cultivated. Some wild species are more popular for their ornamental fruits.

Many species have been described which are now considered as synonyms of *C. melo* (Kirkbride 1993). For instance, among cultivated types, Linnaeus himself described two other species (*C. dudaim* and *C. flexuosus*); Hasselquist described *C. chate*; Roxburgh described *C. momordica, C. utilissimus* and other species. Wild melons are found from Africa to India to Japan and Australia. Among the wild types, *C. maderaspatanus* L., *C. pubescens* Willdenow, *C. trigonus* Roxburgh, and *C. microcarpus* (Alefeld) Pangalo are synonyms of *C. melo*.

Despite many attempts to introduce diversity from other species into melon for breeding purposes, no trait has been introduced in melon from other species by interspecific crossing or protoplast fusion. For instance, some accessions of *C. metuliferus* or *C. anguria* are resistant to root-knot nematodes, but up to now, no melon cultivar resistant to *Meloidogyne* has been released (Granberry and Norton 1980; Norton 1969). The usefulness for melon breeding of *Cucumis picrocarpus* F. Muell., which has been recently described as the most closely related species (Sebastian et al. 2010), has to be clarified, as its cross-compatibility with *C. melo* has not been documented. So, in this review, we will focus on genetic resources of *C. melo* and not of other *Cucumis sp*.

Traditionally, Africa was considered as the centre of origin of melon as most of the wild species (e.g., *C. pustulatus*, *C. carolinus*, *C. dipsaceus*, *C. humifructus*, *C. heptadacylus*) are found in Africa. But it has been suggested that the genus *Cucumis* including melon could be of Asian origin (Sebastian et al. 2010) (see Chapter "Evolution and phylogeny of the cucurbits" in this book).

What are Accessions and Groups?

Genetic resources are a collection of accessions, physically represented by seed lots. Genetic resources will be considered here not as a collection of genes, but as a collection of accessions or genotypes, i.e. of combinations of genes. It supposes that

accessions are quite homogeneous. Scientific material such as introgression lines, recombinant inbred lines, and monosomic or trisomic, or other synthetic research materials will not be considered in this paper.

Different types of accessions have been defined:

Cultivars According to the International Code of Nomenclature for Cultivated Plants (ICNCP), (Brickell et al. 2009) a cultivar is "an assemblage of plants that (a) has been selected for a particular character or combination of characters, (b) is distinct, uniform and stable in these characters, and (c) when propagated by appropriate means, retains those characters."

Landrace A landrace can be more heterogeneous than a cultivar, as *C. melo* is an outcrossing species.

Wild or Feral Genotypes The word "wild" will be used here in the sense of non-cultivated, having apparently evolved under natural selection without agricultural care provided by men. "Domesticated" will be used for melons which cannot grow and reproduce (or maintain themselves) in adequate soil and climatic conditions without appropriate horticultural practices. After domestication, diversification by human selection has developed the different landraces and cultivars. The limit between "domestication" and "diversification" or "selection" is not clear-cut and is better represented by a continuum. "Feral" will be used for melons which return to a wild status from domesticated forms.

Two taxonomic levels will be used above the accessions:

Sub-group or cultigroup is a taxonomic rank above the accession and represents a group of accessions with common characters: for instance "charentais", "ogon", "kirkagac" are the names of some sub-groups.

Group is above the sub-group: for instance the sub-groups "piel de sapo", "amarillo", "tendral", "rochet" belong to the same Group. Some Groups are quite homogeneous and the sub-group level is not necessary, while other Groups are more polymorphic and the sub-group level recognizes this diversity.

An individual accession belongs to only one sub-group, and each sub-group belongs to only one Group. These meanings do not correspond to the ICNCP definitions. Indeed, according to the ICNCP, a cultivar may belong to several Groups according to the definition of the Groups. For instance, one can define an "orange flesh" Group and a "green flesh" Group, but one can also define a "netted skin" Group and a "smooth skin" Group. And a cultivar can belong to the orange flesh Group as well as the smooth skin Group.

Phenotypic Diversity in Wild, Feral and Cultivated Melons

Cultivated melons differ from wild melons (Fig. 1) by some domestication traits such as larger fruits, non-bitter taste, thicker flesh, larger seeds and larger leaves. Other traits are diversification or selection traits, as they can be present or absent in cultivars and landraces. The taxonomy at the infraspecific level is based mainly on flower and fruit traits.

Flowers

Sex Expression Wild melons are monoecious and cultivated melons could be monoecious, andromonoecious (the majority of cultivars), or hermaphrodite (a few accessions from Eastern Asia).

Sepals Sepals are small in most types, but can be large in some Asian accessions.

Hypanthium and ovary hairs Wild and cultivated melons can have very short, sericeous appressed hairs, or long hairs.

Fruit Size and Shape

Wild melons are small (about 30–50 g) and oval or egg-shaped (Fig. 1a). Cultivated melons are larger (up to 35 kg), and vary considerably in shape from flat (length:diameter = 0.6) to very long (length:diameter > 10) (Fig. 16).

Exocarp or Epicarp Traits

Colour Wild melons at maturity are usually light-green with dark-green spots. Cultivated types have exocarps of different colours (e.g., white, brown, yellow, orange). They can be of uniform colour (Figs. 7, 8 and 22), or with secondary colours in speckles, dots, stripes (Figs. 4, 14 and 19).

Vein Tracts Wild melons have no vein tracts. Some cultivated melons present meridian lines (usually 10) from the peduncle to the flower scar (Figs. 12 and 20).

Ribs Wild melons have no ribs; when present in cultivated melons, they can be superficial or deep (Fig. 39).

Blossom Scar Blossom scar can be very small as in monoecious accessions, or more or less large (Figs. 31 and 39b).

Texture Wild melons have a smooth, thin exocarp, whereas cultivated melons can exhibit smooth and thin exocarp (Figs. 5 and 6), but also can be netted (Figs. 43 and 44), or wrinkled (Figs. 30 and 34), or with warts.

Hairs Young or ripe fruits can be almost glabrous with very short, appressed hairs, or velvety, or with long spreading hairs.

Aroma Usually, aroma is concentrated in the flesh, but in Group dudaim aroma is present only in the exocarp.

Mesocarp Traits

Colour Wild melons have a very thin, light-green flesh. Cultivated melons have many flesh colours, ranging from light-yellow or creamy to light-orange to salmon, to dark-orange and magenta (Figs. 24 and 40), or may be white, like milk (Figs. 5, 9 and 19). In other cases, flesh is classified as green even if sometimes it is light-green only under the exocarp and almost white in the middle and central parts of the mesocarp (Figs. 22 and 29).

Sugar Wild melons have very low sugar content. Cultivated types vary from very low (2–3° Brix) in the melons used as "vegetables" (Groups tibish, flexuosus, conomon) to high (>15°Brix) in sweet melons.

Texture At fruit maturity the flesh can be mealy, with no consistency (in Groups flexuosus or momordica). It can also be crispy, firm, juicy, dry, or fibrous.

Aroma Aroma can be intense or weak. Description of aroma is quite difficult.

Placentas and Seeds

Number of Placentas Usually, there are three placentas (including wild melons) (Figs. 22 and 23), but some groups have five placentas (Figs. 4, 11 and 19).

Colour The colour of the placentas can be white (Figs. 9 and 22), green (Fig. 11) or orange (Figs. 23 and 24), and can be different from the flesh colour.

Fusion of Placentas Placentas can be fused together as in wild melons (Figs. 3 and 5), or independent with more or less empty space between them (Figs. 25 and 40).

Sheath around the seeds A gelatinous sheath is present in wild melons and in some Groups (Fig. 1b).

Seed Size Wild melons have very small seeds (0.4–0.9 g for 100 seeds). Seeds in cultivated types range from small (0.6–2.3 g for 100 seeds) to large (more than 5 g for 100 seeds) (Fujishita 1983; Akashi et al. 2002).

Seed Colour Seed colour can be white, ivory, yellow or brown (in mutant with a red stem) (Fig. 13).

Seed Shape Seeds are usually flat, but can be more or less round like pine seeds (Fig. 13).

Fruit Development and Conservation

The time from pollination to maturity depends on the temperature, and can be short (<30 days) or much longer (>60 days). In some groups, fruit maturity can be easily recognized with a clear abscission of the fruit peduncle (Figs. 20, 22 and 43). In

other groups, there is no peduncle abscission (Figs. 29, 34 and 38). Fruits can be stored at room temperature for several weeks to several months (long shelf life) or not (short shelf life).

Horticultural Taxonomy

A natural phenotypic classification will be used in this paper based on a combination of the above-described characters, which can be under monogenetic or more complex (quantitative) genetic control. Most of them are independent and the number of combinations is almost infinite. Indeed, as we are at the infraspecific level, crosses between any accessions can lead to new types and it seems almost an impossible task to define taxonomic units. Nevertheless some more or less homogeneous groups and sub-groups can be defined using combinations of characters. Before any trait in the following descriptions, one should add "usually" or "generally" because there are often some exceptions, e.g., "honeydew" melons have usually a green flesh but some orange-fleshed honeydews have been bred; "Kirkagac" type is usually without vein tracts but a few accessions have vein tracts.

Groups and sub-groups as described hereafter follow earlier descriptions (Naudin 1859; Pangalo 1958; Grebenšcikov 1986; Pitrat et al. 2000) except for some of them where new groups or sub-groups are proposed.

- The large Group *inodorus* was first described by Jacquin (1832) as "toutes les variétés qui le composent sont absolument dépourvues d'arome.[1]" Later, Naudin (1859) wrote "Faute de pouvoir faire mieux, je suis forcé d'accepter cette classe, très vaste et très arbitraire, telle qu'elle a été proposée par Jacquin. On y trouvera réunies des variétés fort différentes d'aspect et de qualité, mais qui, du moins, auront le caractère commun d'être peu odorantes ou même tout à fait dépourvues d'arome.[2]" This Group is highly polymorphic as mentioned by Naudin himself, and it is proposed here to split it into three Groups, namely "cassaba", "ibericus" and "inodorus", which includes the remaining types: "honeydew" and "Earl's".
- Following the suggestion of Munger and Robinson (1991), the Groups "reticulatus" and "cantalupensis" could be merged as there is a continuum for the netting which is the main difference between these two groups.
- Is it proposed to add two new Groups: "kachri" and "indicus."

The two sub-species defined by the pubescence of the ovary (short and appressed hairs versus long spreading hairs; Kirkbride 1993) are not always relevant, as both types are encountered in several Groups such as "agrestis", "kachri" and "flexuosus".

[1] English translation as "All the varieties of this group are absolutely without aroma."

[2] English translation as "For want of something better, I am forced to maintain this class, very broad and arbitrary, as it was proposed by Jacquin. One will find together varieties very different in appearance and quality, but at least which have the common trait to be of low aroma or even entirely devoid of aroma."

Probably it is not necessary to maintain the sub-species rank in the infraspecific classification of melon; one need only to use Group level and in some cases the sub-group level.

Group Agrestis (Fig. 1)

Group agrestis melons are not cultivated, and can be found in Africa, Asia (from Turkey to Japan) and Australia. They may be found for sale in market places, or sliced open and dried in farm yards for later consumption.

Characteristics Monoecious, with short appressed or long spreading hairs on the ovary, immature fruits usually light-green, very small fruits (20–50 g), round, oval or elliptic fruit shape, no ribs, no vein tracts, smooth fruit surface (neither wrinkled nor netted), light-green uniform colour or with dark-greens spots or stripes, dehiscent or non-dehiscent peduncle, thin exocarp, very thin, light-green flesh, three fused white placentas, no cavity, no sugar, no aroma, presence of a gelatinous sheath around the very small seeds (0.4–0.9 g 100 seed weight), long shelf life, small leaves, small stem diameter, numerous branches.

Group Kachri (Fig. 2)

This group is intermediate between wild melons (Group agrestis), and locally cultivated types belonging to different groups. It is quite heterogeneous and could be best characterized as “large agrestis.” Accessions of this group could be harvested or are cultivated, for instance in the semi-desert area of Rajasthan (India) under the name Kachri; they can be drought resistant. Ex: AHK 119, AHK 200.

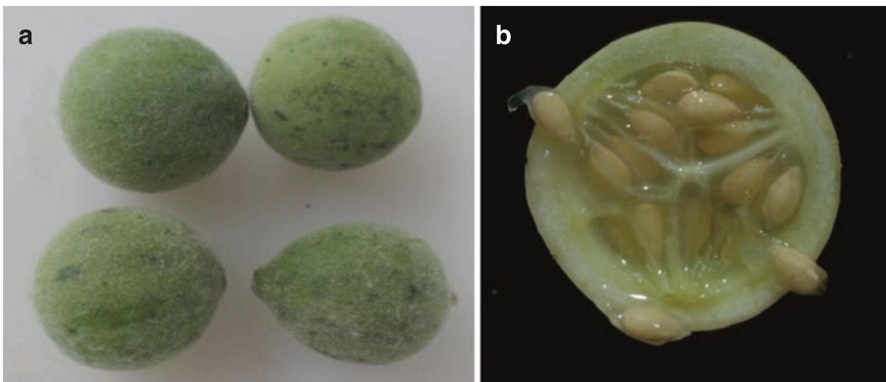

Fig. 1 Group agrestis. Typical very small fruits of a wild melon (**a**) and cross-section (**b**) showing the three fused placentas and the gelatinous sheath around the seeds

Fig. 2 Group kachri. Two accessions of this heterogeneous group intermediate between wild (Group agrestis) and cultivated types: flesh and placentas can be white (**a**) or coloured (**b**)

Characteristics Monoecious, short appressed or long spreading hairs on the ovary, immature fruits usually light-green, small fruits (100–200 g), round, oval or elliptic fruit shape, no ribs, with or without vein tracts, not wrinkled, sometimes slightly netted, light-green, yellow or cream uniform colour or with dark-greens or orange spots or stripes, dehiscent or non-dehiscent peduncle, thin exocarp, thin light-green (sometimes slightly orange) flesh, three fused white placentas, no cavity, no sugar, no aroma, presence of a gelatinous sheath around the small seeds (0.5–2.0 g for 100 seeds), long shelf life, small leaves, small stem diameter, numerous branches.

Group Chito (Fig. 3)

This feral type is present in Central America and the Caribbean Islands.

Characteristics Monoecious, short hairs on the ovary, light-green immature fruit, very small round to ovoid fruits (50–100 g), smooth surface, no ribs, no vein tracts, no netting, not wrinkled, yellow colour of the epicarp, dehiscent peduncle, small blossom scar, thin exocarp, light-green thin flesh, three fused light-green placentas, no sugar, no aroma, gelatinous sheath around the very small seeds (0.4–0.7 g for 100 seeds), long shelf life.

Group Tibish (Fig. 4)

Group tibish fruits are harvested before maturity and eaten raw in salad, like cucumber. It is cultivated and endemic in Sudan. Some accessions are also cultivated for eating the seeds under the name "seinat."

Characteristics Andromonoecious, short hairs on the ovary, small fruit weight, elliptical or pyriform fruit shape, no vein tracts, no ribs, smooth fruit exocarp (not netted, not wrinkled), light-green exocarp colour with dark-green stripes or spots (or the opposite: dark-green main colour with light-green spots or stripes),

Fig. 3 Group chito. Very small round yellow fruits, smooth, without ribs or vein tracts

Fig. 4 Group tibish. (**a**) Very thin flesh and five fused placentas, (**b**) smooth ovoid fruits

Fig. 5 Group acidulus. Bright colour of the fruit epidermis (**a**), very firm white flesh and three fused white placentas (**b**)

non-dehiscent peduncle, thin exocarp, thin light-green flesh, five fused light-green placentas, no sugar, no aroma, small seeds (1.7–3.0 g for 100 seeds) with a gelatinous sheath, long shelf life.

Group Acidulus (Fig. 5)

This group is mainly cultivated in India and Sri Lanka. Several disease resistances (viruses, powdery and downy mildew, *Aphis gossypii*) have been found in some accessions belonging to this group. Ex: PI 313970 (90625), PI 164323, PI 164723, Kekiri.

Characteristics Monoecious, short hairs on the ovary, immature fruits usually light-green, small fruits, oval or elliptic fruit shape, no ribs, no vein tracts, not netted, not wrinkled, smooth fruit surface, yellow or orange or ochre exocarp colour with stripes, non-dehiscent peduncle, thin exocarp, white very firm flesh, three fused white placentas, no cavity, no sugar, no aroma, small seed size (0.9–2.3 g for 100 seeds), presence of a gelatinous sheath around the seeds, long shelf life.

Group Momordica (Fig. 6)

A review of this Group has been recently published by Dhillon et al. (2015) . It is mainly cultivated in India and south-east Asia. Many disease resistances (viruses, powdery and downy mildew, *A. gossypii*) have been described in the *momordica* Group. Ex: MR-1 (PI 124111), PI 414723. This Group is quite heterogeneous and maybe some sub-groups could be defined.

Characteristics Monoecious, short appressed hairs on the ovary, small to medium fruit weight, flat to oval to elongated fruit shape, uniform colour or with speckles, spots or stripes, smooth surface, no vein tracts, not wrinkled, no netting, dehiscent

Fig. 6 Group momordica. Fruits splitting at maturity with white (**a**) or light-orange (**b**) mealy flesh

Fig. 7 Group conomon. Elongated fruit with white or light-green non-sweet flesh

peduncle, very thin exocarp which can sometimes be easily peeled off, splitting at maturity, light-green (sometimes white or slightly orange) flesh, three independent orange or white placentas, low sugar content, mealy flesh, no aroma, small to medium seeds with a gelatinous sheath, early maturing, very short shelf life.

Group Conomon (Fig. 7)

The conomon group is cultivated in the Far-East (China, Japan), but is of decreasing importance. The fruits are harvested before maturity and eaten raw or as pickles, like cucumbers and gherkins. Ex: Tokyo Wase Shiro Uri, Ko Shiro Uri, PI 266935 (Hyogo Shiro Uri).

Characteristics Andromonoecious, short hairs on the ovary, often leaf-like sepals, light-green immature fruit, oval or elongated fruits, light-green or white (sometimes dark-green) exocarp colour, smooth surface, no ribs, with or without vein tracts, no netting, not wrinkled, thin exocarp, light-green or white flesh, no sugar, no aroma, three white or orange placentas, small seeds (1.2–2.2 g for 100 seeds), long shelf life, dark-green small leaves, stiff hairs on the stems and petioles.

Group Makuwa *(Figs. 8, 9, 10, 11, 12, and 13)*

Group makuwa is cultivated in the Far-East, but is declining. Many disease resistances have been identified in this group (viruses, Fusarium wilt race 1.2, *A. gossypii*). A few accessions in different sub-groups have brown seeds (Fig. 13, bottom line, first from left) and red stem.

Characteristics Andromonoecious (few accessions are hermaphrodite), short appressed hairs on the ovary, often leaf-like sepals, medium fruit weight, round to oval fruit shape, not wrinkled, no netting, dehiscent peduncle, usually green (sometimes white or orange) flesh, sometimes five placentas, medium sugar content, small seeds (0.5–2.3 g for 100 seeds), early maturing, short shelf life, dark-green small leaves, stiff hairs on the stems and petioles.

According to the exocarp colour, the presence/absence of vein tracts and the seed shape, the Group makuwa can be divided in six sub-groups (Katsumata and Yasui 1964).

Sub-group Ogon (Fig. 8)

This sub-group is characterized by uniform yellow exocarp colour, no vein tracts, and no ribs. Ex: Ogon 9, Showa Kogane Nahi Makuwa, Mi Tang Thing.

Sub-group Nashi-uri (Fig. 9)

The fruits are oval or round or pear-shaped. They have uniform white-cream exocarp colour, no vein tracts, and no ribs. Ex: Shirokawa Nashi Makuwa 2, Nyumelon, PI 420150.

Sub-group Yuki (Fig. 10)

The fruits are slightly ribbed with a yellow colour of the exocarp and white-cream vein tracts. Ex: Ginsen Makuwa, LJ 90436, SVI 0186.

Fig. 8 Group makuwa sub-group ogon. Uniform yellow skin colour

Fig. 9 Group makuwa sub-group nashi-uri. Uniform white skin colour

Sub-group Kanro (Fig. 11)

The fruits have a uniform colour or with speckles, grey-green vein tracts lighter than the skin, and are slightly ribbed. Ex: Nagata Kim Makuwa, Wasada Uri, Yantai.

Sub-group Ginmakuwa (Fig. 12)

The fruits have a grey/green skin, with green vein tracts darker than the skin, and are slightly ribbed. Ex: Chenggam, Chang bougi.

Fig. 10 Group makuwa sub-group yuki. Yellow skin colour with cream vein tracts

Fig. 11 Group makuwa sub-group kanro. Green skin colour with grey-green vein tracts lighter than the skin

Fig. 12 Group makuwa sub-group ginmakuwa. Grey-green skin colour with green vein tracts darker than the skin

Fig. 13 Group makuwa sub-group seikan. The sub-group seikan is characterized by small yellow round seeds (*bottom line*, second from *left*) compared with seeds of the other sub-groups (*top line*, second and third from *left*)

Sub-group Seikan (Fig. 13)

This sub-group is characterized by small yellow seeds with a more or less round section (like pine seeds). Ex: Lanzhou.

Fig. 14 Group chinensis. Fruits are light-green with dark-green spots and prominent vein tracts (**a**). Flesh is light-green with orange placentas (**b**)

Group Chinensis (Fig. 14)

Group chinensis is cultivated in the Far-East. Several disease resistances have been described in this group (viruses, *A. gossypii*). Ex: PI 161375, PI 255479, PI 266934.

Characteristics Andromonoecious, short hairs on the ovary, often large sepals (leaf-like), medium fruit weight, pear shaped, prominent light-green vein tracts (facets), no netting, not wrinkled, uneven fruit surface, light-green exocarp colour with dark-green spots, non-dehiscent peduncle, large blossom scar, thin exocarp, orange-green flesh, low sugar, low aroma, five fused orange placentas, small (0.8–1.7 g for 100 seeds) yellow seeds with a round section, late maturing, long shelf life, dark-green small leaves, stiff hairs on the stems and petioles.

Group Flexuosus (Figs. 15, 16, and 17)

Group flexuosus fruits are harvested before maturity (about 8–10 days after flowering) and eaten raw in salad or pickled. It is cultivated in a large area from Morocco to India and also sometimes on the northern Mediterranean shore (Spain, Italy, Greece) under the names Adjour, Adzhur, Acur, Faggous, Fakouss, Fqus, Armenian cucumber, Snakemelon, Snake cucumber, Kakri, Cohombro, Tortarello.

Characteristics Monoecious, long (usually) or short (sometimes) hairs on the ovary, very long ovary and fruit (up to 2 m), light-green or dark-green immature fruit colour, at maturity cream (sometimes orange) exocarp usually uniform colour (sometimes with spots or stripes), light-green or slightly orange mealy flesh, no sugar, no aroma, three orange or light-green independent placentas, long ivory seeds with a gelatinous sheath, very short shelf life.

Three main types can be distinguished within the Group flexuosus according to the fruit surface, which can be smooth, wrinkled or with ribs.

Sub-group Adjour (Fig. 15)

Fruits have ribs, usually without vein tracts. This sub-group is the most common and cultivated on a large area from Morocco to India. Fruits can have a light green exocarp (Ex: Silka, Fqus, Meerul), a dark-green exocarp with light-green vein tracts (Ex: Snakemelon), or a dark-green exocarp (Ex: Fakouss CM4, Fegouss 1, Cucumerino siciliano).

Sub-group Tara (Fig. 16)

The fruit exocarp is wrinkled. Fruits are usually longer and thinner than the adjour sub-group. It is mostly cultivated in Afghanistan, Pakistan and India. Ex: PI 222187 (light-green).

Sub-group Arya (Fig. 17)

The ovary has short appressed hairs. The fruit surface is smooth, neither ribbed nor wrinkled. This type is grown in India. Ex: AMES 20662.

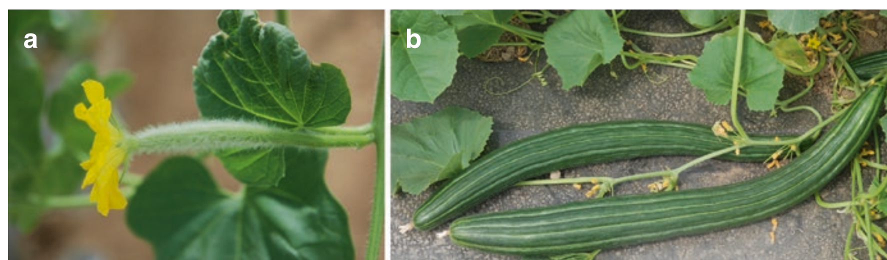

Fig. 15 Group flexuosus sub-group adjour. Very long ovary with long spreading hairs (**a**). Very long fruits with ribs (**b**)

Fig. 16 Group flexuosus sub-group tara. Very long wrinkled fruits

Fig. 17 Group flexuosus sub-group arya. Very long ovary with short appressed hairs (**a**). Very long smooth fruits neither ribbed nor wrinkled (**b**)

Group Chate (Fig. 18)

Like for the Group flexuosus, fruits are harvested about 10 days after flowering and eaten raw as a cucumber. Some carosello accessions produce very early female flowers and present very short first internodes resulting in the "bird-nest" phenotype. Ex: Carosello, Dolmalik.

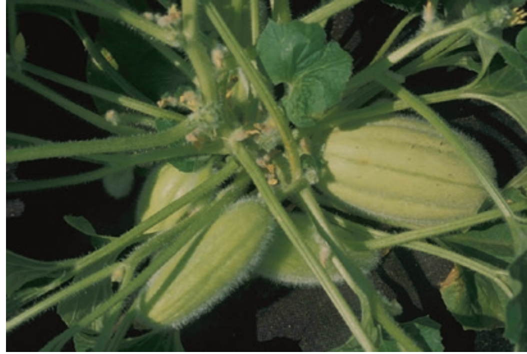

Fig. 18 Group chate. Short first internodes and early female flowers produce the "bird-nest" phenotype with grouped young fruits

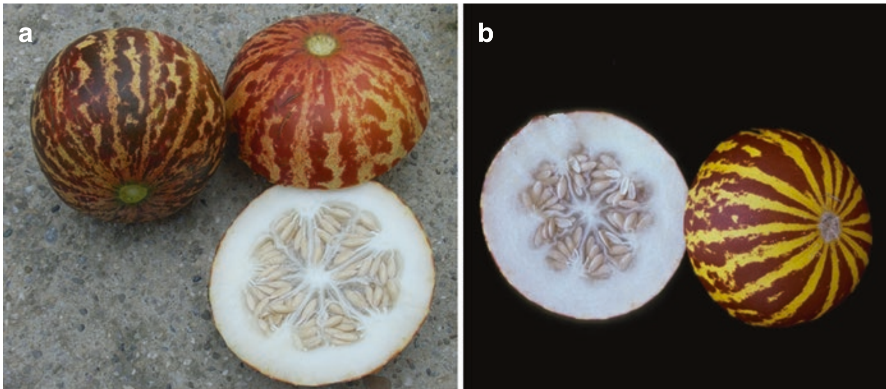

Fig. 19 Group dudaim. Two accessions (**a** and **b**) differing by the exocarp colour, both with five fused placentas and white flesh

Characteristics Monoecious, long hairs on the ovary, hairy fruit surface, medium fruit weight, round to oval fruit shape, usually with vein tracts and ribs, not netted, not wrinkled, no warts, cream exocarp colour, dehiscent peduncle, thin exocarp, light-orange (sometimes white or light-green) flesh colour, three independent orange (sometimes white) placentas, mealy soft flesh at maturity, very low sugar content, no aroma, medium size yellow seeds without a gelatinous sheath, early maturing and short shelf life.

Group Dudaim *(Fig. 19)*

Dudaim melons are cultivated from Turkey to Afghanistan, and north to Turkmenistan. They are not eaten but used as a fragrance in a room. Ex: PI 177362, Queen Anne's Pocket Melon.

Characteristics Andromonoecious, long hairs on the ovary, light-green with dark-green spots immature fruit, round to ovoid fruits the size of an orange, smooth surface, no ribs, no vein tracts, no netting, not wrinkled, yellow colour of the epicarp

with orange/ochre spots or stripes, dehiscent peduncle, thin exocarp, white thin flesh, five fused white placentas, no sugar, very strong aroma in the skin (exocarp) but not in the flesh, medium size ivory seeds embedded in a gelatinous sheath, short shelf life.

Group Chandalak (Figs. 20, 21, 22, and 23)

This Group is mainly cultivated from Central Asia to India.

Characteristics Andromonoecious, long hairs on the ovary, round or flat fruit shape, medium fruit weight, no ribs or slightly ribbed, vein tracts usually present but sometimes absent, netting present or absent, not wrinkled, no warts, uniform cream to orange/brown colour or with speckles, green vein tracts (when present), dehiscent peduncle, often large blossom scar, thin exocarp, usually green (but sometimes orange or white) flesh colour, thin mesocarp, fibrous flesh texture, three independent orange or white placentas, medium sugar content, low aroma, large seeds in a gelatinous sheath, early maturing, short shelf life.

According to the presence/absence of netting and of the vein tracts, four sub-groups can be defined.

Sub-group Zami (Fig. 20)

The fruits have a smooth (not netted) exocarp and are slightly ribbed, with green vein tracts. The exocarp colour can be uniform cream, or with orange/brown speckles or spots. Ex: Zaami 610, Mag Assil, Sorokodnevka, Varanasi local.

Sub-group Tachmi (Fig. 21)

The fruit exocarp is strongly netted, slightly ribbed, with light-green or cream vein tracts. Ex: Persia 202, Samsouri green, Semosouri Varamin, Shahd Shiraz.

In some accessions (e.g., Persia 202), the first internodes of the main stem are short leading to the “bird nest” phenotype.

Sub-group Garma (Fig. 22)

The fruits are smooth or slightly netted without vein tracts. The exocarp colour can be of uniform colour or with spots. Ex: PI 124552.

Sub-group Bucharici (Fig. 23)

The fruits are larger than the other sub-groups, netted, uniform cream with light-green vein tracts, slightly ribbed. The flesh is orange and of better quality (texture and sugar content) than the other sub-groups.

Fig. 20 Group chandalak sub-group zami. Smooth exocarp, green vein tracts, no ribs, thin orange flesh

Fig. 21 Group chandalak sub-group tachmi. Netted exocarp and cream-green vein tracts

Fig. 22 Group chandalak sub-group garma. Smooth exocarp, no vein tracts, no ribs (**a**), thin green flesh (**b**)

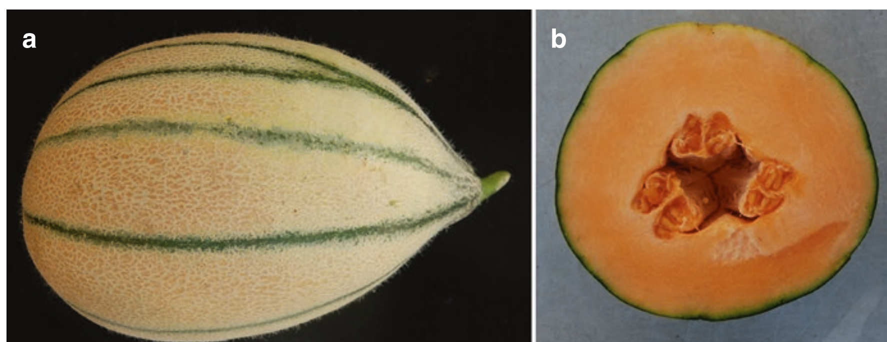

Fig. 23 Group chandalak sub-group bucharici. Ovoid to pyriform fruits, larger than the other chandalak sub-groups (**a**), thick orange flesh (**b**)

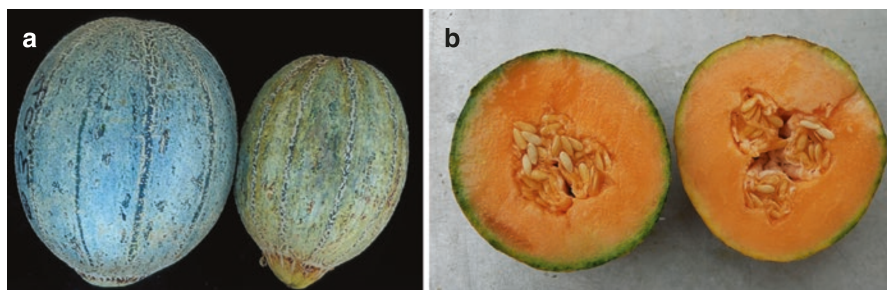

Fig. 24 Group indicus. Elliptical smooth fruits, with vein tracts and protruding blossom scar (**a**), thick orange flesh (**b**)

Group Indicus *(Fig. 24)*

This good quality melon is cultivated in central India (Maharashtra, Telangana, Andhra Pradesh). Ex: landraces from Nagpur, Aurangabad, Hyderabad, Cuddapah.

Characteristics Andromonoecious, light-green immature fruit, medium fruit weight, elliptical fruit shape, dark-green vein tracts, no ribs, sparse long hairs, smooth to slightly netted, not wrinkled, grey/orange/brown/cream exocarp colour, dehiscent peduncle, large protruding blossom scar, orange (rarely green) flesh colour, three independent orange (rarely green) placentas, sweet (high °Brix) thick flesh, juicy firm flesh, large seeds with a gelatinous sheath, medium shelf life.

Group Ameri *(Figs. 25, 26, 27, and 28)*

Group *ameri* melons are mainly cultivated in Asia from Turkey to western China. Ameri melons can be of excellent quality (very sweet, crisp flesh texture, medium to long shelf-life).

Characteristics Andromonoecious, long hairs on the ovary, medium to high fruit weight, oval or cylindrical fruit shape, presence or absence of netting, not wrinkled, no warts, no ribs, dehiscent peduncle, white, light-green or light-orange flesh colour, three independent white or orange placentas, smooth, juicy and sometimes crispy (like watermelon) flesh texture, high sugar content, large yellow seeds without a gelatinous sheath, medium shelf life, large leaves, thick stems.

According to the colour of the exocarp and the presence/absence of vein tracts, four sub-groups can be defined.

Sub-group Ananas (Fig. 25)

The fruit exocarp is of light-orange uniform colour or with cream speckles, regularly netted, without vein tracts. Ex: Ananas Yokneam, Ananas Dokki, PI 534538, Gorgab 1.

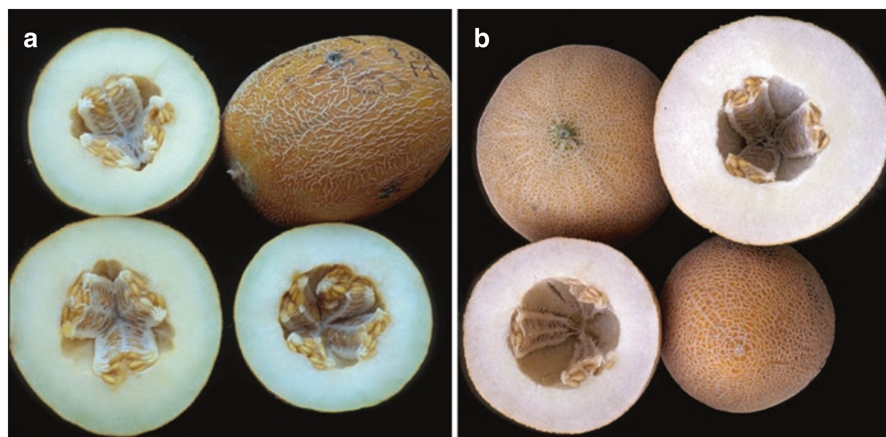

Fig. 25 Group ameri sub-group ananas. Large fruits of orange-brown uniform colour, netted without vein tracts or ribs, light-green (**a**) or white (**b**) flesh

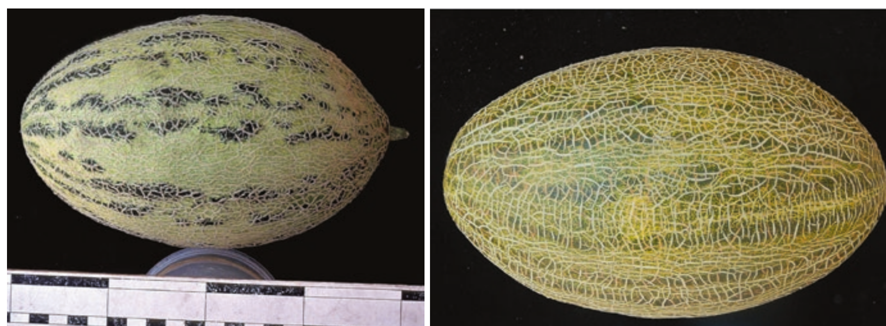

Fig. 26 Group ameri sub-group maculati. Large fruits with dark-green spots, netted exocarp

Sub-group Maculati (Fig. 26)

The fruits have a light-green/yellow exocarp colour with dark-green spots. They are slightly to strongly netted without vein tracts. Ex: Shakar Palaki, Ak Uruk, Red Cotton Rose Melon, Baba Kharman, Chanjo.

Sub-group Bargi (Fig. 27)

The fruit exocarp is uniform cream/yellow or with light-green speckles, slightly to strongly netted without vein tracts. Ex: Evankey 2, Koktcha 588, Twaghermez Isfahan.

Sub-group Mashhadi (Fig. 28)

This sub-group is characterized by the presence of green or cream vein tracts. The exocarp is netted. Ex: Ghasri, Jharbezeh Mashadi, Khagkhani.

Fig. 27 Group ameri sub-group bargi. Uniform yellow cream exocarp, slightly netted

Fig. 28 Group ameri sub-group mashhadi. Large cylindrical fruits slightly netted with light-green vein tracts

Group Cassaba (Figs. 29, 30, and 31)

This Group is mainly cultivated in western and central Asia. According to the fruit skin colour, three types can be defined. Sub-groups within cassaba offer parallel variations for the skin colour with the ibericus Group, i.e., kirkagac and piel de sapo*;* hassanbey and tendral, kuscular and amarillo.

Characteristics Andromonoecious, long hairs on the ovary, medium to high fruit weight, pear-shaped fruit shape (flat on the blossom end and pointed on the peduncle end), more or less wrinkled, no ribs, no vein tracts, no netting, non-dehiscent peduncle, thick skin, thick light-green flesh, very often five white placentas, high sugar content, late maturing, long shelf life, large leaves, thick stems.

Sub-group Kirkagac (Fig. 29)

The exocarp colour is yellow with dark-green spots and warts. Ex: Kirkagac 1, Mustapha Kemal Pacha 2, Hidir.

Sub-group Hassanbey (Fig. 30)

The fruits have a uniform dark-green exocarp colour. Ex: Hasan Bey, Yuva.

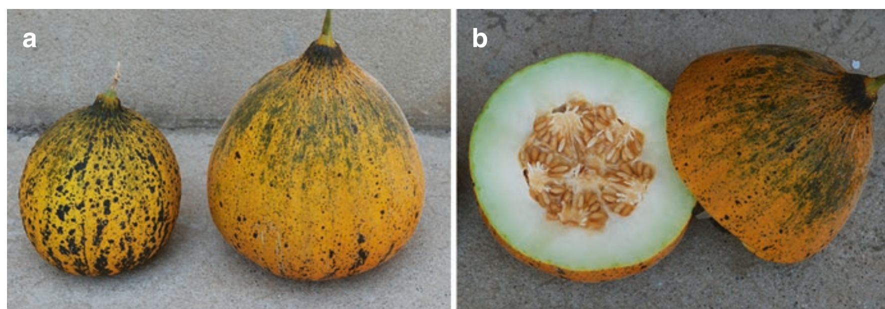

Fig. 29 Group cassaba sub-group kirkagac. Pyriform fruit shape, wrinkled exocarp, yellow colour with dark-green spots (**a**), five placentas, light-green flesh (**b**)

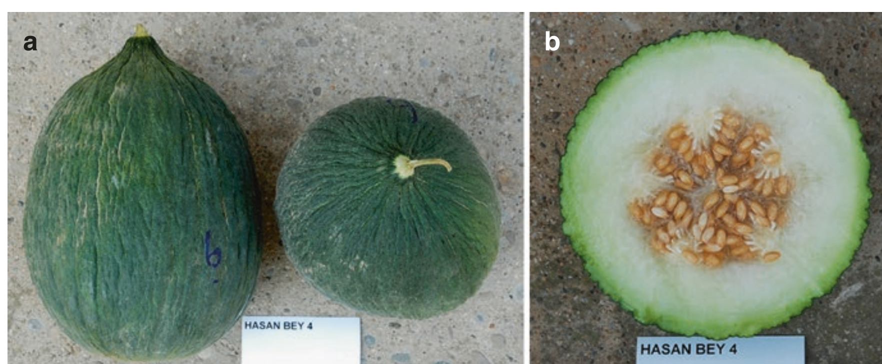

Fig. 30 Group cassaba sub-group hassanbey. Pyriform fruit shape, wrinkled exocarp, dark-green uniform colour (**a**), five placentas, light-green flesh (**b**)

Sub-group Kuscular (Fig. 31)

The fruits have a uniform yellow exocarp colour, sometimes with green speckles at the peduncle end. This sub-group is less wrinkled than the two other sub-groups. Ex: Çumra, Golden Beauty Casaba, Gare ghez.

Group Ibericus (Figs 32, 33, 34, 35, and 36)

This group of melon is mainly cultivated in Spain, but is also popular in all the Mediterranean area, and in North and South America. According to the exocarp colour, five sub-groups can be defined.

Characteristics Andromonoecious, long hairs on the ovary, medium to high fruit weight, elliptical or acorn fruit shape (sometimes round), more or less wrinkled, no

Fig. 31 Group cassaba sub-group kuscular. Wrinkled exocarp, yellow colour, large blossom scar, five placentas

ribs, no vein tracts, no netting or slightly netted, non-dehiscent peduncle, thick skin, thick light-green (sometimes light-orange) juicy flesh, three white or orange placentas, often triangular cavity, high sugar content, low aroma, large yellow seeds without a gelatinous sheath, late maturing, long shelf life, large leaves, thick stems.

Sub-group Piel de Sapo (Fig. 32)

The fruit skin is green with yellow speckles and dark-green spots (warts). Fruits are finely wrinkled, sometimes slightly netted. Some accessions have seeds with a round section named Piñonet. Ex: Piel de Sapo T111.

Sub-group Amarillo (Fig. 33)

The fruits have a uniform yellow exocarp and are more or less wrinkled. Some accessions have round fruits, for instance 'Bola de Oro'. Ex: Amarillo Oro.

Sub-group Tendral (Fig. 34)

The exocarp is uniform dark-green and deeply wrinkled. The sub-group is characterized by a very long shelf life. Ex: Negro, Tendral Verde.

Sub-group Rochet (Fig. 35)

The exocarp is green with yellow speckles, slightly wrinkled, and often with some netting. Ex: Sigura, Cavaillon Espagnol, Escrito.

Fig. 32 Group ibericus sub-group piel de sapo. Oval fruit shape, slightly wrinkled, light-green with yellow speckles and dark-green spots

Fig. 33 Group ibericus sub-group amarillo. Oval to round fruit shape, uniform yellow colour

Fig. 34 Group ibericus sub-group tendral. Oval fruit shape, wrinkled, dark-green uniform colour

Sub-group Branco (Fig. 36)

The fruit skin is uniform white-cream, wrinkled. The flesh colour is white, light-green or light-orange. Ex: Branco de Ribateja.

Fig. 35 Group ibericus sub-group rochet. Oval fruit shape, light-green with yellow speckles

Fig. 36 Group ibericus sub-group branco. Oval fruit shape, wrinkled, white uniform colour

Group Inodorus *(Figs. 37 and 38)*

This Group included accessions with low aroma and long shelf life that do not belong to the Groups *cassaba* or *ibericus* as described above.

Characteristics Andromonoecious, long hairs on the ovary, medium fruit weight, round fruit shape, not wrinkled, no ribs, no vein tracts, with or without netting, non-dehiscent peduncle, thick skin, thick light-green flesh, three white placentas, high sugar content, medium yellow seeds without a gelatinous sheath, late maturing, long shelf life.

Sub-group Honeydew (Fig. 37)

The fruit surface is smooth without netting (sometimes slightly netted) and of a white uniform colour. There is often a large cavity. The flesh is usually green but some orange-flesh accessions are available. This old Mediterranean type is now mainly cultivated in North and South America. Ex: Antibes Blanc, Angel Dew, TAMDew.

Sub-group Earl's (Fig. 38)

The immature fruit is light-green. At maturity, the exocarp is heavily and regularly netted. The yellow-green flesh is very sweet. This old English type (Earl's Favourite) is now a Japanese speciality, which can be very expensive. Ex: Chung Hsing 1, Earl's.

Fig. 37 Group inodorus sub-group honeydew. Round fruit shape, smooth exocarp, white uniform colour

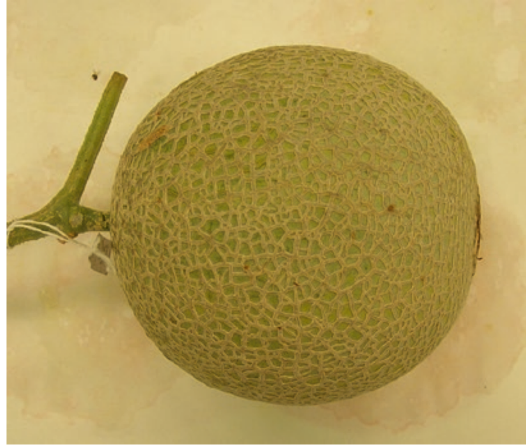

Fig. 38 Group inodorus sub-group earl's. Round fruit shape, no ribs, no vein tracts, uniformly netted

Group Cantalupensis (Figs. 39, 40, 41, 42, 43, and 44)

This Group was first developed in Europe, probably from accessions from eastern present-day Turkey. Later, diversification and selection in the USA led to the American cantaloupes.

Characteristics Usually andromonoecious, long hairs on the ovary, medium fruit weight, flat to round to oval fruit shape, with vein tracts, more or less netted, not wrinkled, warts may be present, white to light-green to dark-green exocarp colour, dehiscent peduncle, orange (sometimes green) flesh colour, three independent orange (sometimes white) placentas, smooth and juicy flesh texture, high sugar content, strong aroma, medium size yellow seeds without a gelatinous sheath, short to medium shelf life.

Sub-group Prescott (Fig. 39)

This sub-group is mainly characterized by very deep ribs. Some accessions are monoecious. The fruits are flat to round, with a cream or dark-green exocarp colour, sometimes netted, with or without warts. The vein tracts are more or less contrasted. The flesh is orange.

Ex: Prescott à Chassis, Cantaloup de Bellegarde, Iopor Menyhert, Noir des Carmes.

Sub-group Saccharinus (Fig. 40)

The fruits are round to oval with a green or yellow/cream exocarp with speckles. They are slightly ribbed, with or without vein tracts, with or without netting. The flesh is usually orange or sometimes green. Ex: Gris de Rennes, Ananas d'Amérique, Leknicki Cukrovi, Madelenen, Aarhus Torg.

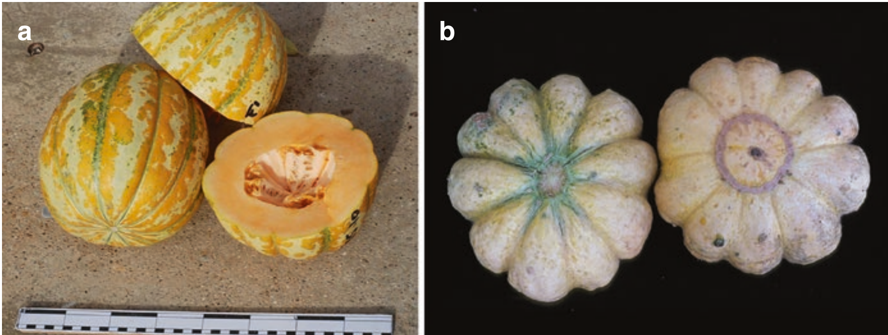

Fig. 39 Group cantalupensis sub-group prescott. Round to flat fruit shape, deeply ribbed with orange warts (**a**) or with large blossom scar (**b**)

Fig. 40 Group cantalupensis sub-group saccharinus. Round fruit shape, slightly ribbed, with vein tracts and speckles, thick orange flesh

Fig. 41 Group cantalupensis sub-group charentais. Round fruit shape, slightly ribbed, grey-light-green uniform colour with green vein tracts, thick orange flesh

Sub-group Charentais (Fig. 41)

The fruits are round, smooth or slightly netted, without warts, slightly ribbed, with green vein tracts. The exocarp colour is uniform light-green to yellow cream. Traditionally this type has a short shelf-life. Modern F_1 hybrids are usually

monoecious, more netted, with longer shelf life and some of them have a deep orange flesh colour. *Charentais* is mainly cultivated in France. Ex: Védrantais, Nantais Oblong.

Sub-group Ogen (Fig. 42)

The fruits are round, slightly netted, with a yellow exocarp and green speckles, with grey/green vein tracts, and are slightly ribbed. The flesh is light-green. This sub-group has a short shelf life. It was first developed in central Europe and later in Israel. Accessions belonging to the *ogen* sub-group are one parent of the Galia type. Ex: Muskotaly, Hemed, Noy Yizre'el.

Sub-group American Western (Fig. 43)

The most typical trait is the very strong netting covering the vein tracts. Fruits are round, slightly ribbed, with an orange flesh colour. This type was developed in the twentieth century in the western part of the USA. Ex: Hale's Best, PMR 45, Jacumba, Top Mark, Perlita, Cinco.

Sub-group American Eastern (Fig. 44)

The fruits have a round to oval shape, are more or less ribbed, have a very strong netting not covering the vein tracts. Flesh is orange. This sub-group was developed in the eastern part of the USA from Italian cantaloupes. Ex: Delicious 51, Harvest Queen, Iroquois, New Yorker.

Conclusions

The 19 Groups briefly described above (section "Horticultural Taxonomy") are defined by a combination of characters. A possible key for the determination of the Groups is suggested:

Fig. 42 Group cantalupensis sub-group ogen. Yellow colour with green speckles, slightly ribbed, green vein tracts

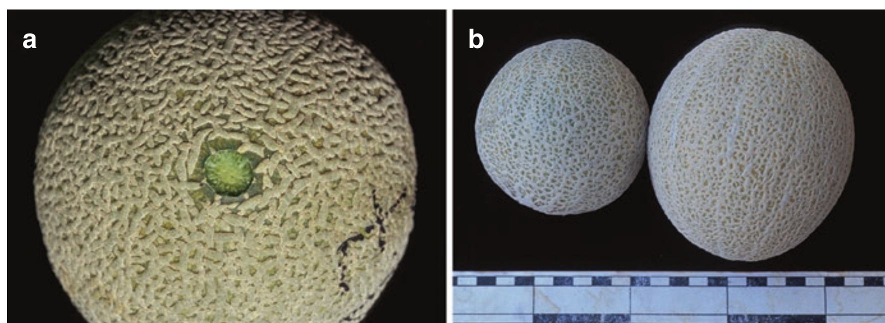

Fig. 43 Group cantalupensis sub-group american western. Round fruit shape, no ribs (**a**) or slightly ribbed (**b**), presence of vein tracts but fully covered by the heavy netting

Fig. 44 Group cantalupensis sub-group american eastern. Round to oval fruit shape, slightly ribbed, light-green to cream vein tracts not covered by the net

1.	Very small round or elliptical fruits (50 g), uniform light-green exocarp or with dark-green spots, wild in the Old World	agrestis
	Other	(2)
2.	Very small round fruits (50–100 g), yellow exocarp, feral in the New World	chito
	Other	(3)
3.	Small elliptical fruits (50–200 g), monoecious, three placentas	kachri
	Other	(4)
4.	Very long fruits (ratio length:diameter > 6), non-sweet mealy flesh at maturity	flexuosus
	Other	(5)
5.	Small seeds, short appressed hairs on the ovary	(6)
	Large seeds, long spreading hairs on the ovary	(11)
6.	Small elliptical or ovate fruits (200 g), andromonoecious, 5 placentas, endemic in Sudan	tibish
	Other	(7)
7.	Monoecious, flat to elongate fruit shape, very thin exocarp, fruit bursting at maturity, mealy flesh	momordica
	Other	(8)
8.	Monoecious, elliptical, ovate or elongate fruit shape, brightly coloured (yellow, orange, brown) exocarp, very firm white acidic flesh	acidulus
	Other	(9)
9.	Pyriform fruit shape, light-green exocarp with dark-green spots, bumps, non-dehiscent peduncle, small round (section) seeds	chinensis
	Other	(10)
10.	Non-sweet flesh at maturity	conomon
	Sweet flesh at maturity	makuwa
11.	Gelatinous sheath around the seeds	(12)
	No gelatinous sheath around the seeds	(14)
12.	Monoecious, round or elongate fruit shape, non-sweet mealy flesh at maturity	chate
	Other	(13)
13.	Small fruit (size of an orange), round fruit shape, colored (yellow, orange, brown) exocarp, highly fragrant exocarp, white flesh, 5 placentas	dudaim
	Other	chandalak
14.	Elliptical fruit shape, dehiscent peduncle, orange/brown exocarp	indicus
	Other	(15)

15.	Large ovate to elongate fruits, dehiscent peduncle, large leaves and stems	ameri
	Other	(16)
16.	Dehiscent fruit peduncle	cantalupensis
	Non-dehiscent fruit peduncle	(17)
17.	Large pyriform fruits pointed at the peduncle, no ribs, no vein tracts, wrinkled exocarp, often large blossom scar, often 5 placentas	cassaba
	Other	(18)
18.	Medium to large elliptical (sometimes round) fruits, more or less wrinkled exocarp	ibericus
	Other	inodorus

This classification into 19 Groups (some of which are divided into sub-groups), based on phenotypic characters of the fruits, does not allow inclusion of all the melon accessions as there are many intermediate types. By adding some genotypic data (molecular markers, sequencing some fractions of the genome, chloroplastic DNA) it may be possible to further clarify relationships among the different Groups.

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Genetic Resources of Cucumber

Rachel P. Naegele and Todd C. Wehner

Abstract The Cucurbitaceae is a monophyletic family without any close relatives. It includes important vegetables such as cucumber, melon, watermelon, squash, pumpkin, and gourd. Within Cucurbitaceae, the genus *Cucumis* includes cultivated species *C. sativus* (cucumber) and *C. melo* (melon), as well as wild species including *C. hystrix*, *C. callosus*, and *C. sativus* L. var. hardwickii. More than 50 species have been identified in *Cucumis* with high levels of phenotypic and genetic diversity found in centers of diversity in Africa, Asia, and India. Primary and secondary centers of diversity can serve as useful sources of variation, and have been widely used to incorporate traits such as disease resistance into cultivated materials. During domestication, cucumber and melon underwent severe bottlenecks, which resulted in low genetic variation despite high phenotypic diversity. Since its domestication, approximately 3000 years ago, cucumber has undergone significant morphological changes from its small-fruited, black spined, seedy progenitor. More than 150 single gene traits have been described in *C. sativus*, including powdery mildew and virus resistance, sex expression, leaf morphology, and parthenocarpy, and molecular markers continue to be rapidly developed.

Keywords Cucumber • *Cucumis sativus* • Gene • Germplasm resources • Plant breeding

Introduction

The Cucurbitaceae or vine crop family is a distinct family without any close relatives (Sikdar et al. 2010). It includes important vegetables such as cucumber, melon, watermelon, squash and pumpkin. Cucumber (*Cucumis sativus* var. *sativus*), grown for fresh and processing markets, is one of the most important cultivated cucurbits with a global production of 70 million tonnes in 2013 (FAOSTAT). Approximately

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Table 1 Top eight producers of cucumbers (fresh and processed) in the world

Production of cucumbers (fresh-market and processing)		
Rank	Country	Production (tons)
1	China	54,315,900
2	Turkey	1,754,613
3	Iran (Islamic Republic of)	1,570,078
4	Russian Federation	1,068,000
5	Ukraine	1,044,300
6	Spain	754,400
7	United States of America	747,610
8	Mexico	637,395
9	Egypt	631,129
10	Uzbekistan	607,397

WorldAtlas.com 2016
http://www.worldatlas.com/articles/the-world-leaders-in-cucumber-production.html

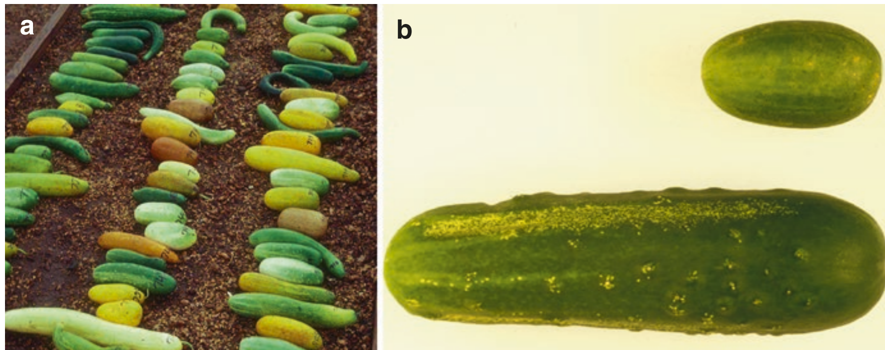

Fig. 1 (**a**) Cultivated cucumber morphological diversity and (**b**) *C.s. var. hardwickii* (*top*) and *C. sativus* (*bottom*)

70 % of the world's production of cucumber is in Asia, with China being the leading producer followed by Turkey, Iran, and Russia (Table 1).

Cucumber probably originated in India, where highly diverse wild as well as cultivated forms are found (Sebastian et al. 2010). Cultivated cucumber and its wild relatives, including *Cucumis sativus* var. *hardwickii*, exhibit large variation in traits such as fruit skin (ridges, colors, speckling), spines (size, density and color), growth habit (vine length and branching), fruit size, sex expression, and flesh bitterness (Fig. 1a). India is the center of diversity for cultivated cucumber. Secondary centers of diversity for cucumber exist in China and the Near East (Meglic et al. 1996; Staub et al. 1999). Accessions of *C. s. var. hardwickii,* which may be more closely related to the original ancestors of cucumber, are found in one of these secondary centers of diversity in the foothills of the Yunnan Province of Southern China (Fig. 1b) (Staub et al. 1999). Other close relatives of cucumber are *Cucumis hystrix* from China and

the African *Cucumis* species, such as melon (*Cucumis melo*) and West Indian gherkin (*Cucumis anguria*) (Chen and Kirkbride 2000; Kerje and Grum 2000; Meeuse 1958). Cucumber was domesticated in Asia, and introduced into Europe where the first cultivars were selected in the 1700s (Staub et al. 2008). These early cucumber cultivars were brought to the Americas by Christopher Columbus, and grown by Native Americans from Florida to Canada by the early sixteenth century. Since this time, cucumber has spread across the globe becoming a major vegetable crop.

Cucumber Production

Cucumber is typically eaten fresh, or as a processed product (processing or pickling types) (Staub and Bacher 1997; Staub et al. 2008). The major cucumber market types are the American processing and fresh market types, the Dutch gherkin and greenhouse types, the German Schalgurken type, the Middle Eastern Beit Alpha type, and the Oriental trellis (burpless) type (Shetty and Wehner 2001). Fresh market cucumbers are field or greenhouse grown, and are usually between 15 (U.S. and Mediterranean) to 40 (European) cm in length. In addition to major market types, regionally preferred types also exist, but are less common. These include fresh market like the Persian cucumber (short fruit types grown in high tunnels or greenhouses) mainly marketed in the Middle East, and hermaphroditic ‘Lemon’ cucumber (shaped similar to a lemon with pale, greenish-yellow skin) (Robinson 2010).

Production practices vary widely according to market type (processing vs. fresh), profit margin, geographic region, and cultivar. Pickling cucumber (*C. s.* var. *sativus*) is an immature cucumber used for processing (brining or pasteurizing). Unless mechanized, harvest is labor intensive due to small fruit size, which is generally <15 cm long. India has low labor costs, and has become a major producer of the small size (<8 cm long) for export to Europe, the U.S., and Russia (Ranjan et al. 2008). Nearly 60 processing companies in the states of Karnataka, Tamil Nadu and Maharashtra grow pickling cucumbers on 12,000 ha. Ajax and Sparta (Nunhems) are some of the dominant cultivars, accounting for 2.5 billion seeds in India (P. Arul Murugan, IAP Farm Services Pvt. Ltd., Tamil Nadu, India, personal communication, 2012).

Pickling or processing cucumbers in the U.S. usually grown flat on bare ground, often with overhead or furrow irrigation, with machine harvest to reduce labor and other input costs (Ando and Grumet 2006; Schultheis 2000). This system, while requiring fewer expenses often results in greater disease incidence and more defects (shape/size/color) than slicing cucumbers (Fig. 2). A significant portion of the pickling cucumbers in the southern U.S. is hand-harvested, with 2 or 3 harvests per week for several weeks per season. Harvest begins in early spring in the southernmost states, and then returns in late fall for a second crop.

In the U.S., fresh market cucumbers are grown on raised beds, often with drip irrigation and plastic mulch, to improve fruit quality and reduce disease incidence (Schultheis 2000). Slicing cucumbers are hand-harvested and stored in forced-air cooling until distribution.

Fig. 2 American pickling cucumber graded from right to left based on a diameter scale of 1 (0–25 mm) to 3 (39–51 mm)

Centers of Diversity for Cucumber

The center of origin for cucumber has been a subject of debate for decades. The center of origin and diversity for wild *Cucumis* is likely Africa (Staub et al. 1992). However, the initial domestication of melon and cucumber occurred in the Middle East and Southern Asia, respectively (Dane et al. 1980). Cucumber was previously thought to have originated in Africa (Tapley et al. 1937), China, India, or in the Near East (Vavilov 1926, 1951; Harlan 1975; De Candolle as cited by Hedrick 1919), with domestication occurring later throughout Europe. Recent molecular assessments of *Cucumis* species have suggested that melon and cucumber are, however, of Asian origin and have numerous species-level relatives in Australia and around the Indian Ocean (Renner et al. 2007; Sebastian et al. 2010). Regardless of its origin, cucumber was domesticated about 3000 years ago, and is indigenous to India, which is a primary center of diversity, if not origin (Jeffrey 1980).

Cucumber Taxonomy

Cucumber belongs to the genus *Cucumis* in the subfamily Cucurbitoideae. *Cucumis* includes the cultivated species *C. sativus* (cucumber) and *C. melo* (melon) as well as many wild species, including *C. hystrix*, *C. callosus*, and *C. sativus var. hardwickii*. More than 50 species have been identified within *Cucumis* and there is a large phenotypic and genetic diversity in the populations collected in Africa, Asia, and India (Lv et al. 2012; Kacar et al. 2012; Weng 2010; Zhang et al. 2012a; Qi et al. 2013).

Crosses have been attempted between cultivated cucumber (*C. sativus*) and its relatives (*Cucumis* spp.) but have rarely been successful. The wild *C. hystrix* from

Yunnan Province of Southern China has been crossed with cucumber and progeny with limited fertility were generated (Chen et al. 1995; 1997). From those progeny, fertile amphidiploids were produced to create the synthetic species *C. hytivus* (Chen et al. 1997; Chen and Kirkbride 2000; Sebastian et al. 2010). Furthermore, the development of the fully fertile *C. hytivus*–derived fertile diploids ($2n=2x=14$) from *C. hytivus* and *C. s.* var. *sativus* cross resulted in potentially useful germplasm for plant improvement (Staub and Delannay 2011).

The 1320 *C. s.* var. *sativus* and var. *hardwickii* accessions currently resident in the U.S. National Plant Germplasm System represent the primary cucumber gene pool. These accessions include elite cultivars, breeding lines, heirlooms, collections from the centers of diversity, and exchange accessions from other collections. Within this collection inbreds, hybrids, monoecious, gynoecious, hermaphroditic, parthenocarpic, male sterile, disease resistant, tall, dwarf, determinate, seed dormant, and photoperiodic flowering types are represented.

The secondary gene pool of *C. sativus* includes cross incompatible (e.g. wild African) or sparingly cross compatible (e.g., *C. hystrix*) species (Chen et al. 1997, Chung et al. 2006). The tertiary gene pool of cucumber consists of distantly related species from other genera or sub-genera (e.g., *Cucumis melo* L. and *Cucurbita* L.), which do not hybridize with cucumber (Chung et al. 2006, Staub et al. 1997b, c). Attempts to exploit resources beyond the secondary cucumber gene pool, e.g., *Cucumis metuliferus*, *C. melo,* have been unsuccessful.

In cultivated cucumber and its closely related *C. sativus* var. *hardwickii*, researchers in India, China, Turkey, and the U.S. have shown that genetic diversity is relatively low despite the apparent diversity in morphology (Aydemir 2009; Horejsi and Staub 1999; Innark et al. 2013; Munshi et al. 2007; Lv et al. 2012; Pandey et al. 2013; Staub et al. 1997a, 1999; Zhang et al. 2012a). In local evaluations, high morphological variation was evident in fruit shape, size, color, sex expression, vine growth habit, and seed traits. Lv et al. (2012) evaluated over 3000 accessions representing cultivated, wild and landrace individuals from Asia, Europe and the U.S. using simple sequence repeat (SSR) markers. They reported little genetic diversity among cultivars collected from Europe, West/Central Asia and the U.S. Most of the genetic differentiation was between geographic or market classes. Accessions from China, East Asia, India and the Xishuangbanna province of China had the highest levels of diversity, and were genetically distinct from accessions from the U.S., Europe and West/Central China. These differentiated groups have potential for bringing in novel alleles and haplotypes to broaden the existing genetic pool for specific cucumber market classes. From this study, a core collection representing approximately 80 % of the genetic diversity was developed. This core collection was later re-sequenced using next generation sequencing technologies (Qi et al. 2013).

Singe nucleotide polymorphism (SNP)-based markers confirmed a low genetic diversity within *C. sativus* and homogenous populations across Eurasian, East Asian, and Xishuangbanna regions (Qi et al. 2013). Between the Eurasian and East Asian populations, non-synonymous SNPs in genes associated with resistance to fungi were highly differentiated, particularly in the Eurasian populations. Highest

genetic diversity and admixed population structure was found in Indian populations for cucumber, consistent with India serving as a center of diversity for cucumber. In cultivated cucumber, this reduction in genetic diversity is likely due to extreme selection pressure during domestication and small initial population sizes (Qi et al. 2013). As a vining crop, one cucumber plant can cover a large surface area (if not trained on a trellis) and produce many fruit over weeks of harvest. Thus, fewer plants are required to produce enough yield for a community or family compared to grain crops (rice, corn, wheat), or root crops (carrot or beet), which have a lower yield per plant. This comparative increase in yield per plant would allow for smaller populations to be maintained resulting in bottlenecks that may have limited the genetic pool of this self-compatible crop. A smaller genetic pool has made finding agronomically useful traits such as disease and stress resistance in cultivated germplasm more difficult.

Because of low genetic diversity, related species with limited crossability, have been evaluated as sources for new traits of interest. SSR markers developed in *C. sativus* have been transferred, with limited success, and used to characterize genetic diversity in *C. melo, C. hystrix*, *C. s. var. hardwickii*, *C. metuliferus,* and *Lagenaria siceraria* (Bhawna et al. 2015; Weng et al. 2010; Kacar et al. 2012). Genetic diversity varied among these species, but was reported to be low (2–5 alleles per locus). These and other closely related species may serve as additional sources for traits such as disease resistance.

Cucumber Cultivar Improvement

A wide array of breeding and genetic resources for cucumber exist in the cucumber germplasm repositories maintained by the U.S. National Plant Germplasm System, Institute of Vegetables and Flowers at the Chinese Academy of Agricultural Sciences, and the Centre for Genetic Resources in Netherlands. Cucumber was grown in the early 1300s in England, using the earliest greenhouses and was known as “cowcumbers” (Boswell 1949). These early cultivars were planted in Haiti in 1494, and brought to the U.S. soon afterward (Sturtevant 1887). Perhaps the first important American-bred cucumber cultivar of the nineteenth century was ‘Tailby’s Hybrid’, developed by Joseph Tailby of Massachusetts, which was derived from a cross between American and English cultivars and introduced in 1872 (Tapley et al. 1937). The success of ‘Tailby’s Hybrid’ encouraged plant breeders to develop new, early generation cultivars such as ‘Arlington White Spine’, ‘Boston Pickling’, ‘Chicago Pickling’, and ‘Snow’s Pickling’ (Fig. 3).

Some cultivars still available today were introduced to the U.S. more than a century ago. ‘Early Russian’, for example, was described by Naudin in France in 1859 (Naudin 1859) while ‘Early Cluster’ was introduced prior to 1800. Boswell in 1949 concluded that all of the distinct types of cucumber in use at that time were known at least 400 years before (Boswell 1949). Among the market types (American processing and fresh market, Dutch gherkin and greenhouse, German Schalgurken,

Fig. 3 'Snow's Pickling' an early American cultivar (ca. 1905) with poor shape, black spines and light skin color

middle eastern Beit Alpha, and Oriental trellis), there is variation in fruit morphology, growth habit, and disease resistance. Unlike some of their more colorful relatives, cucumbers have few vitamins and minerals in their fruit (Table 2), with the exception of lutein, a carotenoid (Perry et al. 2009; Granado et al. 2003). To date, no studies have examined the variation for lutein content among the cucumber market types. However, work has been done on the inheritance of beta-carotene in cucumber and germplasm released (Cuevas et al. 2010; Staub et al. 2011). In general, cultivated cucumber fruit have few spines, a large mesocarp, bitterfree fruit, and few or no seeds. This is in contrast to its wild relative, *C. s. var. hardwickii* that has small and bitter fruits, a large seed cell, and many seeds citation (Walters et al. 1996). More than 150 single-gene traits have been described in cucumber, and molecular markers are being developed for use in selection (Table 3). For a more comprehensive list of cucumber genes and sources, see the Cucurbit Genetics Cooperative list (http://ars.usda.gov/sotheast-area/charleston-sc/vegetable-research/docs/cgc).

Attempts to incorporate useful genes from secondary cucurbit gene pools (*C. metuliferus* and *C. hystrix*) into cucumber have had limited success (Staub et al. 2008). However, Chen et al. (1995, 1997) successfully made an interspecific cross between cucumber (*C. sativus* var. *sativus* primary gene pool) and *C. hystrix* (H; $2n=2x=24$; secondary gene pool). The F_1 progeny ($2n=2x=19$) derived from this mating were both male and female sterile; chromosome doubling was, therefore,

Table 2 Nutritional composition of cucurbits (amounts per 100 g fresh product)

Cucurbit	Water (%)	Protein (g)	Fat (g)	Carbohydrate (g)	Fiber (g)	P (mg)	Na (mg)	K (mg)	Vitamin A (IU)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B_6 (mg)
Cucumber	96	0.5	0.1	2.9	0.6	17	2	149	45	0.03	0.02	0.30	0.05
Melon, casaba	92	0.9	0.1	6.2	0.5	7	12	210	30	0.06	0.02	0.40	–
Melon, honeydew	90	0.5	0.1	9.2	0.6	10	10	271	40	0.08	0.02	0.60	0.06
Pumpkin	92	1.0	0.1	6.5	1.1	44	1	340	1600	0.05	0.11	0.60	–
Squash, acorn	88	0.8	0.1	10.4	1.5	36	3	347	340	0.14	0.01	0.70	0.15
Squash, summer	94	1.2	0.2	4.4	0.6	35	2	195	196	0.06	0.04	0.55	0.11
Squash, winter	89	1.5	0.2	8.8	1.4	32	4	350	4060	0.10	0.03	0.80	0.08
Squash, zucchini	96	1.2	0.1	2.9	0.5	32	3	248	340	0.07	0.03	0.40	0.09
Watermelon	93	05	0.2	6.4	–	10	1	100	590	0.03	0.03	0.20	–

http://www.nal.usda.gov/

Table 3 A selection of cucumber markers associated with phenotypic traits

Tissue	Trait	Gene	Chromosome	Source	Reference
Fruit	Tuberculate	*Tu*	5	S52, S94, S110	Zhang et al. (2010)
	Fruit shape				
	Uniform immature fruit color	*u*	5	9930	Maio et al. (2011), Yang et al. (2014)
	Glossy fruit skin	*d*	5	9110Gt, S06, S23, S76	Maio et al. (2011), Yang et al. (2014)
	Netted fruit	*H*	5	9110Gt	Maio et al. (2011)
	Fruit ribbing	*Fr*	5	9930	Maio et al. (2011)
	Yellow flesh color	*yf*	7	PI 120815	Lu et al. (2015)
	Orange flesh color	*R*	4		Bo et al. (2012)
	Orange skin color	*B*	4	WI7200	Li et al. (2010)
	Black spines	*B*	4	WI7200	Li et al. (2010), Cavagnaro et al. (2010), Yang et al. (2012, 2013)
	Bitterness	*bi-1*	5	9930	Zhang et al. (2012a, b)
	White skin	*w*	3		Dong et al. (2012)
	Weight	*fw*	1,2,3,4,6		Cavagnaro et al. (2010), Yang et al. (2012, 2013)
	Flesh thickness	*fft*	2	D8	Xu et al. (2015, 2014)
	Neck length	*fnl*	3,4,5,6		Xu et al. (2014)
	Spine density	*Fsc*	4,5,6		Cavagnaro et al. (2010), Yang et al. (2012, 2013)
	Diameter	*fd*	1,3,4,5,6		Cavagnaro et al. (2010), Yang et al. (2012, 2013)
Leaf					
	Little leaf	*ll*		H-19	Cavagnaro et al. (2010), Yang et al. (2012, 2013)
	Virescent	*v-1, vl*	6	9930	Maio et al. (2011), Yang et al. (2012, 2013), Cavagnaro et al. (2010)
	Bitterness	*BI, Bt-1*	6	9930	Maio et al. (2011), Zhang et al. (2012a, b)
	Glabrous –2, Glabrous –3	*gl-2, gl-3*	2		Cavagnaro et al. (2010), Yang et al. (2011, 2013), Pan et al. 2015)

(continued)

Table 3 (continued)

Tissue	Trait	Gene	Chromosome	Source	Reference
Growth habit					
	Determinate	*de*	6	H-19	Weng (2014)
	No lateral branch	*nlb*	1		Jiang et al. (2008)
	Compact	*cp*	4	PI 308915	Li et al. (2011)
	Cotyledon length/width	*cl/cw*	1,3		Miao et al. (2012)
Flower	Gynoecious	*F*	6	9110Gt	Maio et al. (2011), Win et al. (2015)
	Time to flower	*FT*	1	Muromskij	Lu et al. (2014)
	Sex ratio	*sex*	5,6	H-19	Fazio et al. (2003)
Disease	Angular leaf spot	*psl*		H 603	Olczak-Woltman et al. (2009)
	Powdery mildew	*pm*	1,3,5	CS-PMR1, Santou	Fukino et al. (2013), He et al. (2013)
	Zucchini yellow mosaic virus	*Zym*	6	A192-18	Amano et al. (2013)
	Downy mildew	*dm*	1,3,4,5,6,7	*C. hystrix*, K8	Bai et al. (2008); Ding et al. (2007); Zhang et al. (2013)
	Fusarium wilt	*Foc2.1*	2	*9110Gt*	Zhang et al. (2014)

performed to produce a fertile amphidiploid (HHCC, $2n=4x=38$) using embryo culture (Chen et al. 1998). This amphidiploid was subsequently self-pollinated for several generations resulting in fertile germplasm that was designated a new species, *C. hytivus* (Chen and Kirkbride 2000). The incorporation of genes from the secondary gene pool of cucumber such as *C. hystrix* is potentially useful to cucumber breeding, especially given that *C. hystrix* has novel genes for disease resistance, such as gummy stem blight caused by *Didymella bryoniae*, that are not present in cultivated cucumber (Chen et al. 2003).

Plant Architecture

Manipulation of plant architecture, stem length, and sex expression, with adjustments in plant population density have resulted in higher yield (Lower and Edwards 1986; Staub et al. 2008). For example, cultivars used in once-over mechanical harvest perform better if they have concentrated fruit set. Predominantly gynoecious and completely gynoecious types are preferred over monoecious for that reason. Determinate plant types also have a concentrated fruit set compared with indeterminate types, requiring fewer harvests (George 1970; Kauffman and Lower 1976). With stressful (low fertility, low water) production conditions, however, indeterminate plant type is better yielding than determinate.

Seedling Traits

Early evaluation of populations at the seed or seedling stage is extremely useful for reducing population size and minimizing undesirable individuals. This evaluation can occur by testing for the presence of molecular markers associated with traits of interest, or by using phenotypic markers. In cucumber, phenotypic markers have been identified for fifteen traits including non-lethal and lethal color mutants, growth habit and bitterfree leaves. The five non-lethal color mutants include virescent (*v*) (Poole 1944; Tkachenko 1935), variegated virescence (*vvi*) (Abul-Hayja and Williams 1976), yellow cotyledons-1 (*yc-1*) (Aalders 1959), yellow cotyledons-2 (*yc-2*) (Whelan and Chubey 1973; Whelan et al. 1975), and yellow plant (*yp*) (Abul-Hayja and Williams 1976). Four of the color mutants cause seedling lethality: chlorophyll deficient (*cd*) (Burnham et al. 1966), golden cotyledon (*gc*) (Whelan 1971), light sensitive (*ls*) (Whelan 1972), and pale lethal (*pl*) (Whelan 1973). Other seedling phenotypic traits include bitterfree foliage (*bi*), with no cucurbitacins in the leaves (Andeweg and DeBruyn 1959), and blind (*bl*) (Carlsson 1961), with no growing point on the seedlings. Delayed growth (*dl*) (Miller and George 1979), long hypocotyl (*lh*) (Robinson and Shail 1981), revolute cotyledons (*rc*) (Whelan et al. 1975), stunted cotyledons (*sc*) (Shanmugasundarum and Williams 1971;

Shanmugasundarum et al. 1972), and nuclear (*Ch*) (Kozik and Wehner 2006, 2008) and chloroplast (Chung et al. 2007; Gordon and Staub 2011) derived chilling resistance are other seedling traits.

Stem and Leaf Traits

Most cucumber cultivars have indeterminate plant habit, where the stem elongates continuously, and 1–2 primary lateral branches originating from the main stem (Lower and Edwards 1986; Staub et al. 2008). Some cultivars also produce secondary lateral branches (originating from primary lateral branches) under some growing conditions, which is under polygenic control (Fazio et al. 2003). More branching occurs when plants are grown at low density. Cucumber plants can be indeterminate, determinate (*de*), or compact (*cp*) (Lower and Edwards 1986). Determinate cultivars have the stem terminating in flowers, and are dwarf as well. Determinate plants are not as short as compact plants. Leaf size is also controlled by a major gene designated *ll*. Plants with *Ll Ll* have large leaves (80–100 cm^2) and plants with *ll ll* have little leaves (25–40 cm^2) (Pierce and Wehner 1990; Fazio et al. 2003). Intermediate leaf types have been also identified in progeny from crosses between normal and little leaf types.

The *C. s.* var. *sativus* line H-19, a mutant type referred to as “Arkansas Little Leaf” (originally AR 79–75), and *C. s.* var. *hardwickii* differ from typical *C. s.* var. *sativus* commercial types in their multiple fruiting, i.e., the sequential setting of fruit without inhibition) and multilateral branching habit (Fazio et al. 2003). Although line H-19 bears processing type fruit (12–15 cm in length) that are similar to normal-leafed *C. s. sativus* types on an indeterminate multilateral branching habit, fruit of *C. s.* var. *hardwickii* are relatively small (3–5 cm in length). Yield and quality of H-19 was optimum when grown at 300,000 plants/ha and harvested at 10 % oversized fruit (Schultheis et al. 1998).

Cucumber stem or vine length can be modified by seven genes: bush (*bu*) (Pyzenkov and Kosareva 1981), compact (*cp*) (Kauffman and Lower 1976), determinate (*de*) (Denna 1971; George 1970; Hutchins 1940), dwarf (*dw*) (Robinson and Mishanec 1965), tall height (*T*) (Hutchins 1940), and *In-de* that behaves as an intensifier for *de* (George 1970). These genes can also have pleiotropic effects on leaf size, shape, or fruit production. Rosette (*ro*), which can affect height, is characterized by muskmelon-like leaves (de Ruiter et al. 1980).

Leaf and foliage characteristics (shape, color, glabrousness, size) can also be affected by genes not affecting stem length. Eight in particular are responsible for leaf shape: blunt leaf apex (*bla*) (Robinson 1987a), cordate leaves-1 (*cor-1)* (Gornitskaya 1967), cordate leaves-2 (*cor-2*) (Robinson 1987b), crinkled leaf (*cr*) (Nazavari et al. 1963; Odland and Groff 1963), divided leaf (*dvl*) (den Nijs and Mackiewicz 1980), ginko leaf (*gi*) (John and Wilson 1952), littleleaf (*ll*), (Goode et al. 1980; Wehner et al. 1987) and umbrella leaf (*ul*) (den Nijs and de Ponti 1983).

Fig. 4 Locule number variation from 2 (*left*) to 5 (*right*)

Leaf arrangement (opposite vs. alternate), color (golden vs. green) and glabrousness (trichomes vs. no trichomes) exhibit variation, and have been linked to single genes. Golden leaves (g), not to be confused with golden cotyledons, results in a golden color on the lower leaves of the plant (Tkachenko 1935). Opposite leaf arrangement (*opp*) is inherited as a single recessive gene that is linked to *m* (andromonoecious flowers) and *l* (locule number) (Fig. 4). Unfortunately, incomplete penetrance makes the opposite leaf arrangement difficult to distinguish from normal plants with alternate leaf arrangement (Robinson 1987c). Glabrous leaves (the absence of trichomes) is controlled by two independently inherited genes, glabrate (glb) and glabrous (Inggamer and de Ponti 1980; Robinson and Mishanec 1964; Whelan 1973). Genes for short petiole (*sp.*) (den Nijs and Boukema 1985) and tendrilless (*td*) (Rowe and Bowers 1965) have also been identified.

Flower Femaleness and Parthenocarpy

Most cucumber cultivars are monoecious (staminate and pistillate flowers) or gynoecious (pistillate flowers only). However, androecious (staminate flowers), hermaphroditic (perfect flowers), andromonoecious (staminate and perfect flowers), and trimonoecious (staminate, perfect, and pistillate flowers) types also exist (Lower and Edwards 1986). Monoecious hybrid cultivars have been available since 1945, when Oved Shifriss developed 'Burpee Hybrid', but the high cost of hybrid seed limited their commercial use. Plants having all pistillate or all perfect flowers are commonly used in hybrid production (Kubicki 1969). Typically, flowering cucumber plants begin producing staminate flowers, transitioning to perfect or pistillate flower production as the plant matures. Sex expression varies within the cucumber germplasm and has been successfully incorporated by plant breeders into cultivars with improved fruit yield and quality (Staub et al. 2008). Development of gynoecious cultivars reduced the cost of producing hybrid seed and improved earliness and adaptation to mechanical harvesting. Germplasm with the gynoecious gene was brought from Korea to the U.S. by E. Meader and distributed by the U.S.D.A. Plant Introduction system as PI 220860. Peterson (1960) backcrossed the gynoecious gene into 'Wisconsin SMR 18' to develop MSU 713–5, the female parent of the first

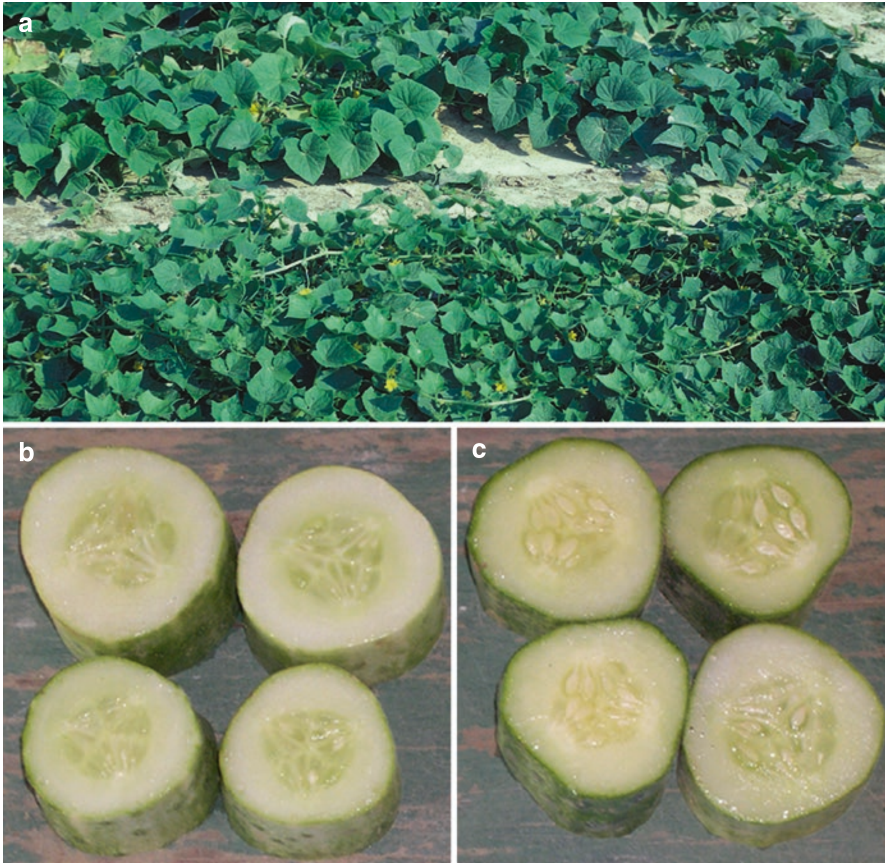

Fig. 5 Regular cucumber leaves (**a**, *top*) compared to little-leaf (**a**, *bottom*) and seed cell of (**b**) parthenocarpic and (**c**) seeded cucumbers

gynoecious hybrid cultivar, 'Spartan Dawn'. Gynoecy has replaced the need for male sterility in hybrid production. However, at least five genes for male sterility have been described (Robinson and Mishanec 1967; Shanmugasundarum and Williams 1971; Whelan 1972).

One of the more important traits to be incorporated into recent cultivars is parthenocarpy, or fruit set without pollination. Gynoecious cucumbers require the addition of a pollenizer to provide pollen for fruit set. Often, 15 % pollenizer (a monoecious hybrid cultivar) seeds are mixed with 85 % gynoecious hybrid for sale to the grower as a blend. Parthenocarpic cucumbers do not need a pollenizer to be grown in the production field or greenhouse, do not need bees or other pollinators, and may also have a concentrated fruit set (Fig. 5) (Staub et al. 2008). Parthenocarpy, first discovered in the early 1900s, is controlled by a single incompletely dominant gene *Pc* (Pike and Peterson 1969). Other genes are involved in controlling the trait, producing a range of parthenocarpic fruit set, with narrow-sense heritability of

0.33–0.62, and 5–13 effective factors (Sun et al. 2006a). Parthenocarpy was first available in greenhouse slicers, then in greenhouse Beit Alpha type for production in high tunnels, and in pickling type for field production. The parthenocarpic trait can be transferred to new types with a few backcrosses from a donor line (Sun et al. 2006b). Slicing cucumbers for open field production are now becoming available. Parthenocarpy results in high yield, seedless fruit, which provide for easier slicing. Although there have been problems with the fruit skin (exocarp) becoming tough in the large sizes at harvest.

Yield

Cucumis sativus var. *hardwickii*, including accessions LJ 90430, PI 183967 and PI 215589, has been used to increase genetic diversity for yield in commercial cucumber (Staub and Kupper 1985). Its fruit quality characteristics (small, bitter, seedy fruit) and lack of disease resistance have limited its use however (Horst and Lower 1978; Staub et al. 2008). Germplasm based on *C. s.* var. *hardwickii* has been released, but their poor internal characteristics and brining quality have precluded their widespread use so far (Staub et al. 1992).

Marker-assisted selection for fruit yield and quality has been an effective tool in cucumber improvement (Behera et al. 2011; Fan et al. 2006; Fazio et al. 2003; Robbins and Staub 2009). Yield heritability is believed to be relatively low ($R^2 = 0.17–0.56$; number of green fruit) depending on the study, and recurrent selection for yield has resulted in small gains (Robbins and Staub 2009; Wehner and Cramer 1996; Wehner 1989). Despite the small, but significant gains, yield comparisons of cultivars developed from 1969 to 1987 have demonstrated a consistent increase in production across locations and years (Wehner 1989). Backcrossing with molecular-based genotyping, along with selection for genetic diversity in *C. sativus* populations has increased diversity (phenotypic and genotypic) in cucumber (Delannay and Staub 2010), and resulted in the release of 94 inbred backcross lines (IBL) for use in cucumber improvement (Staub and Delannay 2011). Unlike crops such as maize, heterosis has been shown to have only a small effect in cucumber (Cramer and Wehner 1999).

Disease Resistance

Pre- and post-harvest diseases are a limitation for cucumber production. It has been estimated that diseases result in economic losses of 30–100 % each year (St. Amand and Wehner, 1991). In the U.S. alone, it has been estimated that $20 million is spent annually to control just *Pseudoperonospora cubensis*. Economically important diseases worldwide include bacterial wilt (*Erwinia tracheiphila*; principally home garden), anthracnose (*Colletotrichum lagenarium*), angular leaf spot (*Pseudomonas*

lachrymans), downy mildew (*Pseudoperonospora cubensis*), Fusarium wilt (*Fusarium oxysporum* f. sp. *cucumerinum*), gummy stem blight (*Didymella bryoniae*), powdery mildew (*Podosphaera xanthii*), scab (*Cladosporium cucumerinum*), and target leaf spot (*Corynespora cassiicola*). There are also several important viruses (CMV, PRSV, WMV, ZYMV) and fruit rots (*Pythium* spp., *Phytophthora capsici*, *Rhizoctonia solani*) that can be controlled using genes for resistance.

Development of cucumber cultivars with improved disease resistance in the U.S. began in the late 1920s, when R.H. Porter brought cucumber mosaic virus resistant germplasm back from China (Porter 1929). He bred the cultivar Shamrock in 1943, which was derived from the cross 'Chinese Long' × 'Davis Slicer' (Anonymous 1957). Disease resistance has been moved into commercial cucumber cultivars from PI accessions as follows: leaf spot (PI 1970888, India), anthracnose (PI 175111, India), bacterial wilt (PI 200818, Burma), target leafspot (PI 109484, Turkey), and powdery and downy mildew (PI 197087, India; PI 197085, India; and PI 212233, India). Recent acquisitions from China, Japan, Pakistan, the Philippines, and Taiwan have added new resources disease resistance (Block and Reitsma 2005; Staub et al. 2002).

Cucumber Downy Mildew

Cucumber downy mildew, caused by *Pseudoperonospora cubensis*, is a foliar disease of cucumber. Prior to 2004, the disease was controlled through a single recessive gene, *dm-1,* identified in a cucumber PI accession collected in India (PI 197087) (van Vliet and Meysing 1974). The *dm-1* gene was incorporated in the 1960s into two cultivars: Pixie and Poinsett. After 2004, the *dm*-1 gene was less effective in maintaining resistance to the pathogen. Additional sources of resistance have been identified in cucumber accessions PI 197088 and 197085, and cultivars Chinese Long and Yuanfeng. Each of these sources of resistance have undesirable fruit quality traits for slicing, pickling and European greenhouse markets (Criswell et al. 2010; Call et al. 2012). Quantitative trait loci (QTL) mapping has identified five or more genes contributing to resistance from Chinese long, and Yanfeng (Pang et al. 2013). Three QTL for resistance were identified in PI 197085, but no genetic mapping has been done on PI 197088 to date (Szczechura et al. 2015). Sources of resistance have also been identified in *C. melo* (PI 124111), but attempts to move those into *C. sativus* have failed (Lebeda et al. 1996). In *C. hystrix*, resistance QTL co-localized with those detected in 'Chinese Long', suggesting that these may be allelic variants of existing resistance genes and not new loci. The main QTL in these studies appear to be located on chromosome 5, with a smaller-effect QTL located on chromosomes 6.

Powdery Mildew

Powdery mildew, caused by the pathogen *Podosphaera fusca*, is a foliar pathogen of cucurbits causing reduction in yield and fruit quality. Three genes have been described for powdery mildew resistance (*pm-1*, *pm-2*, and *pm-3*), with a possible

fourth (*pm-h*) contributing to seedling hypocotyl resistance (Fukino et al. 2013; He et al. 2013; Sakata et al. 2006). Accession PI 197088, in addition to downy mildew resistance, has genes for resistance to powdery mildew. A QTL study identified four QTL, including one with major effects. However, the QTL were not linked to a specific chromosome, nor was the possible connection between powdery and downy mildew resistance evaluated. A study in *C. melo* reported linkage between downy and powdery mildew resistance using the resistant accession PI 124112 (McCreight et al. 2013; Olczak-Wotman et al. 2011; Perchepied et al. 2005). This linkage was also observed in cucumber by van Vliet and Meysing (1977) in the downy and powdery resistant accession PI 197087. In WI 2757, a line with moderate levels of downy mildew resistance, six QTL for powdery mildew resistance were identified (Call et al. 2012; He et al. 2013). These QTL were located on chromosomes 1, 3, 4, and 5. In addition to downy and powdery mildew resistance, PI accessions 197085, 197087, and 197088 also have moderate resistance to angular leaf spot and anthracnose, making them useful parents during plant breeding.

Fruit Rots and Seedling Diseases

Bacterial, fungal and oomycete pathogens can cause fruit rot and seedling disease on cucumber. *Phytophthora* fruit rot caused by the oomycete pathogen *Phytophthora capsici*, is a serious disease in field-grown cucumbers. Small, dark, water soaked lesions develop on infected fruit, eventually encompassing the whole fruit with white sporangia resembling powdered sugar (Hausbeck and Lamour 2004). The disease reduces yield in the field, and, if infected cucumbers are not detected early, can also spoil shipments after harvest (Hausbeck and Lamour 2004). An age-related resistance was detected during the course of screening a cucumber core collection for resistance (Gevens et al. 2006). More recently, screening of the full U.S. cucumber PI collection led to identification of three possible sources of young fruit resistance, PIs 109483, 178884, and 214049 (Colle et al. 2014). Resistance to other *Phytophthora* species also has been identified. In a greenhouse evaluation for seedling damping off using *Phytophthora dreschsleri*, a single resistant cultivar, PS 547, was identified (Nazavari et al. 2016). Another oomycete pathogen, *Pythium aphanidermatum*, can cause fruit rot (cottony leak), as well as seedling damping off. Similar to *P. capsici*, *P. aphanidermatum* starts as small water soaked lesions on the fruit, eventually turning into large fluffy-white lesions (Favrin et al. 1988). Cottony leak is primarily managed by fungicide applications, since no sources of resistance have been reported.

Belly rot, caused by the soilborne pathogen *Rhizoctonia solani*, is a minor disease of cucumber. In optimal conditions, the disease results in small water-soaked lesions on the lower surface of the fruit that reduce yield and quality (Uchneat and Wehner 1998). Sources of resistance have been identified in pickling and slicing cucumber backgrounds to belly rot (Uchneat and Wehner 1998; Wehner et al. 2004). In one study, four sources of resistance were identified from PI accessions 163216, 197088, 357852, and 280096 in field and lab-based evaluations (Wehner et al.

2004). However, no studies to date have determined the genetic inheritance of resistance to this disease. Infection by *R. solani* can also result in seedling damping off, though no sources of resistance have been identified for this disease. Likewise, *Fusarium spp.* also cause fruit rot, damping off, and wilt in cucumber (Zitter 1998). Sources of resistance have been identified for specific *Fusarium sp.* (Rose and Punja 2004). Using the resistant inbred line "9110Gt", a single QTL (*Foc2.1*) was found associated with resistance (Zhang et al. 2014).

Viruses

Several viruses of cucumber cause serious yield loss, leaf damage, or fruit defects. *Cmv* is a single dominant gene controlling resistance to cucumber mosaic virus found in the cucumber cultivar Chinese Long. Multiple potyviruses, including zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), the watermelon strain of papaya ringspot virus (PRSV-W) and Moroccan watermelon mosaic virus (MWMV) all infect cucumber. Several sources of resistance have been identified to these viruses, often within the same genetic material (Wang et al. 1984; Provvidenti 1987). ZYMV resistance in TMG-1 is controlled by a single recessive gene (Provvidenti 1987). Watermelon mosaic virus resistance was controlled by two genes, *wmv-1-1* ('Surinam') and *wmv* ('Kyoto 3 Feet') (Cohen et al. 1971). Further studies have suggested that this may be a quantitative trait, with *wmv-2*, *wmv-3*, and *wmv-4* being identified in TMG-1 (Wang et al. 1984). Additional QTL reported in melon accession PI 161375 provide further support that WMV resistance is polygenic (Guin-Aragones et al. 2014). Analyses of relationships among resistances to the different potyviruses and allelism among different sources of resistance, suggest that multiple resistances may be conferred by either a single gene or tightly linked loci (Wai et al. 1997; Grumet et al. 2000). *Zucchini yellow mosaic virus* (ZYMV) resistance is controlled by a single recessive gene $zym^{A192-18}$ located on chromosome 6 (Amano et al, 2013). *Papaya ringspot virus* (PRSV) resistance was mapped in a segregating F_2 population and a single recessive gene $prsv^{02245}$ was also identified on chromosome 6 (Tian et al. 2015).

Future Research Needed

Much progress has been made in describing phenotypic traits of cucumber, and determining their heritability (quantitative traits) and the genes involved in their control (qualitative traits) since the initial cultivars were developed in the 1700s. A genome sequence is now available for cucumber, as well as genetic information on population structure and diversity, and molecular markers for fruit quality and disease resistance traits. As molecular and sequencing technologies continue to improve, and molecular markers become increasingly economical to use, we can look forward to faster or more efficient selection of traits for use in cultivar development.

In order to continue this trend, genetic resources need to be continually developed, maintained and utilized. Germplasm from primary, secondary and tertiary centers of origin have been collected, stored, and evaluated in the national and international germplasm centers. That includes the heirloom and elite cultivars, as well as some of the gene mutant type-lines. It is imperative that more type-lines are included in the germplasm collections, as traits are identified. This may also encourage researchers to find or generate novel or alternate gene mutants. Useful genes, such as those for fruit quality and disease resistance, have been incorporated from wild or unadapted backgrounds into elite inbreds such as Marketmore, Poinsett, and WI 2757. This germplasm enhancement work makes use of these genes easier for cultivar development, and we hope that prebreeders will continue to do so. In addition, there is a need for continued collection and exchange of cucumber germplasm all over the world. Expanded collections will help feed a growing population, and counter new pest, disease and weed problems.

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Genetic Resources of Watermelon

Amnon Levi, Robert Jarret, Shaker Kousik, W. Patrick Wechter, Padma Nimmakayala, and Umesh K. Reddy

Abstract As a result of many years of domestication and selection for desirable fruit quality, most of the modern dessert watermelon cultivars share a narrow genetic base. Africa is the center of origin and diversity of the genus *Citrullus* and is thus the focus of efforts to collect and conserve germplasm for enhancing dessert watermelons with resistance to diseases and pests. In addition to *C. lanatus*, accessions of several other species of *Citrullus* have been used as sources of disease and pest resistance. These are *C. amarus* (citron watermelon), which is native to southern Africa, *C. mucosospermus* (egusi watermelon) of sub-Saharan/western Africa origin, and *C. colocynthis* (colocynth) native to the deserts of northern Africa, the Middle East and Asia. *Citrullus amarus*, *C. lanatus*, and *C. mucosospermus* are readily intercrossed with one another and thus *C. amarus* and *C. mucosospermus* have at times been classified as subspecies or botanical varieties within *C. lanatus*. Genetic resources within *Citrullus* contain genes conferring resistance to a broad range of fungal diseases such as Fusarium wilt, anthracnose, gummy stem blight; oomycete diseases including *Phytophthora capsici*, powdery mildew, downy mildew; viruses such as the watermelon strain of *Papaya ringspot virus*, *Zucchini yellow mosaic virus*, and *Squash vein yellowing virus* (SqVYV); and insect pests such as root-knot nematodes, whiteflies, and mites. Watermelon germplasm collections are maintained in China, South Africa and Zimbabwe. The United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Plant Germplasm System (NPGS), maintains a large collection of watermelon and related *Citrullus* spp. germplasm. The USDA/ARS/NPGS, Germplasm Resources Information Network (GRIN) http://www.ars-grin.gov/npgs contains general information on accessions held within the USDA/NPGS collection.

Keywords *Citrullus*, colocynth, Crop Wild Relative (CWR), genetic diversity, disease resistance, molecular markers

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Introduction

Watermelon is an important vegetable fruit crop for human consumption. Over the years, watermelon production has increased steadily. According to the Food and Agricultural Organization of the United Nations (FAO), global watermelon production was 105.37 million tons in 2012. China is the largest producer of watermelon with 70 million tons (66.43% of total global production). Turkey and Iran are the second and third highest producers with 4.04 and 3.80 million tons (3.84 and 3.60% of the world total), respectively. Brazil and Egypt are the fourth and fifth high producers with 2.08 million tons (1.97% of the world total) and 1.88 million tons (1.78% of the world total), respectively. The United States ranks sixth in terms of production with 1.77 million tons (1.68% of the world total). This is followed by Algeria (1.50 million tons – 1.42% of the world total), Russia (1.45 million tons – 1.38% of the world total), Uzbekistan (1.35 million tons – 1.28% of the world total), Kazakhstan (1.16 million tons – 1.1% of the world's total), and Mexico (1.03 million tons – 0.98% of the world's total production) (Food and Agricultural Organization-FAO; http://faostat.fao.org).

There is a long history of watermelon production in China. Watermelon was introduced to China and India around 1100 and 800 AD, respectively (Zhao 2015). Most of the modern watermelon cultivars grown in China are derived from local, older cultivars crossed with cultivars introduced from Japan and the USA. The most prevalent local Chinese watermelon varieties include 'San Bai Gua' and 'He Tao Wen' from Shaanxi province near the Yellow River, 'Xiao Hua Li Hu' from Henan province, 'La Ma Gua' from Shandong province, and 'Ma Ling Gua' from eastern China. Unique watermelon germplasm developed and commercially grown (on at least a half million acres annually) in China is produced for seed consumption – the "edible-seed watermelon". Popular edible-seed watermelon cultivars are 'Gao Lan Zi Gua' with very large seed (1000 seed weight >250 g), and 'Lanzhou Da Bang Hong Zi Gua' with a deep red seed coat (1000 seed weight >150 g). Both of these are local cultivars in Gansu province on the Silk Road. The edible-seed watermelons can grow on marginal land and are drought tolerant, with small thin leaves, thin vines and a large number of branches. However, they are highly susceptible to common watermelon diseases that occur in China (Dr. Xingping Zhang, personal communication 2015).

Watermelon was introduced to North America from Africa and Spain. A large number of cultivars have been developed in the U.S. over the last 250 years and many of these cultivars share a similar genetic base (Levi et al. 2001a, b). The narrow genetic base among cultivars may be due to 'founder effect', whereby they were derived from a few accessions introduced to the continent by early immigrants (Mayr 1954; Nimmakayala 2014). The USDA/ARS Plant Genetic Resources Unit (PGRU), Griffin, GA, maintains about 400 USA-developed watermelon cultivars.

According to the USDA Economic Research Service, the fresh market value of watermelon in the United States was around $460 million in 2014, with production

of over 1.45 billion kg grown on over 48,000 hectares. Florida, Georgia, Texas, Arizona, and California are the major watermelon-producing states and account for about 44% of US watermelon production. The average watermelon yield per acre in the USA in 2014 was 14,424 kg. Still, only 24% of the watermelons consumed in the USA are produced by domestic growers; the remainder is imported from Central America and Mexico (http://www.agmrc.org).

Since the early years of the twenty-first century, seedless watermelons (triploid hybrid cultivars) have been the primary type grown commercially for the U.S. market (United States Department of Agriculture, National Agricultural Statistics Service 2009). However, seeded (diploid) watermelons are still produced and consumed throughout the world, mainly in Asia, Africa, the Middle East, and South America. The American cultivars 'Crimson Sweet' and 'Allsweet' have high quality fruit-flesh and small seeds. These cultivars, which were developed at Kansas State University by Charles V. Hall and released in 1966 and 1972, respectively, are perhaps the leading diploid cultivars in terms of current world production, and are in the parentage of many of today's seedless cultivars.

Demand for seedless varieties continues to increase in the USA and throughout the world. Seedless watermelons accounted for 51% and 85% of total watermelon shipments in the United States in 2003 and 2014, respectively. A seedless watermelon is a triploid hybrid, derived from crossing a diploid variety as the male parent with a tetraploid line as the female parent (Kihara 1951). This process of hybridization for triploid seed production is tedious and consequently the cost of triploid seeds is relatively high, reaching 20–80 cents per seed. Production of triploid watermelons involves planting a diploid (seeded) watermelon variety as a pollen source (referred to as the "pollenizer"). The presence of a pollenizer is essential for pollination and fruit-setting of the seedless watermelons in the field. The pollenizer is interspersed throughout the field in a ratio of 1 pollenizer to 3 seedless watermelon plants. Because of the high cost of the seed and the difficulty in establishing a stand, seedless watermelon plants are traditionally transplanted into the field, as opposed to direct seeding.

Center of Origin and Genetic Diversity of Watermelon

Watermelons belong to the xerophytic genus *Citrullus* Schrad. ex Eckl. & Zeyh. They are cultivated in temperate and tropical regions of the world, serving as a water and food source for animals and humans (Wehner 2008; Paris 2015). The center of origin of *Citrullus* is Africa, where wild, feral and landrace populations of this genus thrive in the vast arid and semi-arid regions of the continent. Numerous types of *Citrullus* also exist in the wild and desolate places of the Middle East, central and southern Asia, and Asia Minor (Anatolia). Plant materials in many of the remote areas are poorly characterized and additional studies are needed to properly collect, classify and evaluate them (Levi et al. 2013; Nimmakayala et al. 2010; Reddy et al. 2013).

The annual *Citrullus lanatus* (Thunb.) Matsum. & Nakai, the dessert watermelon, is the best known among all *Citrullus* species. Native to Sudan and Egypt, it includes wild, feral, and cultivated forms (Paris 2015). The sweet flesh of *C. lanatus* has resulted in its spread throughout the world and it has become one of the most extensively consumed vegetable fruit crops. The genus *Citrullus* includes six additional diploid species, three of which are also of regional importance (Jarret et al. 1997). These are the egusi watermelon (*C. mucosospermus* (Fursa) Fursa), the citron watermelon (*C. amarus* Schrad.) and the colocynth (*C. colocynthis* (L.) Schrad.). *Citrullus mucosospermus* is native to sub-Saharan western Africa and is cultivated for its oily seeds (Jarret et al. 1997; Achigan-Dako et al. 2006; Dahl Jensen et al. 2011; Akusu and Kiin-Kabari 2015). *Citrullus lanatus* and *C. mucosospermus* share similar genome sequences (Guo et al. 2013) and can usually be hybridized with one another to produce highly fertile progeny (Levi et al. 2011b). However, exceptions to this have been reported depending on the direction of the cross (Fursa 1983; Gusmini et al. 2004).

Citrullus amarus is native to southern Africa and is cultivated for its edible but typically hard fruit flesh, which is often cooked or pickled (Bush 1978). Known widely as the citron, tsamma or preserving melon, it was given the taxonomic name *C. lanatus* var. *citroides* by L.H. Bailey (1930). *Citrullus amarus* is readily crossed with *C. lanatus* and with *C. mucosospermus.* However, wide differences exist in the genome sequences of *C. amarus* when compared to *C. lanatus* or *C. mucosospermus* (Guo et al. 2013). The genetic populations derived from these crosses with *C. amarus* produce skewed (non-Mendelian) segregation ratios for most genomic regions (Levi et al. 2002).

Citrullus colocynthis, also known as bitter apple, is cultivated for its numerous medicinal properties and the oil of its seeds (Hussain et al. 2014). *Citrullus colocynthis* is native to the deserts and semi-arid regions of northern Africa and southwestern and central Asia, including the Mediterranean islands eastwards to Afghanistan, Pakistan, and India (Jeffrey 1967; Burkill 1985; Dane and Liu 2007; Paris 2015). Additional related species include the desert annual *C. rehmii* (De Winter 1990; Jarret and Newman 2000) and the desert perennials *C. ecirrhosus* (Cogn.) and *C. naudinianus* Sond. (Meeuse 1962).

A review of the genus *Citrullus*, with nomenclatural revisions, was recently presented by Chomicki and Renner (2015) (Table 1). A summary of nomenclatural equivalents across taxonomic ranks was summarized by Paris (2015). However, the taxonomy and nomenclature of *Citrullus* spp. has not been fully resolved (Paris 2016). There are genetic and genomic indications suggesting that several species (i.e. *C. amarus*, *C. mucosospermus* and *C. lanatus*) represent natural admixtures (Guo et al. 2013; Levi et al. 2013; Reddy et al. 2014a, b) thus obscuring the separation among these species.

Archaeological evidence indicates that the dessert watermelon, *Citrullus lanatus*, was domesticated in northeastern Africa, Egypt and Sudan, over 4000 years ago and was most likely spread by the nomadic peoples of the deserts of Africa, the Middle East and Asia as a source of water and nutrients (Paris 2015). The many years of domestication and selection by early agrarians, and later by breeders, for

Table 1 Known names of counterpart *Citrullus* specific, sub-specific, botanical-variety, and cultivar group (non-inclusive)

English name	Species[a]	Subspecies[b]	Botanical variety[c]	Cultivar-group(s)[d]
Dessert watermelon	*C. lanatus* (Thunb.) Matsum. & Nakai	*vulgaris* (Schrad.) Fursa; *cordophanus* Ter-Avan.	*lanatus*	Dessert Cordophanus
Citron watermelon	*C. amarus* Schrad.	*lanatus*	*citroides* Bailey	Citroides
Egusi watermelon	*C. mucosospermus* (Fursa) Fursa	*mucosospermus* Fursa	*mucosospermus* Fursa	Mucosospermus
Colocynth	*C. colocynthis* (L.) Schrad.	—	—	—

Adapted from Paris (2015)
[a]After Renner et al. (2014); Chomicki and Renner (2015)
[b]After Fursa (1972)
[c]There are many designated botanical varieties; only three are listed here
[d]After Jeffrey (2001)

desirable fruit quality resulted in a genetic bottleneck and a narrow genetic base among watermelon cultivars (Levi et al. 2001a, b; Guo et al. 2013). Long-term selection for desirable fruit quality is reflected in the low levels of polymorphism associated with the chromosomal regions flanking selected alleles (Nimmakayala et al. 2010, 2014; Reddy et al. 2014a, b). The narrow genetic base among watermelon cultivars and the loss of alleles associated with resistance may have contributed to the susceptibility of today's cultivated watermelon to a wide range of diseases and pests (Levi et al. 2001a).

The recent sequencing and assembly of the elite Chinese watermelon accession 97,103 (Guo et al. 2013) and the American 'Charleston Gray' (Levi et al. 2011a) confirmed the existence of a low number of single nucleotide polymorphisms (SNPs) (about 1 SNP per 1300 bp) between these two morphologically distinct watermelon types. 'Charleston Gray' was developed and released by Charles Fredric Andrus at the USDA/ARS, U.S. Vegetable Laboratory (USVL) in 1954 and was the first cultivar to have resistance to both of the major diseases, fusarium wilt and anthracnose. 'Charleston Gray' has been used in numerous breeding programs and appears in the pedigrees of many watermelon cultivars throughout the world (Wehner and Barrett 1996), including the very popular 'Crimson Sweet' and 'Allsweet'. In contrast with the Chinese 97,103 elite line that has small to mid-size globular watermelons with red flesh and dark green rind, 'Charleston Gray' produces large, elongated fruits with a pink flesh and a hard gray rind and was considered a "good shipper" suitable for the market conditions in the 1950s. It is still a popular cultivar.

The narrow genetic base of dessert watermelon (*Citrullus lanatus*) cultivars creates a continuous challenge for researchers and breeders aiming to improve the crop for disease resistance. High yield, high fruit quality and early maturity are the principle objectives for most of today's watermelon breeders (Gusmini and Wehner 2005a). Due to the overall low genetic diversity among watermelon cultivars, vigor (heterosis) might be expected to be relatively low in F_1 hybrid watermelon lines. Diallel experiments using several watermelon lines indicated inconsistencies in estimates of heterosis across experiments (Gusmini and Wehner 2005a). Primitive landraces and similar materials might be expected to be a useful source of genes for enhancing genetic diversity and possibly increasing hybrid vigor in diploid and triploid seedless watermelon varieties. Next generation sequencing (NGS) technologies are expected to provide the necessary tools for studying genetic diversity, population structure and the identification of gene loci contributing to heterosis and those conferring resistance to biotic and abiotic stresses. NGS technologies will also facilitate the selective incorporation and utilization of diverse germplasm into watermelon breeding programs (Reddy et al. 2014a, b; Ren et al. 2014; Lambel et al. 2014; Branham et al. 2016).

Citrullus mucosospermus (egusi watermelons) and *C. lanatus* (dessert watermelons) are closely related, and are estimated to have diverged from one another approximately 3.1 million years ago (Chomicki and Renner 2015). *Citrullus mucosospermus* is highly diverse in leaf shape and size, and in seed shape and size (Figs. 1, 2 and 3). The fresh seeds of *C. mucosospermus* are thick with a fleshy pericarp, a trait controlled by a single recessive gene mutation (Gusmini et al. 2004; Prothro et al. 2012). *Citrullus mucosospermus* PIs are an important source of disease resistance genes or alleles. Studies at the USDA/ARS/USVL evaluated a large number of watermelon PIs and identified resistance to *Phytophthora* fruit rot (Kousik et al. 2012a). Fruits of disease resistant egusi watermelons (*C. mucosospermus*) were resistant to *Phytophtora capsici* infection at all stages of growth, when compared to susceptible *C. lanatus* 'Sugar Baby' and 'Mickylee' (Kousik et al. 2014). Several *C. lanatus* and *C. mucosospermus* PIs have been selected and released as germplasm lines for use in watermelon breeding programs (Gillaspie and Wright 1993; Kousik et al. 2014).

Genebank accessions of *Citrullus lanatus*, *C. mucosospermus* and *C. amarus* have been a valuable source of resistance to powdery mildew (PM), a major disease of watermelon (Davis et al. 2007; Tetteh et al. 2010; Kousik et al. 2012a, 2014). Sources of resistance to race 1 or 2W of the powdery mildew pathogen have been identified among various *Citrullus* PIs (Davis et al. 2007; Tetteh et al. 2010) and the mode of resistance inheritance has been determined in several watermelon PIs (Ben-Naim and Cohen 2015; Tetteh et al. 2013). Studies at the USDA/ARS/USVL have identified and developed *C. lanatus* and *C. mucosospermus* germplasm lines with multiple disease resistance (Powdery mildew and Phytophthora fruit rot), useful in watermelon breeding programs (Table 2). Several *C. colocynthis* and *C. lanatus* PIs are a potential source for resistance to Squash vein yellowing virus (SqVYV) that causes watermelon vine decline (Kousik et al. 2009, 2012b).

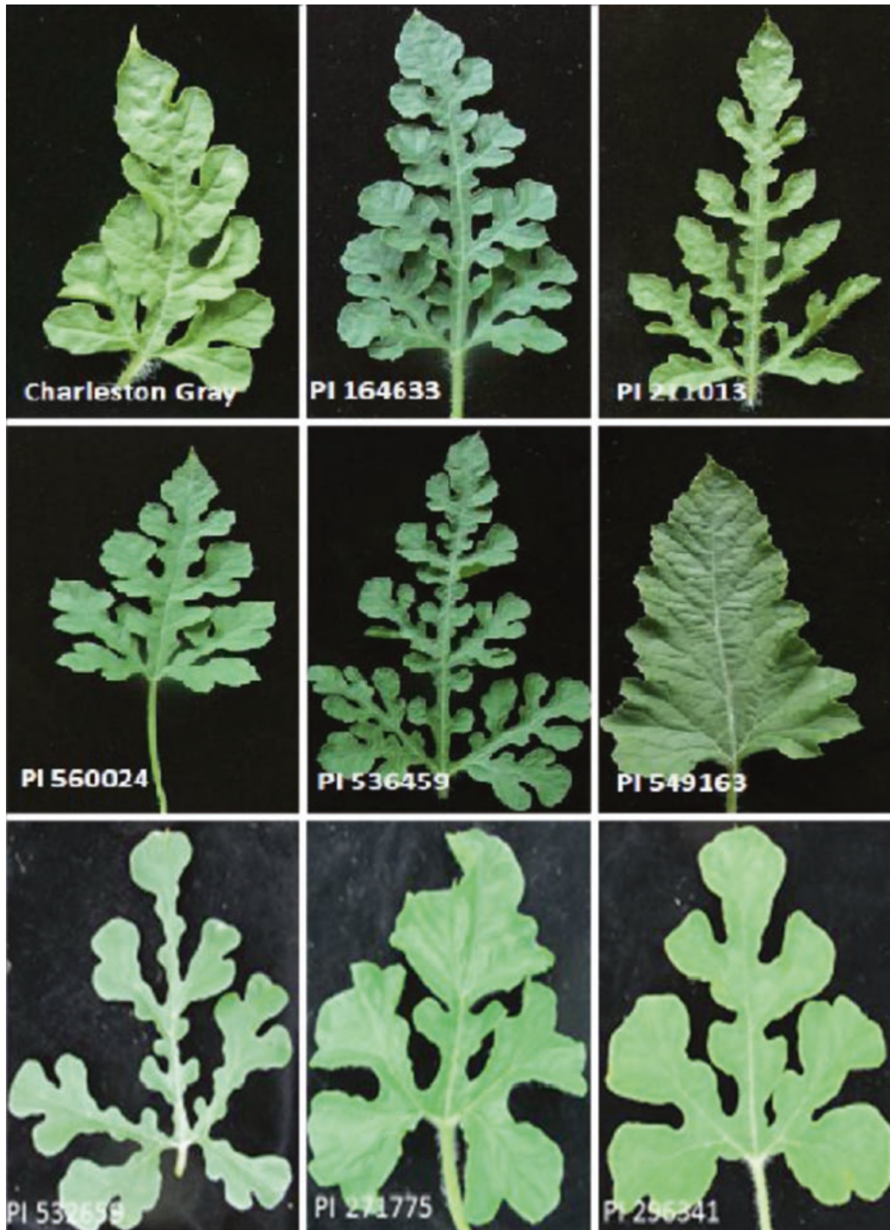

Fig. 1 Leaves of the watermelon cultivar Charleston Gray (*Upper left*) and United States plant introductions (PIs) representing the wide phenotypic diversity to be found in leaf shape, including lobe shape and overall configuration in the genus. PI 164633 (India) – *C. lanatus*. PI 211013 – *C. lanatus*. PI 560024 (Nigeria) – *C. mucosospermus*. PI 536459 (Maldives) – *C. lanatus*. PI 549163 (Chad) – *C. lanatus*. PI 532659 (Zimbabwe) – *C. amarus*. PI 271775 (South Africa) – *C. amarus*. PI 296341 (South Africa) – *C. amarus*

Fig. 2 Seeds of United States plant introductions (PIs) and 'Jubilee' showing wide phenotypic diversity in seed size, shape, and seed coat color and texture. PI 560006 (Nigeria) – *C. mucosospermus*. PI 326516 (Ghana) – *C. mucosospermus*. PI 490375 (Mali) – *C. mucosospermus*. PI 537461 (Spain) – *C. lanatus*. PI 482248 (Zimbabwe) – *C. amarus*

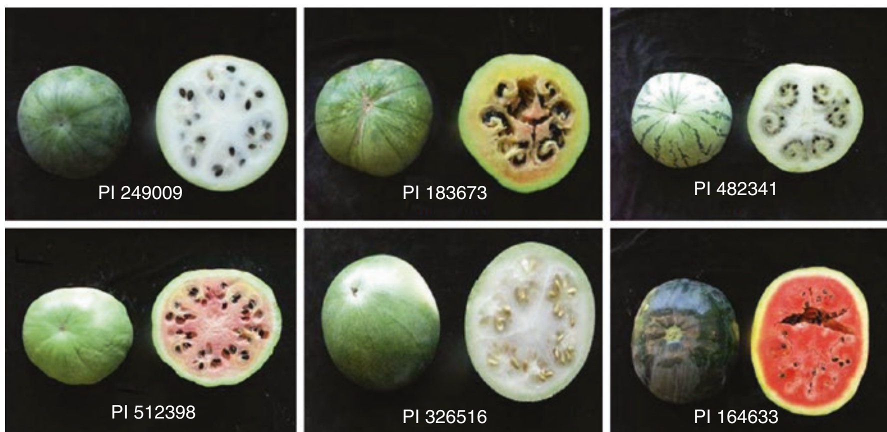

Fig. 3 Watermelon fruits of United States plant introductions (PIs) with wide phenotypic diversity in rind and flesh color, texture, and in shape of placenta and seed cavities. PI 249009 (Nigeria) – *C. mucosospermus*. PI 183673 (Turkey) – *C. lanatus*. PI 482341 (Zimbabwe) – *C. lanatus*. PI 512398 (Spain) – *C. lanatus*. PI 326516 (Ghana) – *C. mucosospermus*. PI 164633 (India) – *C. lanatus*

Wide genetic diversity exists among *Citrullus amarus* accessions collected in southern Africa and they can be differentiated into at least two distinct groups based on allele frequencies (Levi et al. 2013). Various *C. amarus* PIs possess resistance to root-knot nematodes (Thies and Levi 2003, 2007; Table 2), Fusarium wilt race 2 (Netzer and Martyn 1989; Dane et al. 1998; Wechter et al. 2012; Table 2), gummy stem blight (Sowell 1975; Sowell and Pointer 1962; Gusmini et al. 2005; Table 2), anthracnose (races 1, 2 or 3) (Sowell et al. 1980; Boyhan et al. 1994; Table 2),

Table 2 United States Plant Introductions (PIs) with resistance to diseases or pests of watermelon

Disease of watermelon	United States plant introductions (PIs) with disease or pest resistance
Fusarium wilt races 1 and 2	PI 482246, PI 482252, PI 271769, PI 299378, PI 482273, PI 482299, PI 482308, PI 296341
Gummy stem blight	PI 271778, PI 244019, PI 271771, PI 482276, PI 482379, PI 500334,
	PI 500335, PI 296332, PI 505590 and PI 526233
Anthracnose	PI 248774, PI 270562, PI 270563, PI 271775, PI 271778, PI 271779
	PI 299379, PI 500315, PI 500329, PI 505592 and PI 505593
Phytophthora capsici	PI 306782, PI 595203, PI 560020PI, 560002, PI 494531, PI 482328, PI 189225 and PI 532738
Powdery mildew	PI 494531, PI 482326, PI 482328, PI 189225, PI 532738
Watermelon mosaic virus (WMV)	PI 244018, PI 244019, PI 255137 and PI 482252
Papaya ringspot virus	PI 244018, PI 244019, PI 255137, PI 482252, PI 244017, PI 482318
Watermelon strain (PRSV-W)	PI 482342, PI 482299, PI 482315, PI 482322, PI 482379, PI 485583 PI 595203
Squash vein yellowing virus (SqVYV)	PI 392291, PI 482266, PI 386015, PI 386024, PI 459074, PI 500354
Zucchini yellow mosaic virus (ZYMV-FL)	PI 482322, PI 482299, PI 482261, PI 482308, PI 244018, PI 482276, PI 485580, PI 596662, PI 595203
Root knot nematodes	PI 482324, PI 482333, PI 482379, PI 189225, PI 482298
Broad mites	PI357708, PI 500354, PI 386015, PI 386016, PI 525082
Whiteflies	PI 537277, PI 386024

powdery mildew (Davis et al. 2007; Tetteh et al. 2010; Table 2), and potyviruses (Guner 2004; Strange et al. 2002; Table 2). *Citrullus amarus* accessions possess resistance genes or alleles that do not occur or that are not expressed in *C. lanatus* cultivars (Levi et al. 2013). Recent studies (Levi et al. 2013; Thies et al. 2010, 2015) indicate that *C. amarus* could be valuable as a source of germplasm for the development of rootstocks for grafted watermelon cultivars because they produce large root systems (Fig. 4) and significantly higher yields than other cucurbit rootstocks in fields infested with root-knot nematodes. Some *C. amarus* accessions are also a viable source of tolerance to broad mites (Kousik et al. 2007).

Another important species in terms of its potential for contributing to the improvement of dessert watermelons is *Citrullus colocynthis*. This species thrives in diverse desert regions and possesses considerable genetic diversity (Fig. 5) (Levi et al. 2001a). A population structure analysis based on 431 HFO-TAG markers identified several subgroups within the *C. colocynthis* accessions examined and these were correlated with the geographic origins of the plant materials (Levi et al. 2017). Despite the reproductive barriers resulting from extensive differences in genome structure between *C. colocynthis* and *C. lanatus* or *C. mucosospermus* (Levi et al. 2006, 2013), PIs representing these quite distinct *Citrullus* species can be crossed

Fig. 4 Roots of 'Charleston Gray' (7 days post germination), PI 271775 (8 days post germination) and PI 482263 (9 days post germination). The WinRHIZO Root Scanner instrument calculates root capacity, root surface and various other root features (Regent Instruments, Quebec, Canada). 'Charleston Gray – *C. lanatus*. PI 271775 (South Africa) – *C. amarus*. PI 482263 (Zimbabwe) – *C. lanatus*

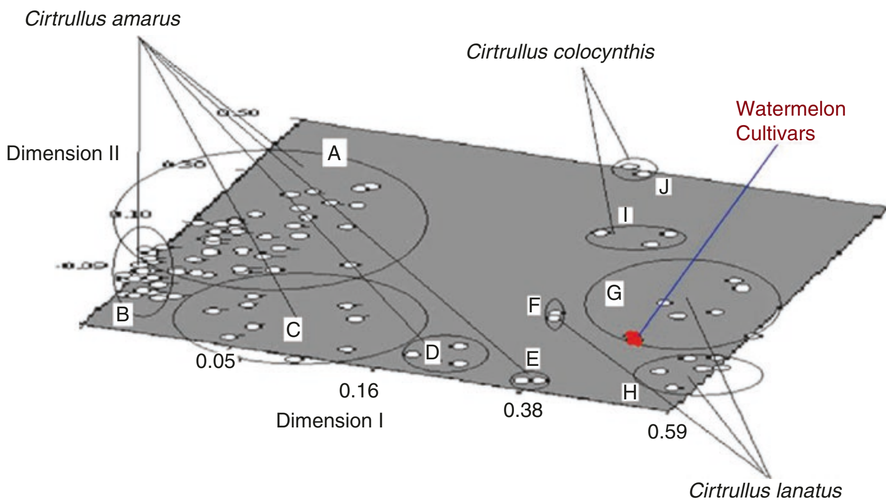

Fig. 5 Two-dimensional plot of *Citrullus amarus*, *C. lanatus* and *C. colocynthis* accessions, and an admixture of watermelon cultivars, using multi-dimensional scaling based on 470 high frequency oligonucleotide – targeting active gene (HFO–TAG) markers.

with one another (Levi et al. 2006, 2017). *C. colocynthis* PIs represent a useful source of genes for enhancing biotic and abiotic stresses, such as drought resistance, resistance to whiteflies (Coffey et al. 2015; Table 2), potyviruses (Levi et al. 2016)

and broad mites (Kousik et al. 2007), in cultivated watermelons. The various *Citrullus* spp. have distinct cytoplasms with large differences in their mitochondrial and chloroplast genomes (Chomicki and Renner 2015; Levi and Thomas 2005; Levi et al. 2006).

The tendril-less *Citrullus ecirrhosus*. and *C. rehmii* are desert-adapted species which are endemic to southern Africa (Meeuse 1962; Robinson and Decker-Walters 1997; Chomicki and Renner 2015). These species possess a tap root (*C. rehmii*) or a water-conserving caudex (*C. ecirrhosus*). *Citrullus naudinianus*, commonly referred to as the gemsbok cucumber, is the most morphologically unique *Citrullus* species, producing a large underground storage root and fruits resembling some *Cucumis* spp. This member of the genus is common in sub-Saharan Africa and has been considered to be an important source of food and water for humans and animals (Chomicki and Renner 2015). Interspecific crosses among *Citrullus* species are possible to various degrees (Robinson and Decker-Walters 1997), and frequently are successful but with low fruit and seed set and/or low pollen viability (Shimotsuma 1963; De Winter 1990; Sain et al. 2002; Sain and Joshi 2003). Little is known about the extent of genetic/morphological diversity within these species or their resistance to various watermelon diseases or pests.

In addition to their potential for enhancing disease and pest resistance, *C. amarus, C. colocynthis, C. mucosopermus,* and possibly other spp. as well, are a valuable source of genes for use in breeding programs aiming to enhance dessert watermelon yields via more efficient root systems that better tolerate drought and extreme temperatures (McGregor 2012). As noted previously, *C. rehmii*, *C. ecirrhosus* and *C. naudinianus* all have root systems that are well adapted to desert conditions. A study of drought tolerance using citron watermelon, *C. amarus*, plants collected in the wild in Botswana indicated that in response to drought, the root system served to store water and extended deeper into the soil (Larcher 1995; Yoshimura et al. 2008). Furthermore, root growth is slow in watermelon cultivars during drought conditions, while in *C. amarus* accessions, vigorous root growth follows drought conditions (Yoshimura et al. 2008). Thus, various *Citrullus* spp. might be expected to be useful in efforts to enhance watermelon cultivars with resistance to abiotic stresses such as heat or cold tolerance, drought tolerance and/or water use efficiency (Akashi et al. 2001; Huh et al. 2002; Kawasaki et al. 2000; Rivero et al. 2001; Zhang et al. 2011).

A variety of studies have been conducted to examine the morphological and genetic (molecular) variation within and among the principal species; *C. lanatus*, *C. amarus, C. colocynthis* and *C. mucosospermus*. However, most of these studies (Tables 3 and 4) examined a relatively few (<100) genotypes and often used specific methods/procedures that make it difficult or impossible to compare results across studies.

Table 3 Publications on the morphological characterization of *Citrullus* germplasm

Presumed sp. (spp.)	No. accessions	Origin	Reference(s)
C. mucosospermus	8	Nigeria	Oyulu (1977)
C. amarus	2	Botswana	Taylor (1985)
C. lanatus, Citrullus spp.	8	Sudan, USA	El Mekki (1991, 1992)
Citrullus spp.	100	USA, Germany, Netherlands, Romania, Yugoslavia, Israel, Turkey, Libya, Taiwan, Korea	Krasteva (2000)
Citrullus spp.	7	Nambia, USA	Maggs-Kolling et al. (2000), Maggs-Kolling and Christiansen (2003)
C. lanatus	—[a]	Hungary	Nagy (2005)
C. lanatus, Citrulus spp.	—	USA (see publication)	Wehner et al. (2001)
C. lanatus	43	Brazil, USA	de Silva et al. (2006)
C. lanatus, C. amarus	30	Sudan	Goda (2007)
C. mucosospermus	20	Nigeria	Idehen et al. (2007)
C. amarus	3	Corsica	Laghetti and Hammer (2007)
Citrullus spp.	134	Turkey	Sari et al. (2006, 2008), Szamosi et al. (2008), Solmaz and Sari (2009)
C. lanatus, undetermined	67	Korea & Turkey	Huh et al. (2008)
C. lanatus	8	Tunisia	Elbekkay et al. (2009)
Citrullus spp.	6	Kenya, USA	Gichimu et al. (2009)
C. lanatus	134	Turkey	Solmaz and Sari (2009)
Citrullus spp.	39	Hungary, Turkey	Szamosi et al. (2009)
C. mucosospermus, C. lanatus	Unknown	Mali	Jensen et al. (2011)
C. lanatus, C. mucosospermus, C. amarus	132	Mali	Nantoumé et al. (2012)
C. lanatus	327	Turkey and others	Solmaz et al. (2012)
Citrullus spp.	213	Africa, Asia, EU, USA	Achigan-Dako et al. (2015)
C. lanatus	8	Morocco	Hakimi and El Madidi (2015)
C. mucosospermus	171	Benin, Cote d'Ivoire, France, Ghana, Nigeria, Togo, Turkey	Gbotto et al. (2016)

[a]No information

Table 4 Publications on the molecular characterization of *Citrullus* germplasm

Presumed sp. (spp.)	No. accessions	Origin	Marker	Reference(s)
C. lanatus, C. colocynthis	44	Israel	Isozymes	Zamir et al. (1984)
C. lanatus, C. ecirrhosus, C. naudinianus, C. colocynthis	5	Israel, southern Africa	Isozymes	Navot and Zamir (1987)
C. lanatus	8	USA	Isozymes	Biles et al. (1989)
C. lanatus, C. amarus	4	USA, South Africa	RAPD	Zhang et al. (1994)
C. lanatus	39	USA South Korea	RAPD	Lee et al. (1996)
C. lanatus, C. amarus, C. mucosospermus	32	Africa, Europe, Asia, and Mexico	SSR	Levi et al. (2000)
C. lanatus, C. amarus, C. colocynthis	34	Various (see publications)	RAPD	Levi et al. (2000)
C. lanatus, C. amarus, C. colocynthis	42	Various (see publications)	RAPD	Levi et al. (2001a)
C. lanatus	30	Various (see publications)	AFLP	Che et al. (2003)
C. lanatus, C. amarus, C. colocynthis, C. rehmii	70	Various (see publications)	cpDNA	Dane and Lang (2004)
Citrullus lanatus	44	USA	ISSR & AFLP	Levi et al. (2004)
C. lanatus	43	Brazil, USA	RAPD	de Silva et al. (2006)
C. lanatus, C. amarus, C. lanatus	90	Numerous (see publications)	Non-coding cpDNA	Dane and Liu (2007)
C. lanatus, C. amarus	30	Sudan	RAPD, SSR	Goda (2007)
C. lanatus	24	USA	RAPD	Levi and Thomas (2007)
C. lanatus	24	Korea	SSR	Kwon et al. 2007)
C. lanatus, C. amarus, C. colocynthis	38	USA, various	EST-PCR	Levi et al. (2008)
C. lanatus	7	India	EST-SSR	Verma and Arya (2008)
C. lanatus	49	USA, Korea	SSR	Kwon et al. (2010)
C. lanatus, C. amarus	10	Zimbabwe	RAPD, SSR	Mujaju et al. (2010)
C. lanatus, C. amarus, C. colocynthis	31	Various (see publications)	AFLP, SSR	Nimmakayala et al. (2010)
C. lanatus and others	303	Turkey (94)	RAPD	Solmaz et al. (2010)

(continued)

Table 4 (continued)

Presumed sp. (spp.)	No. accessions	Origin	Marker	Reference(s)
C. lanatus, C. colocynthis, C. rehmii, C. amarus	27	Korea, France, Thailand, Japan, Taiwan, China, India, USA, Zaire, Senegal, Iran, Zambia	EST-SSRs & AFLP	Hwang et al. (2011a)
C. lanatus, C. colocynthis, C. amarus	8	Korea, France, Thailand, Japan, Taiwan, China, India, USA, Zaire, Senegal, Iran, Zambia	EST-SSRs and high-resolution melting analysis	Hwang et al. (2011b)
C. amarus, C. lanatus	25	Botswana, Namibia, S. Africa, Zambia, Zimbabwe, USA	SSR	Mujaju et al. (2011)
C. mucosospermus	4	Ivory Coast/West Africa	SSR	Minsart et al. (2011)
C. lanatus	47	USA	Ms-AFLP	Nimmakayala et al. (2011)
Citrullus lanatus, C. amarus	90	Turkey, USA, India, Japan, West Africa, South Africa, West Azerbaijan, Iran, Phillipines, Guatemala, Zaire	SRAP	Uluturk et al. (2011)
Citrullus lanatus	20	Korea	EST-SSR	Kwon (Kwon 2013)
Citrullus amarus, C. lanatus, C. colocynthis	96	Various (see publications)	HFO-TAG	Levi et al. (2013)
Citrullus lanatus, C. amarus	—[a]	Zimbabwe, USA	EST-SSR	Mujaju et al. (2013)
Citrullus lanatus, C. amarus, C. mucosospermus	134	Mali	SSR	Nantoumé et al. (2013)
Citrullus lanatus, C. colocynthis	18	Brazil, USA	SSR	Gama et al. (2013)
Citrullus lanatus, "wild"	37	Not specified	DAtTseq-based SNPs	Yang et al. (2016)
Citrullus spp.	1197	*Numerous (see publications)*	SSR	Zhang et al. (2016a, b)

Abbreviations: *RAPD* random amplified polymorphic DNA, *SSR* simple sequence repeat, *AFLP* amplified fragment length polymorphism, *SRAP* sequence-related amplified polymorphism, *EST* expressed sequence tag, *ISSR* inter-simple sequence repeat, *DArTseq* diversity array technology,

(continued)

Table 4 (continued)

cpDNA chloroplast DNA, *Ms-AFLP* methylation sensitive AFLP, *HFO-TAG* high frequency oligonucleotides targeting active genes
[a]No information

Watermelon Fruit Quality and Morphology

Following their domestication thousands of years ago, dessert watermelons, *Citrullus lanatus*, were selected for sweetness of the fruit flesh. However, records of sweetness of dessert watermelons date only to the end of the second century CE (Paris 2015). Over the centuries, selection and breeding of watermelons have focused primarily on improving fruit quality. Flesh color, sugar content and rind pattern in the dessert watermelons are qualitative traits (Gusmini and Wehner 2005b). The inheritance of the important fruit quality characteristic, flesh color (scarlet red, red, yellow, canary yellow, salmon yellow, orange, and white), has not been fully elucidated (Bang et al. 2010). A recent study identified a novel chromoplast phosphate transporter (CLPHT4;2) associated with flesh color development in watermelon (Zhang et al. 2016a, b). The sugar content of modern commercial watermelons is relatively high and consistent with soluble solids content (with a 10-14° Brix value) (Maynard 2001; Wehner et al. 2001). Large quantities of sugars, mainly sucrose, accumulate in the cell vacuoles of the fruit flesh by a yet unknown molecular mechanism. A recent study identified a major sugar content QTL on chromosome 2 with a Tonoplast Sugar Transport gene responsible for the uptake of sucrose, fructose and glucose into the vacuoles of the dessert watermelon (Zhang et al. 2017). Primitive dessert watermelon (*C. lanatus*) landraces, which have low sugar content and Brix values, might be a valuable source of bioactive compounds with health attributes, including citrulline, ascorbic acid, potassium, flavonoids, and carotenoides, such as alpha and beta carotene or lycopene (Davis et al. 2007).

Watermelon Germplasm Resources Useful for Enhancing Watermelon Cultivars

The assembly and the conservation of genetically and morphologically diverse watermelon germplasm are essential activities to ensure the current and future success of watermelon breeding programs. This is particularly true in that genebanks are the principle source of plant material used for the identification of sources of resistance to diseases and pests. Given the ever-increasing human population and the simultaneous reduction in land area appropriated for agricultural use, there is an increasing need for high-yielding, disease resistant crops, including watermelon.

For this reason, on-going efforts are needed to collect, maintain and evaluate *Citrullus* germplasm.

Several genetic and genomic resources are available for watermelon. The USDA/ARS/ARS maintains a collection of *Citrullus* germplasm in Griffin, Georgia. The collection contains about 1800 accessions and includes representative examples of all seven *Citrullus* species. The collection contains numerous heirloom varieties and primitive landraces collected over a period or more than 75 years. The PGRU *Citrullus* collection has been evaluated for resistance to major diseases and pests of watermelon. Many resistance-containing PIs have been further selected and developed into enhanced germplasm or breeding lines that have proven useful in genetic studies and in programs aiming to incorporate or enhance resistance in the watermelon crop. This collection has been used extensively to study trait inheritance and also for the study of *Citrullus* taxonomy and evolution (Reddy et al. 2014a, b; Levi et al. 2013, 2017, Branham et al. 2016). In recent years, material in the PGRU collection has been used for the development of robust rootstocks with resistance to nematodes and soil-borne diseases, traits of value for grafted watermelons (Levi et al. 2013; Thies et al. 2015). Information about the US watermelon germplasm collection can be found on the National Plant Germplasm System (NPGS), Germplasm Resources Information Network's (GRIN) website at: http://www.ars-grin.gov/npgs.

Watermelon germplasm collections rich in genetic and phenotypic diversity are also maintained in Turkey and China. Southern Africa is considered a center of origin and a source of several *Citrullus* spp. In recent years, the Southern African Development Community (SADC) Plant Genetic Resources Centre (SPGRC) and National Plant Resources Centre Regional Network have placed a priority on collecting and conserving watermelon germplasm (Mujaju et al. 2010; Munyenyembe 2009). Also, the National Botanical Research Institute (NBRI), National Plant Genetic Resources Centre (Namibia) and the Zambia Agriculture Research Institute (ZARI) maintain many *Citrullus* entries (McGregor 2012). The N.I. Vavilov Research Institute of Plant Industry (VIR; http://www.vir.nw.ru) (Russian Federation) maintains very many *Citrullus* accessions collected in southern Africa (McGregor 2012). Turkey is the second largest watermelon producing country after China and has extensive watermelon genetic resources. Due to its central location between Asia, Europe and the Middle East, Turkey holds diverse collections of watermelon germplasm, especially materials from the Southeastern, Aegean, Thrace and Middle Anatolia regions (Sari et al. 2006). In 1993, Cukurova University initiated a program to collect and maintain watermelon genetic resources. About 400 watermelon accessions were collected across most of Turkey. A genetic analysis (Solmaz et al. 2016) indicated that many of these watermelon accessions, including open-pollinated and F_1 hybrid cultivars, share a similar genetic background.

In addition to the previous, a large TILLING (Targeting Induced Local Lesions in Genomes) population of 'Charleston Gray' was developed by researchers at INRA in collaboration with the private company BENCHBIO. The TILLING mutant population was developed using ethyl methanesulfonate (EMS) for chemical mutagenesis under controlled conditions, followed by high-throughput screen-

ing for point mutations (Christelle Troadec, troadec@evry.inra.fr). The TILLING population is a useful tool for functional genomics and is a valuable source for watermelon genetic and genomic research and breeding for desirable mutants.

Conclusions and Future Prospective for Genetic Resources of Watermelon

Modern dessert watermelon cultivars share a narrow genetic base, and are susceptible to a large number of diseases and pests, particularly in tropical and subtropical regions of the world with high rainfall and high humidity. Environmental concerns by regulatory agencies and the general public favor a reduction in the use of chemical pesticides. This emphasizes the importance of identifying and incorporating new sources of disease and pest resistance genes into the cultivated crop. We suggest that utilizing the plethora of genes available within the *Citrullus* germplasm that has been collected (and that which is yet to be collected) provides a means to accomplish this goal. Enhancing genetic diversity and host plant resistance in watermelon cultivars is a priority that will enable the maintenance and improvement of the current levels of production. The USDA/ARS/PGRU maintains a large watermelon germplasm collection. This collection is accessible by breeders and researchers throughout the world. Still, there is an urgent need to collect watermelon germplasm in the wild and conserve and utilize the genetic resources to continue developing robust watermelon breeding lines and cultivars.

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Genetic Resources of Pumpkins and Squash, *Cucurbita* spp.

Harry S. Paris

Abstract Pumpkins and squash, *Cucurbita* species, can be found in fruit and vegetable markets almost everywhere. *Cucurbita* is native to the Americas and was first domesticated there approximately 10,000 years ago. Immediately subsequent to the first European contacts with the Americas, *Cucurbita* was dispersed by people to other continents. Five species of *Cucurbita* have been domesticated, the most widely cultivated of these being *C. pepo*, *C. maxima*, and *C. moschata. Cucurbita* plants are large and develop rapidly, and are primarily grown for consumption of their young or mature fruits, seeds, extraction of oil from the seeds, and ornament. *Cucurbita* contains a wealth of genetic variation in fruit size, shape, color, flavor, and nutritional value. Although many cultivar-groups and market types of *Cucurbita* originated in the Americas, some originated in Europe. Modern breeding and genetic enhancement of *Cucurbita* is focused mainly on increased yield, disease resistance, and improved immature and mature fruit quality. *Cucurbita* genetic resources are maintained in large collections in a dozen countries. Enhancement of fruit flavor and quality has been a long-time primary focus of breeding at publicly funded institutions but further achievements are in danger of being lost due to non-replacement of retiring personnel combined with a lack of organized conservation, description, and deposition of their enhanced-germplasm collections.

Keywords Consumer-oriented breeding • Crop diversity • Disease resistance • Evolution under domestication • Fruit quality • Future of genetic resources • Genetic enhancement • Genetic resource conservation • Genetic resource deposition • Interspecific hybridization • Wild relatives

Introduction

Pumpkins and squash, *Cucurbita* L. species, are familiar to the general public of almost all countries (Ferriol and Pico 2008). They are easily grown in tropical, sub-tropical, warm-temperate and temperate climates, and in cool-temperate

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climates can be successfully grown if provided early-season protection from low temperatures. With a shelf life spanning several weeks or months, mature pumpkin and squash fruits can be offered for sale over an extended period of time and shipped long distances. Moreover, they often have an attractive appearance and people everywhere seem to associate them with warmth, vitality, sexuality, fertility, abundance, and well-being (Norrman and Haarberg 1980; Goldman 2004). As a result of their wide adaptability and popularity, pumpkins and squash are ubiquitous denizens of fruit and vegetable markets the world over. FAO statistics for the category "Pumpkins, squash, and gourds", which encompasses *Cucurbita* as well as other cucurbit genera of less worldwide importance, show current total worldwide annual production as exceeding 24,000,000 tonnes produced on 1,770,000 hectares with a gross production value in excess of $4,000,000,000.

Pumpkin, Squash, Gourd

Pumpkins and squash are edible fruits borne by domesticated plants of the genus *Cucurbita*, of the gourd family, Cucurbitaceae. *Cucurbita* is native to the New World (Gray and Trumbull 1983; Erwin 1931; Bailey 1943; Nee 1990; Lira et al. 1993). There are no Old World records of it prior to 1492 (Whitaker 1947; Chauvet 2004; Paris et al. 2006). The modern English word "pumpkin" is derived from the Greek word *pepon*, meaning a sun-ripened fruit, usually referring specifically to watermelon, *Citrullus lanatus* (Thunb.) Matsum. & Nakai (Andrews 1958; Paris and Janick 2008; Paris 2015). By the early Renaissance, this word had been modified in English and French to *pompion* and *pompon*. With the arrival of other large, round, fleshy, edible fruits from the New World during the sixteenth century, this word was applied to them as well. Soon after, the word "pompion" became modified to "pumpkin" and was no longer inclusive of watermelon fruits, instead only applied to *Cucurbita* fruits. Based on its history as a name applied to a large, round, edible cucurbit fruit, the word "pumpkin" is best applied to large, round, edible fruits of *Cucurbita*. The modern American English word "squash" has a different origin, derived from the Native American word *asq*, which in plural form is *asquash*, meaning immature fruits (Trumbull 1876). Thus, the word "squash", based on its derivation, is best used as both the singular and the plural form (rather than "squashes"). As non-round, long or flat, edible fruits of *Cucurbita* are often used when immature, "squash" is most appropriately applied them. Some cultivated forms of *Cucurbita* produce small fruits which are inedible, being bitter, toughly fibrous, or tend to desiccate readily, and are grown for purposes other than as food. These are referred to as "gourds". The word "gourd" is derived from the Latin word *cucurbita*, and is also used as a generic term for other members of the Cucurbitaceae.

Origin of the Genus *Cucurbita*

Cucurbita is one of approximately 95 genera of the gourd family, Cucurbitaceae (Schaefer and Renner 2011a). The Cucurbitaceae, one of eight families comprising the order Cucurbitales, are thought to have originated during the late Cretaceous Period, approximately 75,000,000 years ago, in Asia (Schaefer et al. 2009; Schaefer and Renner 2011b). Over time, through numerous long-distance dispersal events, various cucurbit lineages became established on other continents.

The Cucurbitaceae are now considered to consist of 15 tribes (Schaefer and Renner 2011b). The genus *Cucurbita* is one of 11 genera in the tribe Cucurbitae, the distribution of which is almost entirely in the Americas. All species of *Cucurbita* are native to the Americas and are diploids with 20 pairs of chromosomes (Whitaker and Davis 1962). This high chromosome number has fostered the idea that *Cucurbita* has an allopolyploid origin (Weiling 1959). The genus *Sicana* Naudin is also a member of the tribe Cucurbitae and also has a diploid chromosome number of 40, and the same may be true for other genera of this tribe. If so, the polyploidy may have been involved with the origin of the tribe Cucurbitae, prior to the origin of the genus *Cucurbita* (Schaefer and Renner 2011b).

Taxonomy and Wild Distribution

There are 12 or perhaps 13 species of *Cucurbita* (Table 1). They differ among one another in pecularities of texture of the foliage and leaf and corolla shape (Duchesne 1786; Bailey 1943; Nee 1990) and are reproductively isolated by genetic barriers to crossing. They also differ in environmental adaptation, for example, some thrive in humid tropical areas, others are well-adapted to temperate habitats, and yet others are xerophytic. Eight or nine of the 12 or 13 species, *C. pepo* L., *C. foetidissima* Kunth, *C. digitata* Gray, *C. radicans* Naudin, *C. argyrosperma* Huber, *C. okeechobeensis* (Small) Bailey, *C. lundelliana* Bailey, and *C. pedatifolia* Bailey, as well as the unconfirmed *C. galeottii* Cogn., are native to North America. All occur naturally in Mexico and four of them, *C. pepo*, *C. foetidissima*, *C. digitata*, and *C. okeechobeensis*, have also been found growing wild in the United States. Two of the remaining species, *C. maxima* Duchesne and *C. ecuadorensis* Cutler & Whitaker, have been found growing wild in South America. Wild plants of *C. moschata* Duchesne and *C. ficifolia* Bouché have not been described. These two species are thought to have originated in South America, in tropical lowlands and Andean valleys, respectively (Andres 1990; Nee 1990; Wessel-Beaver 2000b). The prehistoric wild distribution of some species of *Cucurbita* is thought to be broader than at present (Heiser 1985; Decker-Walters 1990; Petersen and Sidell 1996; Monaghan et al. 2006). Nonetheless, even today, the wild distribution of several species is extensive.

Table 1 The 12 or 13 species of *Cucurbita* and their modern known wild distribution

Species	Wild distribution
C. pepo L, 1753	Southeastern and central U.S.A., northeastern Mexico
C. maxima Duchesne, 1786	Northern and central South America
C. moschata Duchesne, 1786	Not known
C. foetidissima Kunth, 1817	Arid central and western U.S.A., northwestern Mexico
C. ficifolia Bouché, 1837	Not known
C. digitata Gray, 1853	Arid southwestern U.S.A., northwestern Mexico
C. radicans Naudin, 1866	Central Mexican plateau
C. argyrosperma Huber, 1867	Pacific coast of central to southern Mexico, Gulf coast of northeastern Mexico
?*C. galeottii* Cogn, 1881	Oaxaca, Mexico
C. okeechobeensis Bailey, 1930	Florida, Gulf coast and foothills from northern to southern Mexico
C. lundelliana Bailey, 1943	Lowlands of Yucatan, Guatemala, Belize
C. pedatifolia Bailey, 1943	Queretaro, Mexican plateau
C. ecuadorensis Cutler and Whitaker, 1969	Pacific coast of Ecuador

After Nee (1990)

Morphology

Wild plants of *Cucurbita* are rapidly growing, herbaceous, multi-branched, procumbent or climbing vines. The stems are slender and the leaves are arranged on them alternately. The palmate, pentalobate leaf laminae are 12–15 cm long and borne on petioles of approximately the same length (Bailey 1943; Whitaker and Bohn 1950; Nee 1990; Lira and Montes 1994; Robinson and Decker-Walters 1997). The junctures of petioles with the stems, referred to as leaf axils, are the sites of differentiation of tendrils, flower buds, and root primordia. Usually, one flower bud is initiated per leaf axil. The plants are monoecious and predominantly staminate. Staminate flowers are differentiated at the basipetal leaf axils. Pistillate flowers are differentiated at some of the subsequently developed, acropetal leaf axils. Pistillate flowers, however, develop more rapidly, reaching anthesis sooner after their differentiation than do staminate flowers. The corollas are large, usually ranging from 8 to 12 cm, and consist of five, sometimes six or rarely seven partially fused orange-yellow petals. The fruits, borne on slender peduncles 5–10 cm long, are small and round, 3.5–9.0 cm in diameter, green when fresh, with 10 broad dark stripes alternating with 10 narrow light stripes. The narrow, lighter stripes correspond with the positions of the 10 underlying main carpellary vein tracts. The rinds are thin but lignified, and the fruit flesh is thin, fibrous, light-colored or pale, usually bitter, and desiccates after seed maturity, approximately 50 days after anthesis. These desiccated gourds are typically packed with 200–300 seeds, which are small, 8–11 mm long, oval, and 1–2 mm thick.

Pumpkin and squash plants differ widely in appearance from their wild gourd ancestors (Whitaker and Bemis 1964). They have larger leaves and flowers, thicker

stems, and fewer branches. They bear larger and fewer, non-bitter fruits having thicker rinds, with thicker peduncles, and larger seeds. Moreover, the fruits vary in shape and exterior color, and can have color patterns of green, yellow, and orange. The rinds are less durable, often less hard and non-lignified, and the flesh is thicker, less apt to desiccate, less coarsely fibrous, non-bitter, and can be highly colored.

Domestication

The spherical, attractively striped fruits borne by wild *Cucurbita* plants are especially conspicuous during the dry season, and probably attracted early American hunter-gatherers (Nee 1990). The most likely initial use of these gourds by people was consumption of the seeds (Whitaker and Cutler 1965; Merrick 1995; Cowan 1997). The seeds are nutritious and rich in oil (Jarret et al. 2013), and are easily washed free of the surrounding bitter placental tissue of the fruit (Lira and Montes 1994). Consumption of the immature fruits probably came somewhat later, after initial domestication, as wild gourds are usually nauseatingly and poisoningly bitter. The compounds producing bitterness, called cucurbitacins (Rehm et al. 1957; Chambliss and Jones 1966), can be removed from the fruits by repeated boiling and changing of the water. Occasional encounters by native peoples with plants having non-bitter fruits facilitated the consumption of the fruits, not just the seeds, and the encouragement of such plants by people was likely an early step in the domestication of *Cucurbita*. Even to the present, in some parts of Mexico the seeds and the unripe fruits of wild *Cucurbita* are used as food (Lira and Montes 1994). The mature fruit flesh of wild plants, besides being bitter, is thin, fibrous, and desiccates quickly, and therefore the mature fruit flesh probably became a food source only after additional steps in the domestication process. Wild gourd fruits can remain intact for quite some time after the plant has died, essentially becoming seed bags, consisting merely of a rind enclosing seeds (Robinson and Decker-Walters 1997). Wild gourds are also useful as ornaments or as receptacles or utensils, for example as floats for fishing nets or for use of the saponins contained in the fruits for washing. Flowers and young shoots of gourd plants are also consumed in some areas.

Archaeological findings of seeds larger and peduncles and rinds thicker than those of fruits borne by wild gourd plants provide evidence for the domestication of *Cucurbita*. Such artifacts have been found at sites dating to 10,000 years ago in southern Mexico (Whitaker and Cutler 1971; Lira and Montes 1994; Smith 1997), Ecuador (Piperno and Stothert 2003), and Peru (Dillehay et al. 2007). As these sites are widely scattered and located on two continents, they almost certainly indicate that more than one species of *Cucurbita* was domesticated by this early time. At least six independent domestication events have occurred for the genus (Sanjur et al. 2002). Moreover, as some species of *Cucurbita* have an extensive geographical distribution in the wild, it is possible that two or more conspecific wild gourd populations were domesticated by different indigenous peoples inhabiting widely scattered geographical areas, and for different purposes. Indeed, it was long ago

proposed (Carter 1945; Whitaker and Carter 1946) and there is now overwhelming evidence that shows (Decker 1988; Decker-Walters 1990; Nee 1990; Wilson et al. 1992; Lira and Montes 1994; Katzir et al. 2000; Sanjur et al. 2002; Ferriol et al. 2003a; Paris et al. 2003; Gong et al. 2012) that *C. pepo* was domesticated at least twice, independently, in what are now Mexico and the United States. Moreover, local wild populations tend to be fairly isolated from one another, favoring genetic drift, and diversity has been observed among wild populations of *C. pepo* (Decker-Walters et al. 1993; Decker-Walters et al. 2002). Selection by local peoples from allopatric *Cucurbita* populations led to divergent paths of evolution under domestication. Ten-thousand years later, there is an astonishingly diverse pool of genetic resources within the genus and particularly within four of the five domesticated species (Duchesne 1786; Naudin 1856; Zhiteneva 1930; Goldman 2004).

Archaeological remains, thousands of years old, have been found for all five cultivated species (Cutler and Whitaker 1961; Whitaker and Cutler 1965). They show that *Cucurbita* fruits were a major constituent of the diet of many pre-Columbian American peoples. The seeds were highly prized and the young fruits were harvested for use as vegetables. The mature fruits were roasted or boiled, contributing toward a balanced diet. Moreover, the mature fruits could be kept intact for weeks or even months, and cut strips of mature fruits could be dried for even longer storage and light-weight transport.

The five cultivated species of *Cucurbita* have different native ranges and climactic adaptations (Whitaker and Cutler 1965; Fritz 1994; Lira and Montes 1994). They were distributed in cultivation differently, usually allopatrically, throughout all but the coldest parts of the Americas in pre-Columbian times, from North America through to South America, from coastal lowland regions to interior highland regions. Archaeological remains of *C. pepo* and *C. argyrosperma* have been found at sites in North America and *C. moschata*, *C. maxima*, and *C. ficifolia* in South America. *C. pepo* was grown in various parts of Mexico, Guatemala, the Gulf Coast of the United States, the Atlantic seaboard northward to southeastern Canada, westward to the Mississippi Valley and beyond, into southwestern United States and northwestern Mexico (Table 2). *C. argyrosperma* was grown in warmer areas, in southern and eastern Mexico, arriving at a later time in the southwestern United States and northwestern Mexico, and remains dating to the eleventh century have been found in the central United States. *C. moschata* was grown mostly in areas of humid tropical climate, especially lowland northern South America, Central America, and the Caribbean Islands, also spreading northeastward at a relatively late date. *C. ficifolia* was grown in high altitudes from central Mexico through Central America into the Peruvian Andes. In pre-Columbian times, the cultivation of *C. maxima* was limited to temperate and sub-tropical South America.

There are some obvious parallels in the phenotypic variation found within each of the five cultivated species, for example, increased fruit and seed size as compared with conspecific wild plants (Whitaker and Cutler 1965; Bisognin 2002). The more-or-less round-fruited cultigens, those having a nearly 1:1 ratio of length-to-width ratio and thus similar in shape to their wild antecedents, are primarily grown for eating their mature fruit flesh or seeds. The long- and flat-fruited cultigens, though,

Table 2 Distribution and usage of the cultivated species of *Cucurbita*

Species	Pre-Columbian distribution	Modern distribution[a]	Primary usage[b]
C. pepo	North America from southern Canada to Guatemala, perhaps also Caribbean Islands	Widely cultivated from cool temperate to tropical, including semi-arid, worldwide	Immature fruits, mature fruit flesh, seeds, seed oil, ornament
C. maxima	Northern and central South America	Widely cultivated in temperate regions	Mature fruit flesh, seeds, ornament
C. moschata	Southeast U.S.A., Mexico through tropical Central and South America and Caribbean Islands	Widely cultivated in tropical to sub-tropical regions, including semi-arid	Mature fruit flesh, immature fruits
C. argyrosperma	Lowland and semi-arid Mexico and western and central U.S.A.	Mexico	Seeds
C. ficifolia	Mountainous Mexico through Central America to northern Chile and Argentina	Mountainous Mexico through Central America, northern Chile and Argentina, eastern Asia	Mature fruit flesh

[a]There are many exceptions. Gardeners in many regions can obtain seeds of the various species and plant them
[b]There are secondary minor usages such as immature fruits of *C. maxima* in South America and *C. argyrosperma* in Mexico, or rather limited usage of various plant parts, such as young branches and flowers, in some areas

are primarily grown for consumption of the young fruits (Paris 1989; Merrick 1995) because deviation from the ancestral fruit roundness results in an increased proportion of the colored exocarp and the firm mesocarp at the expense of the soft, seedy placental tissue (Sinnott and Durham 1929; Paris 2000). However, the prehistoric directions and intensities of selection were not equal among species, as is readily observable today when modern cultigens of each of the cultivated species are compared. For example, long- and flat-fruited cultigens of *C. pepo* are ubiquitous and primarily grown for consumption of the young fruits, their mature fruits retaining the ancestral lignified rinds, poor mature fruit-flesh quality, and small seeds (Paris 2000, 2008; Paris and Nerson 2003). *C. moschata* is most often grown for consumption of its mature fruit flesh, which tends to be thick, non-fibrous, and richly flavored, and most cultigens have non-lignified fruit rinds, but the seeds are generally smaller than those of *C. argyrosperma* and *C. maxima* (Andres 2004a; Ferriol and Pico 2008). *C. argyrosperma* is most often grown for the consumption of its seeds, which are larger than those of cultigens of *C. moschata* and *C. pepo* but the mature fruit flesh almost invariably has poor quality (Tapley et al. 1937; Culpepper and Moon 1945; Merrick 1995; Ferriol and Pico 2008). Some *C. maxima* cultigens bear fruits which are ranked as having the utmost quality of the fruit flesh and medium-size seeds but others have pale flesh of poor quality and very large seeds which are

often attractively colored orange with a yellow margin (Tapley et al. 1937; Culpepper and Moon 1945; Ferriol et al. 2004a). *C. ficifolia* has little variation in fruit size, shape, and color. Its fruits can keep for a year or more but invariably have coarse, insipid, white flesh (Andres 1990; Andres 2006).

Cucurbita ecuadorensis was the last species of the genus to be discovered (Cutler and Whitaker 1969) and is found only wild today. However, its fruits are generally larger than those of other wild species, more variable in their coloration, and often non-bitter and non-lignified, leading to the idea that this species was long ago domesticated and then abandoned (Andres and Robinson 2002).

Diversity and Relationships Within Species

Rather modest phenotypic variation has been observed among wild plants of each of the species of *Cucurbita*. Some variants have been reported for corolla color, fruit shape and exterior color, and non-bitter fruit flesh. Interestingly, the earliest-known illustration of *Cucurbita* in Europe depicts what is considered to be an offspring of a wild *C. pepo* subsp. *texana* gourd, but its fruits are atypical of those of wild plants, being both pyriform and light-colored (Paris et al. 2006).

In comparison to the wild plants, though, the phenotypic variation among the cultivated plants within each of four of the five domesticated species is astounding. Not surprisingly, the most striking variation occurs for the fruits, which have been the focus of attention probably since the first human encounters with *Cucurbita*. Independently derived local selections, conducted over a wide geographical area and varying climactic situations, led to the enormous within-species diversity observed today among the fruits of modern pumpkins and squash. Descriptions in delightful prose escorted by professional-grade, high-resolution color photographs of fruits from 150 cultivars of *Cucurbita* were presented in a recent book, *The Compleat Squash* (Goldman 2004).

Cucurbita pepo

This species has long been considered as one of the most variable for fruit characteristics in the plant kingdom (Duchesne 1786; Naudin 1856). A recent investigation focusing on among- and within-species variation, employing simple sequence repeats (SSRs), has shown that *C. pepo* indeed contains by far the greatest genetic variation of the species in the genus *Cucurbita* (Gong et al. 2013). Fruits can weigh 25 kg. and more, can range from round to extremely long to flat, with longitudinal ribs, ridges, furrows, or grooves. Fruit colors include green, orange, and yellow, which can range in darkness from almost black to almost white and in intensity from vivid to pale, and color patterns include longitudinal striping in a range of

broadness and contiguousness, and mottling, as well as the latitudinal bicolor pattern, such that a single fruit can have four colors on its surface. Interior color can range from greenish white through intense yellow or orange. Seeds range from 8 to 25 mm in length and range from 1.5 to 2.5 times long as wide, but the color is invariably beige (Paris 2001; Paris et al. 2012).

Cucurbita pepo has three widely recognized subspecies founded on observed molecular genetic polymorphisms (Wilson et al. 1992; Paris et al. 2003; Gong et al. 2012). *C. pepo* subsp. *fraterna* (Bailey) Lira, Andres & Nee is known from wild plants in seasonally dry northeastern Mexico (Andres 1987) but does not seem to have any cultivated descendants. Subspecies *pepo*, on the other hand, has not been discovered in the wild, but encompasses most of the cultivated germplasm. Subspecies *texana* (Scheele) Filov, also known as subsp. *ovifera* (L.) Decker, grows wild in the southeastern and central United States and encompasses much of the remaining cultivated germplasm. Teppner (2000) has recognized a fourth subspecies, *gumala*, which he considered to be closely related to ancestral subsp. *pepo*. Subsp. *gumala* is typified by the strongly ribbed pumpkins cultivated in Guatemala and Mexico (Lira and Montes 1994). Indeed, Gong et al. (2012) have confirmed that the cultigens matching Teppner's description of *gumala* have a central position within subsp. *pepo*. Interestingly, some cultivated gourds, such as 'Miniature Ball', have an intermediate position between subsp. *pepo* and subsp. *texana* (Gong et al. 2012), suggesting that they are relicts that are ancestral to *C. pepo* as a whole. Their fruits are the smallest of the species, round and broadly striped, with small seeds and highly branched foliage consisting of thin stems and small leaves. Searches in central and southern Mexico for wild ancestors of *C. pepo* and *C. pepo* subsp. *pepo* have not, so far, been fruitful but finding wild populations of *Cucurbita* is very much dependent on the correct timing for searching, which can vary from year-to-year due to climactic fluctuations (Andres 2000).

For millennia, subsp. *pepo* and subsp. *texana* were subjected to selection by people, separately and hundreds or even thousands of kilometers apart, in what are now Mexico and the United States, respectively. Some of the traits that were acquired under cultivation are the same in the two subspecies, and others are different (Paris et al. 2012). Both have increased leaf, flower, fruit and seed size as compared to wild plants. Many cultigens of both subspecies share bush growth habit, which facilitates multiple harvesting of immature fruits, and the same gene, *Bu*, confers this trait in the two subspecies (Paris and Edelstein 2001). However, the two subspecies differ markedly in a number of characteristics possessed by their edible-fruited cultigens. The fruits are larger and the seeds are longer in subsp. *pepo* than in subsp. *texana*, and some alleles, such as those affecting stem color, leaf mottling, and multiple flower-bud initiation in leaf axils, are nearly fixed in one of the subspecies but rare in the other (Paris et al. 2012). The fruits of the two subspecies markedly differ in their topography. In subsp. *pepo*, the fruit surfaces over and adjacent to the 10 longitudinal, subsurface primary carpellary vein tracts protrude in the form of 10 rounded ribs whilst in subsp. *texana* they are depressed in the form of ten angular furrows (Paris et al. 2007).

Fruit shape has served as a basis for intraspecific classification in *Cucurbita pepo* (Paris 1986). Accordingly, eight cultivar-groups (morphotypes) are easily distinguished from one another by their fruit shapes (Fig. 1), four in each of the two subspecies. The fruit shape of six of these groups, three in each of the two subspecies, deviates markedly from the ancestral 1:1 length-to-width ratio. These six cultivar-groups are grown mostly for the consumption of the young fruits. Two of the cultivar-groups, one in each subspecies, do not deviate markedly from the ancestral 1:1 ratio and are grown mostly for use of their mature fruits as food. Three additional groups, gourds grown for autumn decoration, are also recognized (Paris 2000, 2007). These 11 cultivar-groups are each comprised of a host of cultivars, some of which form distinct market types within these groups. Even though subsp. *pepo* has been under cultivation much longer than subsp. *texana*, archaeological evidence (Cowan 1997) as well as SSR polymorphisms (Gong et al. 2012) indicate that the differentiation into distinct fruit shapes occurred over 2000 years earlier in subsp. *texana*. Indeed, the literary evidence is consistent, showing well-differentiated cultivar-groups of subsp. *texana* in sixteenth-century herbals (Paris 2000). Native Mexican and Guatemalan cultigens are almost invariably pumpkins, ranging from oblate to globose, spherical, oval, and short oblong (Zhiteneva 1930; Merrick 1986). Fruit elongation from the pumpkins to the vegetable marrows and cocozelles, and later to the zucchinis, occurred in Europe and Asia Minor, and thus is quite recent (Paris 2000; Gong et al. 2012; Lust et al. 2016).

Cucurbita moschata

This species is probably the next-most variable of the genus (Andres 2004a; Ferriol and Pico 2008; Gong et al. 2013). The variation in this tropical species has, however, been generally under-appreciated, because of its day-length sensitivity, sensitivity to cold, late maturity, and the particularly large size of its plants, all of which contribute to the difficulty in raising a large number of accessions simultaneously in the same field, for phenotypic comparisons and characterization. Although local or regional market types have been designated and described by some authors, there is no cultivar-group treatment for *C. moschata* that adequately encompasses its worldwide genetic variation. Until comprehensive, comparative research of the variation in *C. moschata* is undertaken, probably the most useful tentative intraspecific classification is that presented by Goldman (2004), who recognized four cultivar-groups, Cheese Pumpkin, Japonica, Tropical and Neck (Table 3), and supported them with descriptions and photographs. DNA-sequence polymorphisms have been used in several studies of local or regional *C. moschata* germplasm (Gwanama et al. 2000; Ferriol et al. 2004b; Wu et al. 2011). There are no reports of undertaking the considerably larger task of comparing polymorphisms of a worldwide germplasm representation. The likely center of diversity for *C. moschata* is in lowland Colombia (Wessel-Beaver 2000b). There, some landraces have extremely small (5 mm) dark seeds, and some of the local cultigens have primitive-looking plants, with small

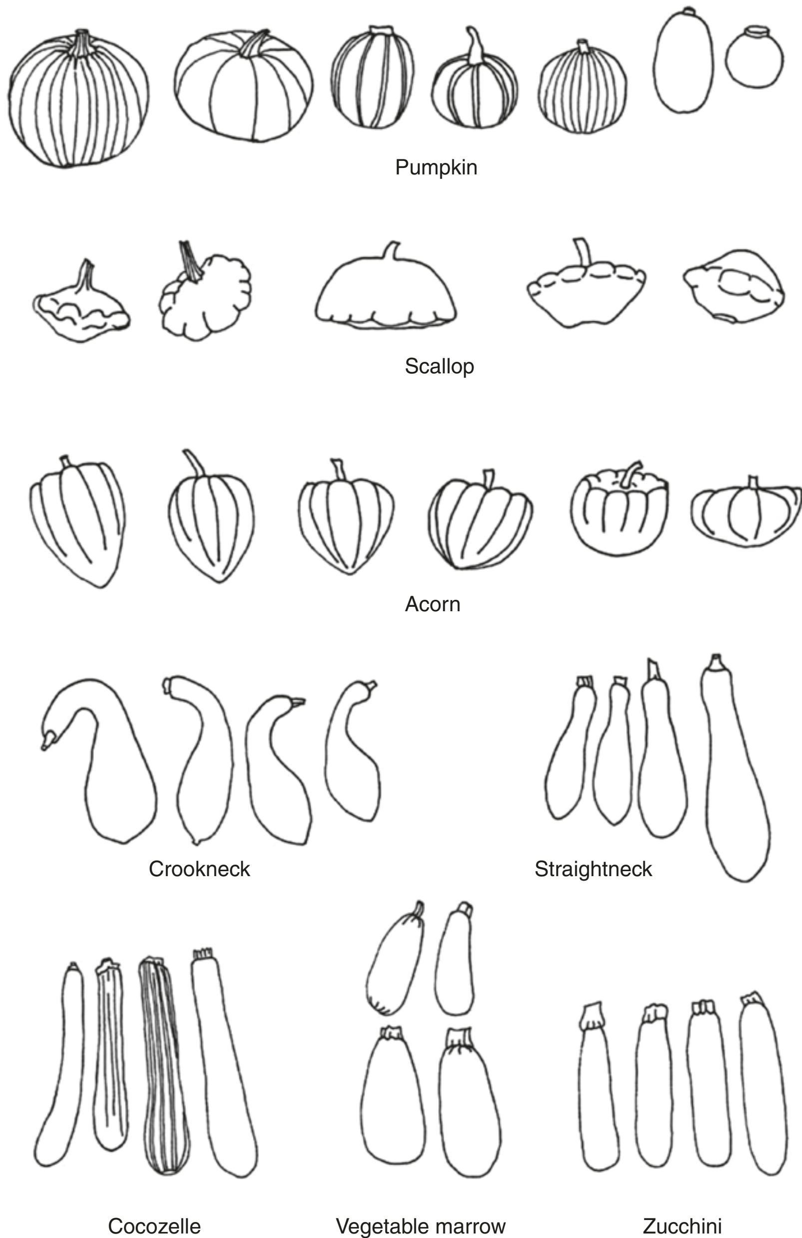

Fig. 1 Schematic representations of fruits of the eight edible-fruited cultivar-groups of *Cucurbita pepo*. The Pumpkin, Cocozelle, Vegetable Marrow, and Zucchini Groups are of subsp. *pepo* and the Scallop, Acorn, Crookneck, and Straightneck Groups are of subsp. *texana* (After Paris et al. 2007, 2012. Diagram by the author. Also on-line at: http://www.google.co.il/url?sa=t&rct=j&q=&esrc=s&frm=1&source=web&cd=4&ved=0CEEQFjAD&url=http%3A%2F%2Fwww.reginfo.gov%2Fpublic%2Fdo%2FDownloadDocument%3FdocumentID%3D122305%26version%3D1&ei=rqtnUqvBJMrJ4gSu5oBg&usg=AFQjCNEW7KFtk9NAvmd5FwU0M_du5To0CQ)

Table 3 Summary of four tentative cultivar-groups for *Cucurbita moschata*

Cultivar-group	Standard cultivar	Description
Cheese Pumpkin	Cutchogue Flat Cheese	Oblate, moderately to deeply furrowed
Japonica	Chirimen	Oblate, sometimes dumbbell, furrowed
Tropical	Seminole	Variously shaped but mostly round, furrowed
Neck	Canada Crookneck	Elongate with bulbous stylar end, non- to slightly furrowed

After Goldman (2004)

Fig. 2 Whole and equatorially cut mature fruits of the furrowed, high-quality pumpkin of *Cucurbita moschata* 'Musquée de Provence' (ruler, at bottom, is 30 cm long) (Photograph by the author)

Fig. 3 Young, extremely long fruits of *Cucurbita moschata* at market, Ventimiglia, Italy, in July 2009 (Photograph by the author)

leaves and very long internodes, bearing small (<500 g) fruits that have lignified rinds. Much larger fruits with horticulturally desireable traits, such as non-lignified rinds and thick, intense orange flesh, are found in Colombia as well. Elsewhere, *C. moschata* fruits are usually globular to oblate, typically smooth or furrowed, have thick, non-bitter, non-fibrous, well-colored flesh, relatively small seeds, and non-lignified rinds, sure indicators that this species has been cultivated primarily for consumption of its mature fruit flesh (Fig. 2). Fruits weighing over 100 kg. have been reported in cultigens from the Middle East and northern Africa. Although fruit shape is most often oblate or otherwise round, it can range to bell-shaped to extremely long with a large bulging end (Fig. 3). Mature fruit exterior color is usually not vibrant, most often being buff, sometimes yellow or dark green, with a waxy bloom, but the flesh color is often intense orange. The fruit flesh has a characteristically strong odor, hence the species name (Duchesne 1786; Paris 2007). The

seeds are of modest size, generally small in relation to the size of the fruit and in comparison with those of other cultivated species. They are usually light brown. Also, unlike other cultivated species of *Cucurbita*, the overwhelming majority of *C. moschata* cultivars have non-lignified fruit rinds, which allow easy slicing of the mature fruits, suggesting that this species has been cultivated for a very long time mostly for the consumption of its mature fruit flesh (Schaffer et al. 1986a; Merrick 1995). There are, though, some long-fruited cultivars in Italy and South Korea that are grown for consumption of their young fruits (Fig. 3).

Cucurbita maxima

Also highly variable, this species was restricted to South America prior to the arrival of Europeans (Cutler and Whitaker 1961; Merrick 1995; Ferriol and Pico 2008). Sailors must have taken fruits or seeds with them from South America to Europe early in the sixteenth century. Large, *C. maxima* show pumpkins (Table 4) of three colors were illustrated in festoons, painted between 1515 and 1518, of the fabulous Villa Farnesina, Rome, Italy (Janick and Paris 2006). The fruits of some show-pumpkin cultivars of *C. maxima* are the largest fruits in the world, some weighing over 600 kg. Besides these show pumpkins, *C. maxima* contains many cultivars which have peculiar turban-like or banana-like shapes, as well as cultivars bearing fruits that are drum-shaped, top-shaped, fusiform, or oblate. Fruit exterior color has a nice range from red-orange to orange to pink-orange, nearly white, bluish gray, intense green, and black-green (Fig. 4). Turban gourds are often bi- or tri-colored. Flesh color can be intense orange-yellow, and this species has a number of cultivars, most of them drum-shaped and dark green or black-green, that are highly esteemed for their quality. In Argentina, some flat-fruited cultivars having bush growth habit are grown for consumption of their young fruits, known locally as *zapallitos*. Other than for local or regional market types, no comprehensive, molecular-genetic investigation of polymorphism in *C. maxima* has been conducted and no cultivar-group treatment of worldwide phenotypic variation, supported with adequate descriptions

Table 4 Summary of eight tentative cultivar-groups for *Cucurbita maxima*

Cultivar-group	Synonym	Standard cultivar	Description
Show Pumpkin	Mammoth	Atlantic Giant	Round though often lopsided
Hubbard		Green Hubbard	Short fusiform
Banana		Banana	Elongate fusiform
Buttercup	Kabocha	Buttercup	Drum-shaped
Delicious		Delicious	Turbinate
Queensland	Australian Blue	Queensland Blue	Oblate
Zapallo			Flat
Turban		Turk's Turban	Bulging stylar end, rest of the fruit flat

After Goldman (2004)

Fig. 4 Mature fruits of various cultigens of *Cucurbita maxima*, with two yellow-striped fruits of *C. argyrosperma* 'Gold Striped Cushaw' (Photograph by the author)

or photographs, has been proposed. As in *C. moschata*, DNA-sequence polymorphisms have been compared in several studies of local or regional *C. maxima* germplasm (Ferriol et al. 2003b, 2004a), but none have attempted the considerably larger task of comparing polymorphisms of a worldwide germplasm representation. Goldman (2004) presented a tentative cultivar-grouping backed by descriptions and beautiful color illustrations, which is modified slightly herein (Table 4). The seeds are usually white, orange in some cultivars, and tend to be rather large, and some cultivars are grown for seed consumption.

Cucurbita argyrosperma

This species, sometimes referred to as the silverseed gourd, also is characterized by a high degree of variation but less than *C. moschata* (Merrick and Bates 1989; Merrick 1990; Ferriol and Pico 2008). Fruit size does not attain the extremes found in *C. maxima* or even *C. moschata* and shape varies from long with bulbing at the stylar end to oblate. Exterior color is not as dull as in *C. moschata* but not as vivid as in *C. pepo* and *C. maxima*, in shades of green, orange, and yellow (Fig. 4). Flesh ranges in color from pale to fairly intense yellow or orange to black-green, the last resulting from the presence of chlorophyll. Regardless of color, the quality of the flesh is usually poor (Culpepper and Moon 1945). The greatest variation in this species is found in its seeds, which can be as long as 30 mm and have a range of colors and color combinations, such as a silver aspect to the seed margin (Merrick and Bates 1989). This species is mostly grown in Mexico, for the consumption of its seeds, and is considered to be comprised of two subspecies, *sororia* and *argyrosperma* (Merrick and Bates 1989; Merrick 1995). Subsp. *sororia*, which encompasses only wild plants, has slender peduncles, small, round fruits averaging 8 cm in diameter that are green with narrow longitudinal irregular stripes or blotches of cream, with thin, fibrous, pale-colored, bitter flesh, and the seeds are light brown, oval, 9–10 mm long. Subsp. *argyrosperma* encompasses the feral var. *palmeri* and several

Table 5 Infraspecific classification of *Cucurbita argyrosperma*, botanical varieties

Subspecies	Cultivar-group	Fruit shape
sororia	(Wild)	Round
argyrosperma var. *palmeri*	(Feral)	Round
argyrosperma	Silverseed	Round, also pyriform and bulbous cylindrical
argyrosperma	Stenosperma	Round to pyriform
argyrosperma	Callicarpa	Round, pyriform, elongate

The credits to Merrick and Bates (1989) and Jeffrey (2001)

cultivar-groups (Table 5). Var. *palmeri* has slightly thickened peduncles and round fruits averaging 12 cm in diameter, green with narrow irregular stripes or blotches of cream, the flesh varying in thickness, fibrousness, and bitterness. The seeds are light brown and 13 mm long. The Silverseed or Argyrosperma Group has moderately thickened peduncles and fairly large fruits, about 20 cm in diameter, smooth, round to pyriform, to bulbous cylindrical sometimes achieving a length of 50 cm or more, cream or striped or netted cream and green, the flesh is rather fibrous and thin, pale yellow or orange. Seeds are elliptical, averaging 24 mm long and 12 mm wide. The Stenosperma Group is distinguished by having markedly thickened peduncles, smooth, oval to pyriform fruits 13–28 cm long, cream or striped or netted cream and green, flesh rather fibrous and thin, pale yellow or orange with green tinting increasing from the outside to the black-green placenta, with large, narrow seeds averaging 24 mm long and 8 mm wide. Both of these groups are grown mainly for consumption of the seeds. The Callicarpa Group is distinguished by having a markedly thickened peduncle, fruit shape varying more than in the other groups, from flattened globe to pyriform to elongate with a bulbous stylar end, fruits ranging in length to 50 cm or more, the surface can be smooth or with five shallow longitudinal furrows or five corky ridges or irregular corky tissue or warts toward the peduncle end, fruit colors like those of the other two groups ranging to completely dark green and sometimes with irregular stripes of intense orange or yellow (Fig. 4). The fruit flesh also is variable in color, thickness, and fibrousness and the seeds are long and narrow, varying in color and smoothness of the surface. This group is grown for consumption of its seeds and fruits.

Cucurbita ficifolia

The fig-leaf gourd has markedly less phenotypic variation than the other cultivated species of the genus (Andres 1990, 2006). It has long been under cultivation in the Americas, from northern Mexico to central Chile and northern Argentina. It is usually grown in communities lying 1000–4000 m above sea level, from 30 N to 30 S latitude and its greatest diversity is found from Peru to Colombia. The plants are rampant growers and often planted on marginal agricultural land, and require a long

growing season to produce and mature their fruits. They can be found in low-latitude, highland habitats ranging from cloud forests to arid. The plants are quite cool tolerant but are killed by frost. Its fruits are of modest size and almost invariably round, with an exterior of green and/or white, the amount of green versus white varying throughout the cultivated range in the Americas. Larger fruits are found in moist areas, where they can weigh as much as 20 kg, but it is not clear what proportion of the variation in size is genetic and what proportion is environmental. The flesh is white with thick fibrous strands, and bland. The fruits have lignified rinds and a particularly long shelf life. Seeds are usually black, occasionally tan. In the Americas, this species is grown for its fruit flesh, which is made into confections and jams or is cooked. Elsewhere, it is grown as a rootstock for other cucurbits, or for use of the fruits as animal fodder or as ornaments.

Diversity and Relationships Among Species

The genus *Cucurbita* has a natural distribution from central North America to central South America, with the greatest diversity in Mexico (Andres 2000; Ferriol and Pico 2008). Wild *Cucurbita* plants have been found in a wide range of habitats, from high-rainfall areas to deserts, from tropical to temperate climatic regions, from lowlands to mountain valleys. The genus was once thought to consist of as many as 27 species (Whitaker 1974; Whitaker and Bemis 1975). However, attempts at crossing among these 27 revealed a number of combinations that gave fully fertile progeny, indicating that the number of biological species is only 12 or 13 (Nee 1990). Wild *Cucurbita* populations that were named as species were subsequently reduced to subspecific status when it was found that they were fully cross-compatible with cultivated *C. pepo*, *C. maxima*, or *C. argyrosperma*. Moreover, *C. okeechobeensis* and plants formerly named *C. martinezii* produce fully fertile progeny; they are, therefore, the same species, the latter properly being referred to as *C. okeechobeensis* subsp. *martinezii* (Bailey) Walters & Decker-Walters (Robinson and Puchalski 1980; Andres and Nabhan 1998; Walters and Decker-Walters 1993). Wild *Cucurbita* plants named *C. californica* Wats., *C. palmata* Wats., *C. cordata* Wats., and *C. cylindrata* Bailey are probably ecotypes conspecific of *C. digitata* (Nee 1990).

Prior to the advent of molecular-genetic techniques, putative relationships among species in the genus *Cucurbita* were based on plant morphology, natural habitat, and degree of crossability. Species were defined and designated by differences in shape of the leaf laminae, foliar trichomes, flowers, fruits, seeds, and locality of collection (Erwin and Haber 1929; Bailey 1943; Whitaker and Davis 1962; Křistkova et al. 2004). Some species were found in desert habitats, others in seasonally dry areas, others in humid tropics, and others in highland or temperate regions (Whitaker and Bemis 1964; Whitaker and Cutler 1965). There were many recorded attempts at interspecific crossing in *Cucurbita*, especially between wild and domesticated species (Whitaker and Davis 1962; Whitaker and Robinson 1986; Lebeda et al. 2007). One motivation of such attempts was to introgress disease resistance into cultigens.

Another motivation was oriented to assessing genetic relationships among the species. Results of attempts at interspecific crossing, through 1960, were exhaustively reviewed by Whitaker and Davis (1962). Later reports have been summarized in reviews by Lebeda et al. (2007) and Ferriol and Pico (2008). Results of the very many reported interspecific crossing attempts in *Cucurbita* have shown that the 12 widely recognized biological species are incompletely isolated from one another. The percentage of fruit set in *Cucurbita* from intraspecific hand pollinations is quite high, often exceeding 90 % (Loy 2012), but is markedly lower in interspecific pollinations. Although barriers to gene exchange among species often appear robust, a number of artificial interspecific pollinations have produced some fertile progeny. Moreover, even in the open field, some species have been observed to have partial compatibility with others (Wilson et al. 1994; Wessel-Beaver 2000c).

The strongest, most readily apparent differences in plant morphology, natural habitat, and cross-compatibility in *Cucurbita* are between the mesophytic and xerophytic species (Whitaker and Davis 1962; Bemis and Whitaker 1969; Robinson and Decker-Walters 1997). Most of the species of *Cucurbita* are mesophytic annuals well-adapted to moist habitats, have fibrous roots, and reproduce by seed. Others are xerophytic perennials having thick storage roots that enable the plants to survive long rainless periods, reproducing themselves by seed as well as vegetatively. Attempts at crossing between mesophytic and xerophytic species almost invariably met with complete failure to produce viable progeny. Interestingly, some of the mesophytes are from seasonally dry areas and contain accessions that are well-adapted to semi-arid climates (Andres 2000; Lira et al. 2009). Wild *C. pepo* (Andres 1987) and wild and cultivated *C. argyrosperma* (Merrick 1995) occur in semi-arid parts of Mexico and cultivars of *C. moschata* are widely grown across semi-arid to arid northern Africa into the Middle East (Ibrahim et al. 1996; Alsadon et al. 1998; Andres 2004b). The xerophytes, therefore, are probably specialized derivatives of ancestral mesophytes (Whitaker and Bemis 1964; Bemis and Whitaker 1969; Whitaker 1974; Merrick 1995).

Another obvious contrast within the genus *Cucurbita* is exhibited by *C. ficifolia*, which differs starkly from the other cultivated species in its morphology and adaptation to high-altitude, cool habitats. Based on its lack of crossability with other *Cucurbita* species, *C. ficifolia* was considered by Whitaker and Bemis (1964) to be the most isolated among the domesticates.

On the other hand, there are among the dozen *Cucurbita* species numerous cases of overlapping traits and adaptations, and some degree of crossability. Two of the cultivated species, *C. arygrosperma* and *C. moschata*, share many features and to such an extent that they were not recognized as separate species until the Russian researcher, K.I. Pangalo, distinguished between them in 1930 (Pangalo 1930; Cutler and Whitaker 1956). They have similar foliar characteristics but differ in various reproductive traits. Both species thrive in warm, humid areas. Early attempts at crossing these two species met with infertility but, overall, were more successful than for other interspecific combinations. More recent crossing attempts produced fertile progeny, mostly when *C. argyrosperma* was used as the female parent (Wessel-Beaver et al. 2004; Ortiz-Alamillo et al. 2007). Interestingly, when two

temperate climate-adapted cultivars, one from each species, were crossed, seeds and fertile F_1 plants were obtained when *C. moschata* was used as the female parent (Connolly 2007). Even under some conditions in the field, these two species have been observed to cross spontaneously (Wessel-Beaver 2000c; Cuevas-Marrero and Wessel-Beaver 2008). There is also evidence of introgression from *C. argyrosperma* into some cultigens of *C. moschata* (Decker-Walters et al. 1990). Of the cultivated species, *C. argyrosperma* and *C. moschata* are clearly the most closely related (Whitaker and Davis 1962; Merrick 1990).

The more successful attempts at interspecific crossing in *Cucurbita* often had *C. lundelliana* as a parent. Among the cultivated species, attempts involving *C. moschata* were generally more successful than those that did not. These observations led T. W. Whitaker and his colleagues to consider *C. lundelliana* as the most centrally placed species of the genus and *C. moschata* as the hub of the cultivated species (Whitaker 1956; Whitaker and Davis 1962; Whitaker and Bemis 1964; Whitaker and Robinson 1986).

Molecular-genetic techniques that have been developed over the past quarter century have been used in efforts to obtain more accurate assessments of genetic relationships within the genus *Cucurbita* (Lebeda et al. 2007; Esteras et al. 2012b). Polymorphisms observed at allozyme loci were studied initially and were soon superseded by polymorphisms observed using various DNA marker systems. Overall, the results obtained through the application of these new techniques have been consistent with the results obtained by comparing morphology, habitat, and interspecific crossability, but have brought them into sharper focus.

Polymorphisms of chloroplast DNA (Wilson et al. 1992; Zheng et al. 2013), mitochondrial DNA (Sanjur et al. 2002), and nuclear DNA (Gong et al. 2013) have confirmed that *Cucurbita foetidissima* and other xerophytic species are distant and outlying to the mesophytes. *C. argyrosperma* and *C. moschata* are closely related, as are *C. ecuadorensis* and *C. maxima,* and the wild species *C. lundelliana* and *C. okeechobeensis*. However, *C. okeechobeensis*, not *C. lundelliana*, is the most central species of the genus (Gong et al. 2013), and *C. pepo* was observed to be relatively outlying by both Wilson et al. (1992) and Gong et al. (2013). Zheng et al. (2013) observed an unexpectedly close relationship between *C. ficifolia* and the xerophytic *C. foetidissima* and *C. pedatifolia*.

Considering the Entire Genus as One Gene Pool

The majority of attempts at interspecific crossing have been met with incompatibility, the extent and expression of which can vary greatly (Whitaker and Davis 1962; Lebeda et al. 2007; Loy 2012). Often, female flowers that receive pollen from other species fail to develop into fruits. Fruits that do develop often lack seeds completely, or have incompletely developed seeds, or fully developed seed coats containing incompletely developed seed embryos. Lebeda et al. (2007) have reviewed the many reports of embryo-rescue techniques that have been applied to allow the

development of interspecific hybrid plants from incompletely developed embryos, but often such plants are feeble or sterile. Sometimes, a few seeds develop completely and can be planted in soil directly. The resulting plants often develop abnormally and are sterile, but sometimes can be sparingly or largely fertile. In a few cases, interspecific hybrid plants are apparently normal and fertile, with abnormalities or decreased fertility becoming apparent in their progeny.

To overcome problems of interspecific incompatibility, three approaches have been implemented. One, the use of heterozygous parents to achieve gametic diversity, was proposed by Wall and York (1960), who used an F_1 hybrid of two *C. pepo* subsp. *texana* Straightneck Group cultivars as one parent and the F_1 hybrid of *C. moschata* 'Butternut' and 'Golden Cushaw' as the other. Another approach has been the use of genetic bridges, such as a genetically intermediate species or subspecies (Rhodes 1959; Whitaker and Robinson 1986; Munger 1990). A third has been the use of accessions within each of the parental species that have previously been identified as imparting greater success in interspecific crossing (Whitaker and Davis 1962; Whitaker and Robinson 1986; Lebeda et al. 2007; Ferriol and Pico 2008). In combination, these approaches can greatly increase the chance of overcoming interspecific incompatibility.

Apparently, there is more than one genetic barrier to interspecific hybridization because there are greatly varying degrees of interspecific incompatibility. This incompatibility can range from failure of fruit set to near complete compatibility, expressed as fertility of the F_1 progeny. The increased success encountered using gametic diversity, genetic bridges, and particular accessions reinforce this view and further suggest that there are a number of unidentified genes that affect the degree and expression of interspecific-cross incompatibility. Gong et al. (2013), in their investigation of SSR polymorphisms among 88 accessions from nine *Cucurbita* species, noticed that particular accessions of one species had unusually low genetic distances from particular accessions of other species. These observations raise the possibility that the success of particular interspecific cross combinations could be predicted by genetic distance values obtained using SSR polymorphisms.

There have been significant efforts to introgress horticulturally valuable traits from one species of *Cucurbita* into another. The bush growth habit of *C. pepo* was introgressed into *C. moschata* (Munger 1959). Most of these attempts, though, have been directed towards disease and pest resistance, especially introgressing resistance to viruses from *C. moschata* and wild species into *C. pepo* (Whitaker and Robinson 1986). Some commercially available hybrids of *C. pepo* are resistant to one or more viruses. However, descendants of interspecific hybrids, even if fully fertile, can have subtle genomic problems. *C. pepo* plants homozygous for the powdery mildew resistance derived from *C. okeechobeensis* have reduced fruit length (Paris and Cohen 2002) and are low yielding (McGrath and Staniszewska 1996). *C. pepo* hybrids carrying genes for resistance to viruses from *C. moschata* and resistance to powdery mildew from *C. okeechobeensis* were observed to be unexpectedly closely related to one another (Formisano et al. 2010), even though they were bred over many years, by different companies and using different elite breeding lines as recurrent parents for backcrossing disease resistance. This unexpectedly

close relationship, despite the high chromosome number of *Cucurbita* and after so many generations of crossing, is a phenomenon referred to as quasi-linkage or genetic drag (Miké 1977; Peng et al. 2000). The causal mechanism is obscure as, compared with the other major cucurbit crops, knowledge of the genomics of pumpkins and squash is in its infancy (Lebeda et al. 2007; Blanca et al. 2015).

Scope and Implications of Genetic Resources of *Cucurbita*

Distribution and Usage of Cucurbita

Pumpkins and squash are primarily food crops (Ferriol and Pico 2008; Paris 2008). The young fruits, mature fruit flesh, seeds, and oil extracted from the seeds are consumed by people. Flowers and even young shoots are consumed in some areas. Often, too, pumpkins and squash are grown for use of their fruits as ornaments. Sometimes they are grown for animal fodder or as a fast-growing cover for unsightly landscapes, or for other purposes.

Pumpkins and squash are found in countries on all continents, and are grown in all areas of suitable climate. They are raised in home gardens and fields, under extensive and intensive conditions, and are conventionally and organically produced. The immature fruits are a popular vegetable in the developed countries, and quality crops fetch a high price at the markets. The economic value of the genus derives mostly from these immature fruits, especially those of *Cucurbita pepo* subsp. *pepo* Zucchini Group (Paris 2008). Flowers, staminate and pistillate, have specialty markets in Italy and elsewhere. Flowers of subsp. *pepo* are larger and meatier than those of subsp. *texana*, and some cultivars of the Cocozelle Group have flowers that excel in these traits (Umiel et al. 2007). The mature fruits, especially of *C. moschata*, are a nutritious, low-priced, staple crop in the economically less-developed countries. The importance of pumpkins to the diet of people living in these countries is inadequately measured by monetary value. Mature pumpkins of *C. pepo* and *C. maxima* have considerable monetary importance, as esculents and ornamentals, in the United States, Europe, and Australia. Mature hull-less-seed *C. pepo* pumpkins, for extraction of oil in the seeds, are economically important in central Europe. Production of ornamental pumpkins and gourds, especially of *C. pepo* and *C. maxima* for Halloween and Oktoberfest activities, is a large, growing industry.

Flowering and Reproduction

Cucurbita plants are monoecious and self-compatible. Each of the staminate and pistillate flowers opens once, at the break of dawn and withers by noon, in the interval being frequented by bees. Under field conditions, some of the pollen transferred to the pistillate flowers by the bees is derived from staminate flowers on the same plant and

some is derived from other plants, that is, both self- and cross-pollination occur naturally. Inbreeding depression is usually not encountered or unappreciable (Bushnell 1922; Erwin and Haber 1929; Scott 1934; Whitaker 1962), though there are exceptions (Loy 2012), most notably of wild *C. pepo* subsp. *texana* (Hayes et al. 2005). On the other hand, heterosis is quite common in *Cucurbita*, especially in *C. pepo*.

Heterosis for fruit production was first observed by Curtis (1939) in a cross between two straightneck cultivars of *Cucurbita pepo*. He recommended that F_1 hybrids of squash be commercially deployed. Implementation occurred first in other *C. pepo* summer squash, especially zucchini. Over time, hybrid pumpkin and winter squash cultivars of *C. pepo*, *C. maxima*, and *C. moschata* were developed and commercialized. Presently, almost all cultivars of *C. pepo* and most of *C. maxima* and *C. moschata* sold by large seed companies are hybrids. In almost all cases, the hybrids outproduce their open-pollinated parents and breeders have worked intensively to improve the quality of their fruits so as to equal or exceed that of the available open-pollinated cultivars.

Environmental Effects on Production of Pumpkins and Squash

Production of pumpkins and squash is affected by a number of environmental factors. Generally, plants of *Cucurbita* thrive at warm temperatures, 25–30 °C, but, clearly, *C. pepo*, *C. maxima*, and *C. ficifolia* are more tolerant of lower temperatures and *C. moschata* and *C. argyrosperma* are more tolerant of higher temperatures. Also, the ability of seeds to germinate and seedlings to develop rapidly at suboptimal temperatures is important for early-season crops in most regions where pumpkins and squash are grown. *Cucurbita* germplasm possesses much variability for the ability to flower and produce fruits in a timely fashion regardless of daylength and in spite of less-than-optimal soil and weather conditions. Adequate soil moisture is important for plant maintenance and optimal fruit production, especially for long-season crops of summer squash. Excessively high temperatures as well as low light intensity often result in increased incidence of pre-anthesis female flower senescence and abortion of newly set fruits (Wien et al. 2002).

Production of Immature Versus Mature Fruits

There is a major conflict in attempting to best exploit the genetic potential of the genus because productivity and quality components of mature fruits are not the same as for immature fruits (Ferriol and Pico 2008; Paris 2008; Loy 2012). Resource allocation is fundamentally different in *Cucurbita* plants on which fruits are allowed to develop to maturity from those on which the fruits are continually harvested when young. The first developing fruit, in proportion to the number of seeds it contains, inhibits the development of subsequent fruits and causes abortion of female

flower buds (Stephenson et al. 1988). Plants having maturing fruits have slowed vegetative development (Zack and Loy 1981; Avila-Sakar et al. 2001) but the continual removal of young fruits, as in cocozelle and zucchini squash, favors continued production of leaves, flowers, and fruits (El-Keblawy and Lovett-Doust 1996). Indeed, for the 10,000 years that *C. pepo* has evolved under domestication, the difference in resource allocation, desired by people for maximum production of immature versus mature fruits, has led to much of the vast diversity that is observed today in this species.

Cucurbita plant morphology ranges widely, from procumbent, rampant viney to erect bushy, from highly branched to non-branched, petioles held at more vertical or horizontal angles, size of laminae and their shape, and number of leaves produced per plant, in total and over time. None of these traits have been closely examined and compared to determine which architectural components give the most efficient light interception. As small-leaved, rampant, highly branched vines are characteristic of wild plants, one can assume that these traits are the most efficient for survival and reproduction in the wild. As people aided and abetted the growth of local wild *Cucurbita* populations, and eventually adopted them adjacent to their living quarters, they favored those plants and fruits that better fit their needs and desires at the expense of plant ability to survive and reproduce in the wild. It seems reasonable to assume that one of the desires of early cultivators was larger seeds and fruits. In traditional *Cucurbita* cultigens being grown to this day in Latin America, all of the plant organs, including the seeds and fruits, are larger than those of wild plants but the procumbent, rampant, viney growth of the plants is preserved. The fruits of most plants are round, similar to those of wild populations, but some elongate variants occur within some otherwise round-fruited cultigens. The fruits borne by wild plants have dark green longitudinal stripes, which presumably contribute photosynthates to fruit development (Bazzaz et al. 1979), but are absent from the fruits of many primitive cultigens.

Over time, step-by-step and unrecorded for generations, people adopted plants and fruits that fit their needs and desires even more. In sixteenth-century herbals, most of the depictions of *Cucurbita pepo* scallop squash show them as having bushy plants (Paris 2000). Selection of bush growth habit, which is conferred by a single gene (Paris and Edelstein 2001), the magnitude of which varies in different genetic backgrounds (Loy 2012), was an important advance for cultigens grown primarily for eating of their immature fruits, because the plants must be harvested numerous times during the growing season, resulting in trampling of viney plants and significant damage to flowers and young developing fruits. Moreover, with bushy plants, growers waste considerably less time searching for randomly scattered fruits in the field. Also documented in the Renaissance herbals are the presence of viney acorn squash as well as a great diversity of pumpkins, some of which closely resemble those grown in the United States to the present day and are consumed when mature. Thus, cultigens of both, *C. pepo* subsp. *texana* and *C. pepo* subsp. *pepo* having reasonably good mature fruit quality also existed already in the sixteenth century.

In modern, large-scale commercial enterprises, *Cucurbita* growers closely monitor factors that affect production efficiency and profit margin. This has fueled further

genetic changes in *Cucurbita*, which for the past 150 years have been concentrated in the hands of publicly as well as privately employed breeders. For more efficient production of immature fruits, bush cultivars have been selected for erect growth, no branching, and more horizontally positioned leaves, traits which constitute "open growth habit" (Baggett 1972; Paris 1996). Plants having an open growth habit expose the fruits to the sight of the pickers, resulting in faster harvesting of the crop and savings on labor to the grower. Plants with medium-length petioles and medium-size laminae are preferred to large plants because they can allow for closer spacing in the field and yet be no less efficient producers of fruits than larger plants (Paris et al. 1986a). Productivity of summer squash is correctly measured by fruit number, not fruit weight, as fruits that are excessively large have poor quality and most markets have premium prices for medium to small size fruits. For zucchini, the preferred size is 40–50 mm diameter. Higher plant densities allow for even higher production per unit area of relatively small fruits, which tend to bring premium prices to the grower.

The immature fruits of *Cucurbita* are most widely enjoyed when they are picked when 2–5 days past anthesis, at the peak of their quality (Culpepper 1937; Lorenz 1949). These tender young fruits are high in water and low in dry matter, vitamin, and mineral contents but, for most constituents, still higher than the other major cucurbit fruit eaten immature, cucumber (*Cucumis sativus* L.) (MacGillivray et al. 1942; Gebhardt and Thomas 2002). The young fruits have a glossy surface and, barring physical or pathogenic damage, glossiness is the most important characteristic foretelling the quality of immature *Cucurbita* fruits (Paris 1996, 2008). Fruits having a matte surface are unattractive, regardless of shape or color, and usually too old to be enjoyed. There are, however, differences in usage of the immature fruits according to their shape. The round-fruited "summer pumpkins", such as 'Tondo di Nizza', as compared with the non-round summer squash, have a higher proportion of the total volume of the fruit constituted by the endocarp, which is the soft placental tissue surrounding the developing seeds. In culinary preparations of summer pumpkins, the endocarp is scooped out and the resulting large cavity is stuffed, often with the same ingredients used to stuff the hollow insides of peppers (*Capsicum annuum* L., Solanaceae). The vegetable marrows, which have a short, tapered cylindrical shape, have less endocarp, proportionately, than the summer pumpkins, but still have markedly more than the longer fruited cocozelle and zucchini squash. The vegetable marrows are quite popular in the Middle East and they, too, are primarily used for stuffing. The cocozelles and zucchinis, due to their large deviation from roundness, have proportionately much less endocarp and much more of the colored exocarp and firm mesocarp (Sinnott and Durham 1929). Fruits of these two cultivar-groups are useful in a wide variety of recipes and are cooked and eaten whole. The cocozelles are often harvested and eaten when quite young, on the day of anthesis or the day following. The long-fruited crookneck and straightnecks are also consumed whole, as are the flat scallops. Similarly, the very long fruited *C. moschata* cultivars (Fig. 3) and the flat zapallitos of *C. maxima*, are usually consumed whole, when young and glossy.

The variations in fruit color in *Cucurbita pepo* are under the control of 13 loci, some of which are multiple-allelic (Paris and Brown 2005). The color of young *Cucurbita* fruits is green or yellow, consisting of two shades of either color if carrying the gene for striping, $l\text{-}1^{BSt}$, or both green and yellow if carrying the bicolor gene, *B*. Harvest of yellow-fruited cultivars is faster than green-fruited cultivars, as the yellow fruits are more obvious within the green foliage (Paris et al. 1986b). There are noticeable differences in flavor between green- and yellow-fruited cultivars and, in some regions, preferences are so strong that consumers would not even consider tasting fruits that have different shapes or colors than what they are used to. There are also differences in flavor among green-fruited cultivars. Some cocozelles, such as 'Romanesco', have a particularly rich flavor not found in the vegetable marrows or zucchinis. Zucchini, crookneck and straightneck squash have more vitamin A than many scallop squash, attributable to their more intense coloration, and zucchini has considerably more potassium than the other three (Paris 2008). One hybrid cocozelle, 'Àroma', was superior to ten other commercial *C. pepo* subsp. *pepo* cultivars in vitamin and mineral content and also rated superior in flavor (Z. Madar and H.S. Paris, unpublished). Summer squash have a shelf life of only 2 or 3 days, and are particularly subject to desiccation and chilling injury (Mencarelli et al. 1982; Sherman et al. 1985; Sherman et al. 1987; McCollum 1990) but some, such as the scallop squash, have a longer shelf life than others (McCollum 1990). A potentially important trait for growing summer squash under protected conditions is parthenocarpy, the ability to produce fruits without pollination. Summer squash cultivars differ greatly in their ability to develop fruits parthenocarpically (Martinez et al. 2014). Production of more than one flower bud per leaf axil, which is common in *C. pepo* subsp. *texana*, has recently been introgressed into cocozelle and zucchini germplasm and, for long-season crops grown under protected conditions, may offer the possibility of greatly increased yields (Paris 2010). The multiple-flowering trait is conferred by a single recessive gene (Paris and Hanan 2010). Other characteristics that need to be considered in collecting, evaluating, and breeding summer squash have been enumerated and discussed elsewhere (Paris 2008).

Fruit flesh and seeds of mature *Cucurbita* fruits form an important part of the diet in many parts of the world, including countries that are not strong economically (Ferriol and Pico 2008). The hundreds of existing cultivars, however, differ widely in their usefulness, particularly with regard to consumption of the fruit flesh. Flowering of pumpkin and winter squash cultivars adapted to temperate regions begins around 6–8 weeks after sowing, a week or two later than summer squash cultivars (Loy 2012). Tropical pumpkins, *C. moschata*, and those of other species adapted to long-season conditions, are mostly rampant vines that tend to flower much later, and can be daylength sensitive. For pumpkins and winter squash, multiple branching is a desirable trait. Competition among fruits for photosynthates appears to be a function of location on the plant (Wien 1997) and is lessened when the fruits are borne on different branches, meaning that more fruits can set and interfere less with their mutual development (H.S. Paris, unpublished observations). Multiple branching also results in a more uniform canopy development (Loy 2012). Bushy rather than viney plants offer a considerable advantage to the commercial

grower not just through earlier production but also less costly, easier management of the field. To ensure that the field has maximum photosynthetic potential, that is, to make the most effective use of bush cultivars, it is necessary to ensure a nearly complete canopy cover of the field at flowering time. This can be accomplished by increasing the number of plants growing per unit area, to fill the empty between- and within-row spaces more quickly (Loy and Broderick 1990; Loy 2004).

The two most important components of good fruit-flesh quality are starch and sugars, which are often measured, respectively, as percent dry matter and percent soluble solids (Haber and Argue 1927; Culpepper and Moon 1945; Merrow and Hopp 1961; Murphy et al. 1966; Harvey et al. 1997; Corrigan et al. 2001). Generally, fruits reach their maximum size by 20–30 days past anthesis, and maximum dry matter content by 30–40 days. For soluble solids content and flesh color, peak values occur at around 50–60 days. During storage, fruit weight, and starch and total carbohydrate contents decrease but sugar content increases over several weeks (Culpepper and Moon 1945; Phillips 1946; Holmes et al. 1954; Hopp et al. 1960; Nerson 1995; Harvey et al. 1997; Loy et al. 2004; Loy 2006a, b; Noseworthy and Loy 2008).

Starch and sugar contents of the fruit flesh are not simply inherited (Loy 2012). The amounts of starch and sugars accumulated in the mesocarp during fruit maturation are subject to wide variation induced by a number of non-genetic factors. Crop productivity, measured as dry weight of fruits or seeds, is ultimately determined by the amount of photosynthetically active radiation intercepted by the plants, the efficiency with which this energy is absorbed and converted to carbohydrate by the plants, the efficiency of transport of these carbohydrates and the proportion which is allocated to the fruits or seeds (Loy 2004). The number of pistillate flowers produced per pumpkin or squash plant far exceeds the number of maturing fruits that the plant can carry, primarily because of the large carbohydrate requirements of developing fruits. Developing fruits out-compete pistillate flowers and more recently set fruits, causing them to abort (Stephenson et al. 1988; El-Keblawy and Lovett-Doust 1996). Competition is especially keen on single-stem, bush plants. Fruits that develop early in the season, when the plants are small, or large fruit loads on bush plants, can result in an insufficient supply of photosynthates for distribution to the fruits, resulting in inadequate accumulation of dry matter, especially starch, in the fruit mesocarp (Loy and Broderick 1990; Loy 2004).

Cultivars having high fresh-weight yields tend to have low contents of dry matter in the fruit flesh (Loy 2004). Fruit dry matter content can be increased through intensive breeding but there are clear negative associations between fruit size and fruit-flesh quality, and yield and fruit-flesh quality (Loy 2012). Therefore, cultivars with good eating quality cannot be expected to produce fresh weight yields equal to those having inferior quality. As grower profits are derived from the fresh weight of the product, attempts to market new high-quality pumpkins and squash through large commercial channels have met with great difficulty. One successful case, though, was the marketing of an acorn squash in Israel, a product that was not present on the market beforehand. There, a sweet acorn squash hybrid, 'Table Sugar', was developed by the Agricultural Research Organization and licensed to a local

company, Origene Ltd., for seed production and marketing. The company sold seeds only to growers who agreed to pick the fruits when they were fully ripe, at the peak of their quality. The prices per unit fresh weight obtained by the growers were far higher than for other pumpkins and winter squash, thereby compensating them for the lower fresh weight yields.

Intensity of flesh color is an important component of *Cucurbita* fruit sensory quality (Schales and Isenberg 1963; Murphy et al. 1966; Carbonell et al. 1990). Carotenoid pigments present in the fruit flesh of *Cucurbita* impart yellow and orange coloration (Paris 1994). Carotenoids are anti-oxidants and some are provitamin A compounds (Goodwin 1980). Often, they are found in appreciable quantities in pumpkins and squash, providing an important source of provitamin A in the diet (Borenstein and Bunnell 1966). Intensity of the yellow or orange coloration of the flesh is a fairly reliable indicator of relative carotenoid content (Francis 1962; Borenstein and Bunnell 1966; Kubicki and Walczak 1976; Itle and Kabelka 2009). Carotenoid content of the fruit flesh varies greatly among *Cucurbita* accessions (Holmes et al. 1945; Borenstein and Bunnell 1966; Schaffer et al. 1986b; Danilchenko et al. 2000; Murkovic et al. 2002; Noseworthy and Loy 2008) and tends to increase during storage (Holmes et al. 1954; Lewis and Merrow 1962). Some cultivars of *C. maxima* and *C. moschata* have been reported as especially high in carotenoid content (Arima and Rodriguez-Amaya 1988; Murkovic et al. 2002; Azevedo-Meleiro and Rodriguez-Amaya 2007). In *C. pepo*, the dominant alleles *B* and *L-2*, in complementary interaction, impart an intense orange coloration to the fruit flesh (Paris 1988) and an increase in carotenoid content of as much as 15 fold (Schaffer et al. 1986b).

Relatively thick fruit mesocarp is a desired trait but genes associated with mesocarp thickness have not been identified. However, deviation from round fruit shape, specifically the 1:1 length-to-width ratio of the fruit, results in greater mesocarp thickness (Sinnott and Durham 1929). Nonetheless, some round-fruited cultivars have thick flesh. ‘Musquée de Provence’, a large French *Cucurbita moschata* pumpkin, has thick, intensely orange flesh of high quality (Fig. 2).

The cultivation of *Cucurbita pepo* pumpkins for the extraction of oil from the seeds dates to at least the early eighteenth century in Styria, Austria (Teppner 2004; Lelley et al. 2010). The great expansion of oil-seed pumpkin production in Austria and neighboring countries began soon after the isolation of the hull-less or “naked” seed mutant that occurred spontaneously in Austria around 1880, which facilitated the industrial-scale extraction of oil from the seeds (Teppner 2000). Today, the oil-seed pumpkin industry is of considerable size and economic importance and concentrated in central Europe (Cook 2000; Konrad 2000; Artyomenko and Chaban 2003). In Austria alone, approximately 18,000 ha are planted to oil-seed pumpkins, with an average yield of 0.61 t/ha of seeds (Lelley et al. 2010). Oil-seed pumpkins are being bred intensively in Austria, Hungary, and Serbia (Berenji and Papp 2000; Winkler 2000; Berenji 2011). Round fruits (pumpkins) are preferred over elongate fruits (squash) because pumpkins have more, larger, and flatter seeds (Nerson et al. 2000; Paris and Nerson 2003; Nerson 2005). A review of genetic relationships of oil-seed pumpkins is provided by Lelley et al. (2010). SSR-sequence polymorphisms

have indicated that, indeed, the oil-seed pumpkins are a genetically rather isolated market type (Gong et al. 2012). Hull-less-seed pumpkins are being bred in the United States not for extraction of the oil but rather as a nutritious snack food (Loy 2000; Loy 2004). Breeding for seed production is different than breeding for improved flesh quality because photosynthate needs to be more efficiently channeled into the growth and maturation of seeds rather than the mesocarp (Loy 1988). In a way, this is a return to the wild gourds, which have thin flesh and are full of seeds. Seed embryos usually do not reach their maximum size until 50 or even 60 days past anthesis (Vining and Loy 1998).

Diseases

Viruses transmitted by aphids, whiteflies, and other insects are the cause of some of the most destructive diseases in *Cucurbita*, but the particular viruses responsible for the most crop damage are in a constant state of flux, tending to vary from region to region and year to year (Nameth et al. 1986; Lecoq et al. 1998; Cohen and Ben-Joseph 2000). Moreover, reports of "new", additional viruses causing damage to cucurbit crops are frequent. Zucchini yellow mosaic virus, a potyvirus vectored by aphids first described in 1981 (Lecoq et al. 1981; Lisa et al. 1981), has for the past 30 years been one of the most destructive viruses of pumpkin and squash crops (Lisa et al. 1984; Desbiez and Lecoq 1997; Gal-On 2007). Squash leaf curl virus, a begomovirus borne by whiteflies (Cohen et al. 1983; Brown et al. 2002), is becoming increasingly important in the warmer production regions (Brown et al. 2002; Abudy et al. 2010).

There are a wide variety of other pathogens that limit production of pumpkins and squash (Zitter et al. 1996; Babadoost and Zitter 2009). In drier climates, the powdery mildew fungi, *Podosphaera xanthii* (Castag.) U. Braun & N. Shish. and *Golovinomyces orontii* (Castag.) Heluta, can be devastating, weakening plants over the course of the season and markedly reducing mature-fruit quality. Powdery mildew is often difficult to control (McGrath 2002a; Lebeda et al. 2016). Various other pathogens, including those causing fruit rots, are favored by rain and high humidity (Zitter and Kyle 1992). The downy mildew fungus, *Pseudoperonospora cubensis* (Berk. & Curt.) Rost., is a resurgent problem (Cohen et al. 2015). Fruit rot caused by *Phytophthora capsici* Leonian has become an increasingly serious problem (Babadoost 2000). Gummy stem blight, *Didymella bryoniae* (Auersw.) Rehm (syn. *Mycosphaerella citrullina* (C.O. Smith) Grossenbacher), and *Fusarium* spp. also cause fruit rots (Kucharek and Schenck 1983; Elmer 1996). Although *Cucurbita* is generally fairly resistant to soil-borne diseases affecting roots, *C. pepo* is susceptible to *Fusarium* (Martyn and McLaughlin 1983; Babadoost and Zitter 2009). The bacterium *Erwinia tracheiphila* (E.F. Smith) Holland also incites plant wilting. The leaf silvering disorder, induced by whiteflies, *Bemisia tabaci* (Gennadius) and exacerbated by drought stress, can be devastating (Paris et al. 1987; Simons et al. 1988; Yokomi et al. 1990; Costa et al. 1993; Paris et al. 1993a; Chen et al. 2004), reducing photosynthesis by as much as 30 % (Burger et al. 1988).

Disease Resistance

Disease resistance, especially virus resistance, has been considered to be the most important goal of breeding pumpkins and squash (Whitaker and Robinson 1986). Moderate resistance or tolerance to viruses has been reported in *Cucurbita maxima* (Provvidenti 1982; Křistkova and Lebeda 2000b) and *C. pepo* (Walkey et al. 1984; Lebeda and Křistkova 1996; Křistkova and Lebeda 2000b). There are also *C. maxima* cultivars with potyvirus resistance derived from *C. ecuadorensis* (Herrington et al. 2001). As summer squash plants are picked continually rather than being allowed to mature their fruits, they continue to grow and differentiate throughout the season, continuously subjecting them to infection by viruses. The tropical species *C. moschata* as well as the other tropical and xerophytic wild species harbor resistances to various viruses, including potyviruses (Provvidenti et al. 1978; Whitaker and Robinson 1986; Provvidenti 1990; Gilbert-Albertini et al. 1993; Brown et al. 2003) but, as discussed earlier, introgression of these resistances to *C. pepo* has often been quite problematic. Today, there are commercially available cultivars of *C. pepo* that carry resistance to ZYMV and other potyviruses that has been introgressed from *C. moschata* (Munger and Provvidenti 1987; Paris et al. 1988; Provvidenti 1997; Paris and Cohen 2000; Pachner et al. 2009). The pyramiding of genes for resistance to various viruses and other diseases and pests is an important ongoing challenge to pumpkin and squash breeders (Robinson and Provvidenti 1997; Pachner et al. 2015).

Cucurbita germplasm encompasses accessions that are less susceptible or even resistant to some fungal and bacterial pathogens as well as insect pests (Loy 2012). Cultivar-groups of *C. pepo* subsp. *texana* are less susceptible to powdery mildew than those of *C. pepo* subsp. *pepo* but the opposite is the case for downy mildew (Křistkova and Lebeda 2000a; Lebeda and Křistkova 2000). A few accessions of *C. moschata* have been identified as having some resistance to powdery mildew (Sowell and Corley 1973; Adeniji and Coyne 1983). Powdery mildew resistance has been introgressed from *C. okeechobeensis* to *C. pepo*, and resistant *C. pepo* hybrids have been commercially deployed (Jahn et al. 2002; Paris and Cohen 2002; Formisano et al. 2010). This same resistance was also introgressed from *C. okeechobeensis* into horticulturally advanced germplasm of *C. moschata* (Cho et al. 2003). Germplasm of *C. pepo* and *C. moschata* has been identified that is less susceptible to the crown rot induced by *Phytophthora capsici* (Padley et al. 2008; Padley et al. 2009; Chavez et al. 2011). There are also differences in susceptibility of pumpkin and squash cultivars to bacterial wilt (McGrath 2002b). Sources of resistance to silverleaf have been identified in *C. pepo* (Paris et al. 1993b; Cardoza et al. 1998) and *C. moschata* (Wessel-Beaver 1998; Wessel-Beaver 2000a).

Biotechnology

Embryo-culture techniques have been employed for decades to rescue incompletely developed embryos of putative interspecific hybrids of *Cucurbita* (Lebeda et al. 2007). The development of *in vitro* protocols for producing haploid plantlets, by

irradiated pollen (Kurtar et al. 2002) and by ovule culture (Shalaby 2007), promise to make possible the rapid generation of homozygous lines which could then be selected for combining ability in the production of hybrids. Tissue-culture protocols for regeneration have undergone successive refinements (Kathiravan et al. 2006; Mookann 2015). Significantly, the ability to regenerate shoots differs markedly among genotypes (Kiss-Baba et al. 2010; Gisbert et al. 2010– 2011).

Genetic Engineering

Genetic engineering has joined the efforts to obtain virus-resistant *Cucurbita*, with much success (Clough and Hamm 1995; Fuchs et al. 1998; Tricoli et al. 2002; Gaba et al. 2004). Transgenic virus-resistant *C. pepo*, notably zucchini and crookneck squash with resistance to cucumber mosaic, watermelon mosaic, and zucchini yellow mosaic, have been deployed commercially in the United States (Fuchs and Gonsalves 2007). The resistances are derived from the insertion of genes coding the coat proteins of the viruses into the *C. pepo* genome. These genes can be introgressed into other elite breeding lines and recombined with other traits. The level of resistance to specific viruses is quite high and has proven effective in both artificial inoculations and in the field (Fuchs et al. 1998). New viruses inflicting heavy losses to cucurbit crops emerge every several years. Genetic engineering methodologies, if they become relatively inexpensive, could offer an economically feasible way to combat destructive new viruses as they appear.

Moreover, if public confidence in the safety of consuming engineered vegetables and fruits increases, the possibilities of genetic engineering would be seemingly endless. Notably, *Cucurbita* and many other cucurbit genera are not known to produce anthocyanins. If the ability to synthesize anthocyanins could be engineered into *Cucurbita*, then it might be possible to introduce new colors in pumpkins, squash, and gourds, as well as increase their anti-oxidant value. The downside of engineered valuable traits is that they would be privately owned and patented, and therefore not available for being absorbed into a general, common pool of genetic resources.

Mapping

Mapping of the *Cucurbita* genome lags far behind that of cucumber, melon (*Cucumis melo* L.), and watermelon. A map based on morphological and random amplified polymorphic DNA (RAPD) markers was constructed by Brown and Myers (2002). Maps for *C. pepo* based on morphological, RAPD, AFLP (amplified fragment length polymorphism), and a few SSR markers were presented by Zraidi and Lelley (2004) and Zraidi et al. (2007). Gong et al. (2008a, b), using many more SSRs, observed a high degree of macrosynteny between *C. pepo* and *C. moschata*. The

SSR markers had conserved orders in the two species, representing orthologous loci. A single-nucleotide polymorphism- (SNP-) based map for *C. pepo* that includes several putative quantitative-trait loci related to vegetative and reproductive traits was constructed for *C. pepo* (Blanca et al. 2011; Esteras et al. 2012a, 2012b). A high-density map was recently constructed for *C. maxima* (Zhang et al. 2015). A draft genome for zucchini (*C. pepo*) has been assembled (Blanca et al. 2015) and is currently being undertaken for several *Cucurbita* species (*pepo, maxima, moschata*) (Pico et al. Chap.11).

Collection, Maintenance, and Characterization of Genetic Resources

Collection and maintenance of crop germplasm is the function of institutions referred to as gene banks, seed depositories, and plant introduction stations. Many of these institutions maintain seeds of wild and cultivated accessions of *Cucurbita*, and particularly large collections are held in the U.S.A., Mexico, Costa Rica, Russia, Italy, Brazil, Colombia, Bolivia, Czech Republic, Spain, Turkey, and Portugal (Clark et al. 1991; Nuez 2000; Diez et al. 2002; Lebeda et al. 2007; Ferriol and Pico 2008; Karlova 2008).

The acquisition of diverse germplasm is the first necessary step for genetic enhancement (Lebeda et al. 2007). Wild, feral, and landrace germplasm can have great value, but the logistics of exploration and collection of such germplasm are often not simple or straightforward (Andres 2000). Old cultivars, sometimes referred to as "heirlooms", often have valuable, underexploited traits but are no longer available through commercial sources (Goldman 2004). Modern cultivars and hybrids contain the traits that facilitate economically efficient, large-scale crop production. Breeders of *Cucurbita* need all of these germplasm categories in order to develop new cultivars that will be advantageous to the entire chain of commerce, from the seed producers, to the growers, produce traders, and consumers. All of these germplasm categories need to be safely maintained and available for future use. Although seeds of the same germplasm are often maintained at more than one gene bank/depository/plant introduction station, that is, much of the germplasm is repetitive, the existence of unique germplasm at more than one facility increases the security of its being successfully maintained over the long term (Esquinaz-Alcazar and Gulick 1983; Fowler 2008).

Maintaining the genetic identity of the many *Cucurbita* germplasm accessions in gene banks is not a simple task (Esquinaz-Alcazar and Gulick 1983; Lebeda et al. 2007). *Cucurbita* are large plants requiring much space. To prevent contamination, each accession to be reproduced must be hand pollinated or grown in its own separate screen cage, with a beehive being placed within. In order to minimize genetic drift, as many plants of the accession as practical are hand pollinated or grown in the cage. The cage itself and its location, however, constitute a selective environment which may favor some variant plants over others. The harvested seeds have to then

be maintained under temperature and moisture conditions that ensure their vitality and prolong their longevity. Under optimal conditions, *Cucurbita* seeds can remain viable for 30 years or more.

To make the most efficient future use of the maintained *Cucurbita* germplasm, the accessions need to be characterized. The species needs to be correctly identified and, if identity is uncertain, indicated as such (Lebeda et al. 2007). In addition to listing by numbers, the germplasm should be accompanied with descriptors and color photographs (Esquinaz-Alcazar and Gulick 1983; Vinter et al. 2004). Whenever appropriate and possible, the subspecies, cultivar-group, and/or market type should be given (Paris 2001). A long list of cultivar differentials are in the Objective Description of Variety of the U.S.D.A., Agricultural Marketing Service, for *Cucurbita pepo* and other pumpkins, squash, and gourds (Paris et al. 2007; also published by the U.S.D.A./A.M.S. on-line; Fig. 1). While such a comprehensive description as appears in this list is not essential for descriptions of germplasm in gene banks, the major descriptors, such as fruit shape and developmental color (Paris and Nerson 1986), and outstanding features of the accession, such as resistance to a particular disease, can provide invaluable assistance to researchers and breeders. The distribution of germplasm samples is a function of gene banks as well. The function of gene banks is not just to collect, maintain, and characterize germplasm, but also to make it available, in reasonable quantities, to all interested researchers and breeders.

Future of Genetic Resources

The fate of *Cucurbita* germplasm existing in gene banks, seed repositories, and plant introduction stations seems to be fairly well assured. The germplasm is usually reproduced to more-or-less maintain the genetic constitution of each accession and the seeds are housed in conditions that allow their long-term viability. Moreover, there is considerable safety duplication among these facilities. However, there likely is still *Cucurbita* germplasm that has not been collected. Outstanding examples are the missing wild *C. moschata*, *C. ficifolia*, and *C. pepo* subsp. *pepo*. Collections of the primitive cultivars, the old and "lost" cultivars, and newer cultivars and hybrids that have been superseded in commerce are either difficult to find or no longer available, and are likely to be incomplete. But there is yet another category of *Cucurbita* germplasm where complete collections have been lost and of which the entire continued existence is in danger. These are the collections of breeder-geneticists at research and academic institutions who have been working with *Cucurbita* for many years. These collections are often fairly large and encompass not only wild, feral, and primitive accessions, old and modern cultivars and hybrids, but also germplasm that these breeder/geneticists have developed that has unique combinations of potentially valuable traits. Lack of financial support, public and private, has led to non-replacement of these plant breeder-geneticists when they retire, ending further fruition of their germplasm enhancements. All too often, no provisions are made for

maintaining, together with descriptive lists, the enhanced germplasm that was developed by these retiring, unreplaced public breeders. Important achievements attained through of years of plant-breeding efforts are thus widely subject to loss by default.

Cucurbita is ever more popular around the world for food and ornament, having a steadily marked increase in production over the years. Private companies and international conglomerates breed for traits important to their customers, the commercial growers, and the competition is keen. Privately employed breeders have done an excellent job in improving crop yield and resistance to pathogens, traits which are of the utmost importance for achieving and insuring maximum profits to growers of squash and pumpkins. Privately employed breeders, though, do not usually focus painstaking efforts on breeding for traits that are important to the consumer.

The largest potential for expansion of *Cucurbita* may lie in the development of cultivars having supreme mature fruit-flesh quality, as this trait can be expected to sharply increase the consumption of pumpkins and winter squash. In fact, this has already occurred in Japan, following the development of the kabocha squash, a derivative of the supreme quality *C. maxima* 'Buttercup' (Ratnayake et al. 2004). It is in this consumer-oriented trait, fruit quality, that the publicly employed breeders have their greatest ongoing legacy. The best-documented achievements in improved mature-fruit quality of pumpkins and squash are from the United States. *C. maxima* 'Buttercup', which was bred at the North Dakota Agricultural Experiment Station (Yeager and Latzke 1932; Tapley et al. 1937), is today the standard by which all other pumpkins and winter squash are rated for quality. Other notable achievements in breeding for high mature fruit-flesh quality include germplasm related to acorn squash (*C. pepo*) developed at Oregon State University by J.R. Baggett (Baggett and Kean 1990), tropical pumpkins (*C. moschata*) at the University of Puerto Rico by L. Wessel-Beaver (Wessel-Beaver 2005; Wessel-Beaver et al. 2006) and at the University of Florida by D.N. Maynard (Maynard et al. 2002), temperate *C. moschata* at the University of Nebraska by D.P. Coyne (Coyne and Hill 1976), and bush *C. maxima* and acorn squash at the University of New Hampshire by J.B. Loy (Loy 2012). The powdery-mildew resistance so necessary to ensure highest fruit quality was introgressed from *C. okeechobeensis* to *C. pepo* at Cornell University by H.M. Munger (Jahn et al. 2002). Indeed, the recorded enhancements of mature-fruit mesocarp quality are mainly the result of the efforts of public breeders, whose ultimate employers are the taxpaying consumers.

Programs for research and breeding of pumpkins and squash have not been major attractions for outside financial support because officials of granting agencies have not considered them to be important-enough crops. Thus, public research for these crops has steadily diminished. Moreover, potential funding for traditional plant breeding has been increasingly awarded to molecular breeding, biotechnology and, recently, the various "-omics", little of which has as yet been applied toward the development of pumpkin and squash cultivars having superior mature fruit-flesh quality. Private companies and international conglomerates usually prefer to devote their financial resources to breeding in-house rather than outsourcing to public breeders. Recent laws

intended to prevent the general public from inadvertently introducing exotic pathogens and pests on imported plant material have stymied the continued collection and characterization of germplasm by scientists devoted to particular crops. These laws include requiring certificates of freedom from disease based on samples of 10,000 seeds and proven facilities for growing the plants in quarantine, even though seed samples of *Cucurbita* typically are much smaller, consisting of 30–50 seeds, and *Cucurbita* plants are so large that they would quickly overrun most quarantine facilities. Therefore, the collection and maintenance of the broad spectrum of potentially valuable germplasm, wild, feral, primitive, old or otherwise commercially unavailable, is becoming less and less possible for the remaining publicly employed plant breeders. Yet, it is this group of scientists that is the most answerable to the taxpaying consumers, tend to have the greatest knowledge of crop genetic resources and, accordingly, the imagination and vision of the possibilities for the future consumer-oriented exploitation of these resources. Dwindling financial support and lack of recognition by government officials of the role of public breeders is resulting in an increasingly tenuous future for consumer-driven enhancement of *Cucurbita* genetic resources.

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Gourds: Bitter, Bottle, Wax, Snake, Sponge and Ridge

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Abstract Bitter gourd, bottle gourd, wax gourd, snake gourd, sponge gourd, and ridge gourd are cultivated and marketed by smallholder farmers, and are important crops in home gardens throughout southern and southeastern Asia. These vegetables provide significant dietary nutrients such as vitamin A and C, iron and calcium. Public sector breeders and germplasm curators release open-pollinated varieties of these cucurbits developed through selection from landraces. Private sector breeders

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develop F_1 hybrid cultivars of these gourds that are popular with growers because of their uniformity, early and total marketable yield, and, in some cases, disease resistance. This chapter reviews the status of germplasm resources for sustained genetic improvement of these cucurbit species. Susceptibility to viruses is currently the major production constraint for these gourds, and systematic evaluation of their germplasm against viruses will be helpful for breeding improved cucurbit lines. The germplasm resources of these gourd species are held in an array of genebanks in several countries and may not be readily available for scientific research or to commercial breeders outside of their respective country. Many accessions of these gourd species listed by the World Vegetable Center and the U.S. Germplasm Resources Information Network are either not available or inactive. More accessions of these gourd species and their relatives need, therefore, to be collected from various regions of the tropics, conserved, and evaluated to ensure continuous genetic gains in breeding programs.

Keywords *Momordica charantia* • *Lagenaria siceraria* • *Benincasa hispida* • *Luffa* spp. • *Trichosanthes* spp. • Genetic resources • Disease resistance • Grafting • Plant breeding

Introduction

"Cucurbits" is a broad term representing all taxa within the highly diverse family known as Cucurbitaceae, which comprises at least 950 species distributed in over 90 genera that are predominantly distributed in the tropics and subtropics (Schaefer and Renner 2011). Major cultivated cucurbit crops of significant economic importance include cucumber (*Cucumis sativus* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], melon (*Cucumis melo* L.) and squash and pumpkin (*Cucurbita* spp.). These cucurbits are widely cultivated globally. Six other cucurbit crops of considerable value to growers and consumers in the Old World tropics are bitter gourd (*Momordica charantia* L.), bottle gourd [*Lagenaria siceraria* (Molina.)

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Standley], wax gourd [*Benincasa hispida* (Thunb.) Cogn.], sponge gourd (*Luffa aegyptiaca* Mill., syn. *Luffa cylindrica* Roem.) and ridge gourds (*Luffa acutangula* (L.) Roxb.), and snake gourd (*Trichosanthes cucumerina* L.). These regionally important cucurbits are grown and marketed by smallholder farmers and remain essential components of home gardens in southern and southeastern Asia. Their fruit contain important nutrients such as vitamin A and C, iron and calcium (Pandit and Acharya 2008). This chapter will provide a brief review of genetic resources of these six cucurbit crops.

Bitter Gourd

Bitter gourd (*Momodica charantia* L.) is an important vegetable in Asia where it is grown on approximately 340,000 ha annually by smallholder farmers (McCreight et al. 2013). The crop is cultivated in some African countries, such as Ghana, and fresh fruit is exported to Europe where it is in great demand among expatriate Asian communities. Cultivation of this cucurbit is expanding in Zambia, Congo and Madagascar for local consumption and export. It is also cultivated in smaller volumes in Australia (Northern Territory, Queensland, New South Wales, Victoria), where Asian varieties are grown and consumption is primarily by ethnic communities from Asia (Morgan and Midmore 2002). Bitter gourd fruit is rich in beta-carotene, vitamin C, folic acid, magnesium, phosphorus, and potassium (Yuwai et al. 1991; Dhillon et al. 2016a). In addition to its use as a vegetable, bitter gourd is often used in folk medicine in Asia to manage type 2 diabetes, a non-communicable disease that affects 347 million people worldwide, with 80 % of these living in low- and middle-income countries (World Health Organization 2016).

The species is monoecious (produces separate male and female flowers on the same plant) and tends to cross-pollinate, a mechanism that tends to promote phenotypic and genotypic diversity. Recent biogeographic analyses suggest that *M. charantia* originated in Africa (Schaefer and Renner 2010) and probably was domesticated in eastern India and southern China (Reyes et al. 1994). *M. balsamina* is a wild bitter gourd in northern and eastern states of India. The fruit of this species are spindle shaped, green with 6–9 regular or irregular rows of cream or yellowish blunt spines. It is genetically diverse from the genepool of *M. charantia* and accession THMC 281 has been found resistant to melon fly, *Bactrocera cucurbitae* (Dhillon et al. 2016b). *M. charantia* × *M. balsamina* hybrids are difficult to obtain (Bharathi et al. 2012).

Consumers prefer bitter gourd fruit at a physiologically immature or unripe stage. Immature fruits have a fresh bright appearance and the seed coats are creamy-white. Mature fruits have yellow flesh with red seed coats and usually split, rendering them inedible and unsalable. Consumer preferences for fruit color, shape, skin pattern, and size vary between and within countries. Fruit colors range from white or cream to light-green to dark-green, and shapes include cylindrical, elliptical, spindle and conical types. Fruits develop irregular longitudinal ridges and warty skin, depending upon the variety. Based on these fruit traits, nearly 20 market types of

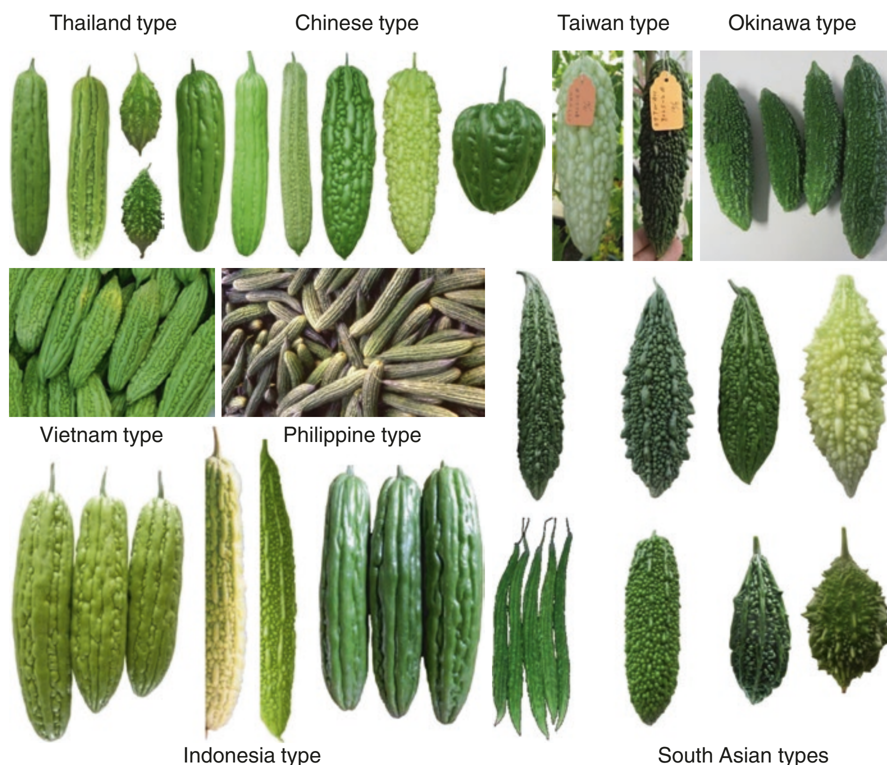

Fig. 1 Bitter gourd: variation within fruit of eight market types

bitter gourd exist in Asia, and nearly half are cultivated in India, China, Nepal, Bangladesh, and Sri Lanka (Fig. 1).

Bitter gourd genetic resources are conserved *in situ* in various genebanks in Asia. Nearly 1000 accessions of bitter gourd are stored in the national genebanks of India at various locations (New Delhi, Jodhpur, Hyderabad, Thrissur, Shillong, Ranchi), and 250 accessions are available in the genebanks of Kasetsart University, Thailand (M. Anil, personal communication). The Chinese Academy of Agricultural Sciences holds 177 bitter gourd accessions in several provinces such as Guangdong, Guangxi and Yunnan (Kai-Lin Hu, personal communication). The World Vegetable Center (WorldVeg) (http://avrdc.org/seed/seeds/) listed 425 bitter gourd accessions in 2016, with 139 (33 %) of them available for distribution, the others were either unavailable (4 %) or inactive (63 %; Table 1). The U.S. Germplasm Resources Information Network (GRIN [Internet]. Beltsville (MD): United States Department of Agriculture, Agricultural Research Service. http://www.ars-grin.gov/) listed 103 *M. charantia* accessions, as seeds, from 22 countries, including 48 from India, but only one accession was available in 2016 (Table 1).

Table 1 Total numbers of accessions of six gourd species listed by the U.S. Germplasm Resources Information Network (GRIN [Internet]. Beltsville (MD): United States Department of Agriculture, Agricultural Research Service. http://www.ars-grin.gov/) and the World Vegetable Center (WorldVeg; http://avrdc.org/seed/seeds/) in 2016, and the respective numbers that are available or not available for distribution, and inactive

		Total		Available		Not available		Inactive	
Common name	Species	GRIN	WorldVeg	GRIN	WorldVeg	GRIN	WorldVeg	GRIN	WorldVeg
Bitter gourd	*Momordica charantia*	103	434	1	139	22	17	80	269
Bottle gourd	*Lagenaria siceraria*	500	329	185	9	254	1	61	319
Ridge gourd	*Luffa acutangula*	67	341	59	18	3	0	5	323
Snake gourd	*Trichosanthes cucumerina*	37	71	0	7	7	0	30	64
Sponge gourd	*Luffa cylindrica*	78	409	64	18	4	0	10	391
Wax gourd	*Benincasa hispida*	106	285	13	36	18	0	75	249

There are few reports of bitter gourd genetic diversity analysis. Random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), and simple sequence repeat (SSR) molecular markers have been used to study genetic diversity of a limited number of bitter gourd accessions that originated from India and China (Dey et al. 2006; Gaikwad et al. 2008; Ji et al. 2012; Yuan et al. 2012). Analysis of 38 Indian bitter gourd landraces from eastern India indicated that genotypes Mohanpur Sel-215 and Jayanagar Sel-1 were highly diverse, based on AFLP markers (Gaikwad et al. 2008). Analysis of 212 bitter gourd accessions from 15 countries in Asia, South America and Africa using 36 SSR markers demonstrated that accessions from China, India and South America were genetically divergent and clustered in three separate subpopulation groups (Kai-Lin Hu, personal communication).

Hybrid bitter gourd cultivars are more popular with Indian farmers than open-pollinated (OP) cultivars. Indian consumers prefer green, shiny, and bitter fruits with spines. Cultivars of different market segments are characterized according to the length of the fruit: short segment (<10 cm), medium segment (10–20 cm), and large segment (> 20 cm). The Indian bitter gourd market is dominated by medium- and long-segment cultivars. Important hybrid cultivars developed in India include Palee and Parachi (East-West Seed), Amanshri (Nunhems), VNR 28 and VNR 32 (VNR Seeds), CT 108 (Chia Tai Seeds), Vivek F_1 (Sun Grow Seeds), Vishesh (Golden Seeds), Abhishek (Seminis), Arjuna, Pallavi, Raja and Parijat (Rasi Seeds), and US1315 (US Agriseeds). Landraces still popular with Indian farmers include Faizabadi Karela, Green long, White long, Jaipur long, Katai, and Jhalari.

In China, popular bitter gourd landraces come in a variety of shapes and sizes. Fruits of cultivars popular in Guangdong, Guangxi, and Hainan provinces of southern China are 20–30 cm long, cylindrical with blunt ends, green and ribbed. Fruits of bitter gourd typical to central China are 30–40 cm long, elongated with pointed ends, warty and light-green to white. One cone-shaped landrace, Dading, is popular in the Pearl River Delta of China. Zhenzhu, another popular landrace, has fruit with pearl-like warts on the rind; its distribution ranges from southwest to central China (Chen et al. 2012; Kang et al. 2010). A miniature-fruited landrace known as Laigua or Laiputao is grown for consumption and ornamental purposes in central China. Resistance to *Fusarium* wilt, powdery mildew and root-knot nematodes have been reported in wild species and landraces that originated from China (Shen et al. 2007; Chen et al. 2014; Tian et al. 2015).

Popular cultivars of bitter gourd derived from landraces in Bangladesh include Ranipukur, Rampali, and Gazkarala. Commercial hybrids are fast replacing landraces in Bangladesh, and important cultivars include BRAC Hybrid 1 and BRAC Hybrid 2 (BRAC Seeds), BARI Karala 1 (BARI), Tia F_1 and Kakoli (Lal Teer Seed), Mukta F_1 (Getco Seed), and Bolder (Metal Seed), Shyama and BT-03 (ACI Seed). Kee Nok ("Bird dropping," which refers to the small size of the fruit) is a bitter gourd landrace-derived cultivar popular with growers and consumers in Thailand. Its fruits are short (5–10 cm), spindle shaped, spiny and green (Fig. 1), but this cultivar captures a small market share because the Thai bitter gourd market is dominated by hybrids with fruit traits such as cylindrical shape, smooth, light-green and blunt ends (Fig. 1). Popular hybrid cultivars include Kiew Yok (East-West Seed),

Morakot (Thai Seed & Agriculture) and Yok Tip (Metro Seeds Thailand). The Philippines bitter gourd market is dominated by hybrid cultivars bred by East-West Seed; popular cultivars include Bonito F_1, Galactica F1, Galaxy F_1 and Jade Star L F_1. The bitter gourd market in Vietnam predominantly belongs to a single market type with cultivars that are slightly spindle shaped, medium-long (10–15 cm), with smooth ridges (Fig. 1). Important cultivars in this market segment include Vino Galaxy B1 (Viet Nong Seed), HN 126 (Vina Seed), Thuy Phi (Known You Seed), Jupiter (En Vang Seed), Apolo – 17 (An Phu Nong Seed) and Sumo 742 (Southern Seed Corporation).

Use of a gynoecious inbred in hybrid development reduces the cost of F_1 hybrid seed production and enhances seed purity. Breeders have developed gynoecious inbreds (DBGy-201, Gy263B, OHB61–5) with better combining ability that improved early and total fruit yield in bitter gourd hybrids (Ram et al. 2006; Iwamoto et al. 2009; Dey et al. 2010). Repeated use of a comparatively small number of closely related bitter gourd lines in commercial breeding has narrowed the genetic diversity within the bitter gourd crop. This is attributed in part to the availability of a few gynoecious bitter gourd lines that are used repeatedly by seed company breeders. Breeders focus primarily on elite × elite crosses to capitalize on previous breeding successes. This has resulted in genetic uniformity among commercial cultivars, which could increase the overall risk to farmers due to pests and diseases. Trait-specific breeding pays rich dividends to commercial breeding programs. Systematic and comprehensive evaluations of the global collection of bitter gourd can provide traits for sustainable production and new genetic diversity. For example, evaluation of the diverse bitter gourd germplasm collection held at the Worldveg led to the development of cucurbit powdery mildew (*Podosphaera xanthii*) resistant lines (Dhillon et al. 2015). Currently, *Cucurbit aphid-borne yellow virus* (CABYV), locally known as “Namamarako,” in the Philippines and “Mara Ba″ in Thailand, has become a grave limitation to bitter gourd production (Relevante et al. 2012). Bitter gourd accession VI049946 from the WorldVeg genebank segregated for resistance to this polerovirus (Fatkhu Rokhman, personal communication).

Bottle Gourd

Bottle gourd [*Lagenaria siceraria* (Molina.) Standley] also known as white-flowered gourd or calabash is native to Africa (Richardson 1972), and archaeological findings suggest its arrival in Asia and the Americas over 10,000 years ago via human migration (Erickson et al. 2005). Long-fruited edible bottle gourds were familiar to the Romans and other Mediterranean civilizations of the first centuries CE (Janick et al. 2007). It is annual and monoecious. The species presents the largest variation in fruit shape resulting, presumably, from thousands of years of selection in isolated areas of the world (Yetişir et al. 2008). Fruit shapes include long and cylindrical, elongate, curved, pyriform, crooked necked, and globular (Fig. 2). The young tender peeled fruits are eaten. Fresh bottle gourd juice is used for its cooling, diuretic, antibilious,

Fig. 2 Bottle gourd: variation of fruit shape and size among landraces, varieties and cultivars

and pectoral properties (Minocha 2015). Young shoots and tendrils are also cooked, and oil is extracted from the seed. Heiser (1979) provided a fascinating account of other uses of mature, dried hard shells of bottle gourd including musical instruments, cups, barrels, milk pails, ladles, fishing floats, penis sheaths, carvings, etc. Fruits are also used as herbal medicines in Asia. Five wild species of *Lagenaria* exist in Africa: *L. breviflora* (Benth.), *L. abyssinica* (Hook f.), *L. rufa* (Gilg.) Jeffrey, *L. sphaerica* (Sonder) Naudin and *L. guineensis* (G. Don) Jeffrey. Wild species produce small round fruits with strong bitter taste (Morimoto et al. 2005). During domestication, selection for non-bitter fruits must have been practiced.

A significant number of bottle gourd accessions collected in different regions of the world are held by the National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India (739 accessions). The WorldVeg listed 329 accessions, only nine were available, the majority (97 %) were inactive (Table 1). GRIN listed 500 accessions of *L. siceraria* from 23 countries in 2016; 185 (37 %) were available for distribution; 51 % were not available (Table 1). Africa accounted for 233 of the GRIN accessions: Burundi (1), Ethiopia (5), Nigeria (1), South Africa (8), Zaire (4), Zambia (106), Zimbabwe (108).

Levi et al. (2009) examined genetic diversity among 57 *L. siceraria* accessions that originated from 16 countries in Asia, Africa, and South America, using sequence-related amplified polymorphism (SRAP) markers; they found that collections of Indian origin were genetically distinct from the collections collected in Southern Africa and the Americas. Simple sequence repeat (SSR) genetic analysis of 60 Turkish and 31 exotic accessions of bottle gourd indicated that Indian accessions were not closely related to bottle gourd accessions from other parts of the world, and Turkish accessions were not clustered according to their geographic origin in Turkey (Gürcan et al. 2015). Comprehensive information about the genetic diversity in bottle gourd germplasm with respect to disease/pest resistance is not available.

In Japan, bottle gourd is increasingly used as a rootstock to manage soil-borne diseases, specifically Fusarium wilt of watermelon (Oda 2002), as most of the rootstocks of bottle gourd are non-hosts to *Fusarium oxysporum* f. sp. *niveum*, the pathogen that causes Fusarium wilt of watermelon (Cohen et al. 2007, Bruton et al. 2009). In the United States, interest in grafting has increased due to the phase-out of methyl bromide for soil fumigation. Indian bottle gourd accession PI 271353 was reported resistant to cucurbit powdery mildew caused by *Podosphaera xanthii* (Kousik et al. 2008). USVL#1–8 and USVL#5–5, two breeding lines of bottle gourd derived from Indian accessions were resistant to *Zucchini yellow mosaic virus* (ZYMV), *Papaya ringspot virus* strain watermelon (PRSV-W), *Watermelon mosaic virus* (WMV), and *Squash vein yellowing virus* (SqVYV) (Ling et al. 2013). Resistance to multiple viruses also has been reported in Cow Leg, a variety from Taiwan (Provvidenti 1981). Narendra Shishir, a cultivar from India, has been observed to be resistant to anthracnose, downy mildew, and an unspecified viral disease complex (Singh 2013). Resistance to crown rot caused by *Phytophthora capsici* has been reported in commercial bottle gourd rootstocks 'FR-Strong', 'Emphasis', 'Marcis', and 'WMXP-3938' (Kousik et al. 2012). Commercial bottle gourd rootstocks 'FR-Gold', 'Skopje', and 'Brecik' were reported tolerant to salinity (Yetişir and Uygur 2010). Important hybrid cultivars developed by the private seed sector in India include Warad (Mahyco Seeds), Sharda (Seminis), Sarita (VNR Seeds), Vidya and Swati (Sungro Seeds), Anmol and Gadda (East-West Seed), Mallika (Bio Seeds), and Anokhi (Nunhems India). These hybrids display high-level resistance to Fusarium wilt and have consistently high yields with better fruit quality than open-pollinated cultivars. Bottle gourd hybrid cultivars FR-Ganggeon and FR-Sinsegye are resistant to Fusarium wilt (Huh et al. 2012).

Wax Gourd

Wax gourd [*Benincasa hispida* (Thunb.) Cogn.] is also known by several other names such as ash gourd, white pumpkin, and white gourd. Mature fruits have a thick waxy cuticle. It is an important vegetable in India, China, Bangladesh, Philippines, Vietnam, Thailand, Indonesia, Turkey and Iraq. Immature and mature fruit are edible (Marr et al. 2007). The fruit flesh is white to pale-green, with a weak

flavor. In China and Southeast Asia, thick pieces of the mature fruit are prepared in soup, and in India, fruit pieces are cooked in curries. Big fruit pieces are “candied” in India, China and Cuba (Heiser 1979). In China, a canned beverage is prepared from the fruit. Immature fruit (“Hairy melon”) is sliced and eaten raw or cooked (Walters 1989). Young leaves, vine tips and flower buds are also consumed as boiled greens.

The Indo-China region is regarded as the center of origin (Robinson and Decker-Walters 1997), and the genus *Benincasa* is considered monotypic. Related wild species of *Benincasa* are not known. Four major cultivar groups are recognized based on the vegetative, floral, fruit and seed traits (Walters and Decker-Walters 1989, Bates and Robinson 1995):

1. *Unridged winter melon group* comprises large cylindrical fruits (50–100 cm) with a dark-green rind that has little or no waxy bloom and unridged seeds. This group, along with the next two groups, is common in China and parts of western Asia.
2. *Ridged winter-melon group* is similar to the first group with the exception of its ridged seeds.
3. *Fuzzy gourd group* cultivars have small, narrowly cylindrical fruits (20–25 cm), light-green to green fruit skin covered with soft white hairs without waxy bloom and ridged seeds. This group is also common in Southeast Asia.
4. *Wax gourd group* predominates in India and other parts of South Asia. Fruits are covered with a white, waxy bloom; seeds are mostly ridged. Marr et al. (2007) proposed 16 cultivar groups based on the wide range of fruit size, color, shape and intensity of waxy bloom.

   India and China hold maximum diversity in terms of fruit traits (Fig. 3) with fruit weight ranging from 1.5 to 50 kg, and various shapes such as round, oval, oblong, long cylindrical, and short cylindrical. Fruit skin color varies from light-green to dark-green, and speckled green. Fruits may carry strong, medium, or weak wax, or be wax-less. There are five categories of seed size: super small seed (90–95 seeds/g,)very small seed (60–65 seeds/g), small seed (35–40 seeds/g), medium seed (20–25 seeds/g), and large seed (10–12 seeds/g).

   Landraces are still grown by local people in different regions of Asia. Mo-kwa, a high yielding landrace, is heat-tolerant. Cultivar Chi-fon is popular in Taiwan, and is highly resistant to ZYMV, *Cucumber mosaic virus*, PRSV-W, and *Melon vein-banding mosaic virus*. Wax gourd is an important crop in Vietnam, where it is cultivated on more than 33,000 ha annually.

   Round or oblong fruits (6–8 kg) of light-green to dark-green color are preferred by Indian consumers, whereas long cylindrical fruits (1–2 kg) with dark-green color and white specks are preferred by consumers in Vietnam. In northeast India, landraces are genetically divergent from those originating from other parts of the country (Pandey et al. 2012). Popular open-pollinated and hybrid cultivars in India include KAU local, Indu (tolerant to begomoviruses), CO-1, Kashi Ujwal, Kashi Dhawal, Kashi Surabhi (best for *petha,* a sweet dish), Virat F1 (tolerant to begomoviruses) and Siddhi F1 (Rasi Seeds), MAH-1 F1 (Mahyco

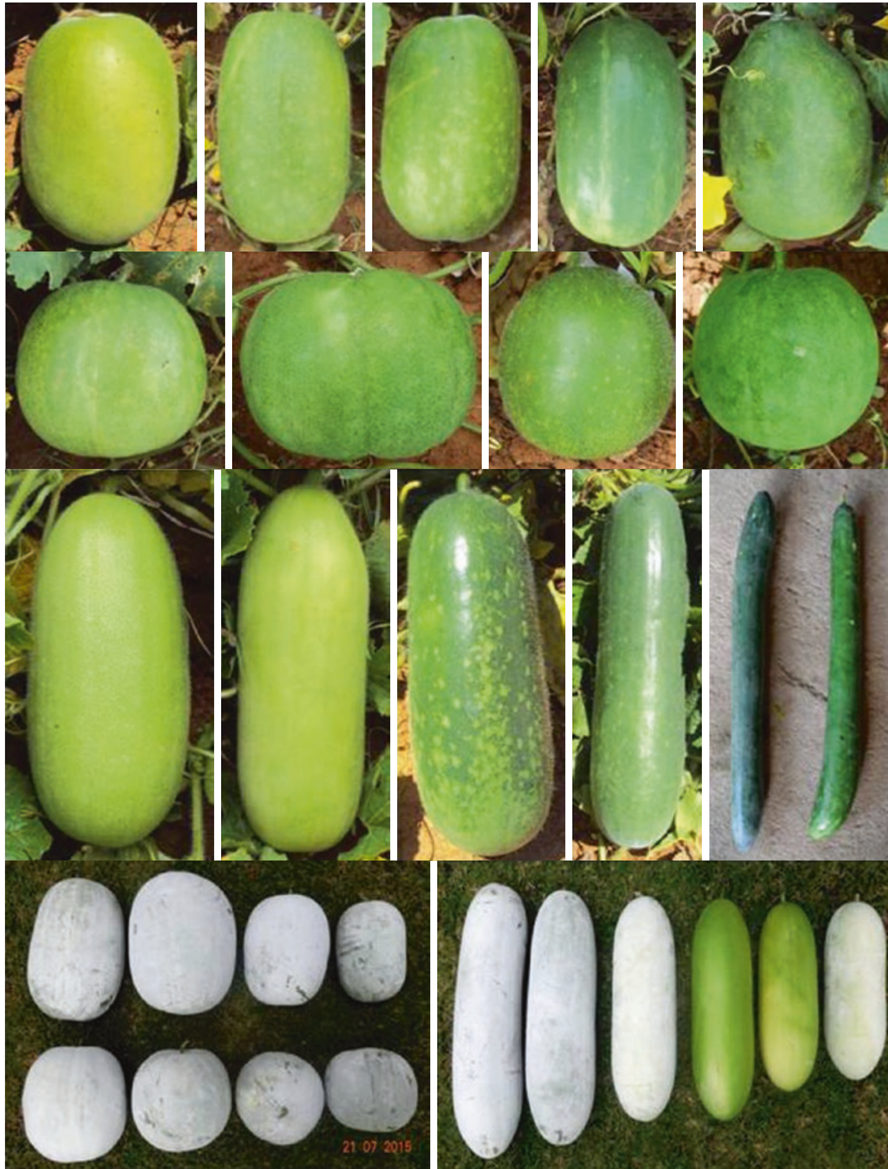

Fig. 3 Wax gourd: variation in fruit among varieties

Seeds), Sowmya F_1 (Beejo Sheetal), No 600 F_1 and No 700 F_1 (Sungro Seeds), Rakhiya F_1 (VNR Seeds), Pearl F_1, Jade F_1 and Gold 195 F_1, a wax-less cultivar (East-West Seeds), Heera F_1 and Greena F_1 (Chia Tai Seed). Wax gourd cultivars popular in Japan are Okinawa No 1 and Kurokawa Early. The Field Crops Research Institute (FCRI) in Vietnam has developed improved lines through

selection from landraces such as Wax gourd No.1, Wax gourd No. 2, Wax gourd Thien Thanh 5, Wax gourd Sac, Wax gourd Chu Thap and Wax gourd Da.

The WorldVeg listed 285 wax gourd accessions in its genebank in 2016; 13 % (36) are available and 87 % are inactive (Table 1). FCRI Vietnam has more than 200 wax gourd accessions stored in its genebank. In India more than 222 accessions are maintained in NBPGR, New Delhi. GRIN listed 106 wax gourd accessions in 2016 from six countries, including 21 from India and 57 from China. Thirteen (12 %) GRIN accessions were available for distribution and 75 (71 %) were inactive (Table 1).

Snake Gourd

Snake gourd [*Trichosanthes cucumerina* L.'Anguina' (L.) K.Pradheep, D.R.Pani&K.C.Bhatt] is an annual, creeping cucurbit well-adapted to humid lowland tropics. The common name refers to the narrow, twisted and elongated fruit that resembles a snake. It is commercially cultivated in India, Sri Lanka, Thailand, China, and Japan. Fully-grown, tender, cooked fruits are eaten. The crop was domesticated in India (Li 1970). The genepool of snake gourd (probably primary) includes *T. cucumerina* (syn. *T. lobata*) and its two subspecies *villosula* (*T. perrottetiana*) and *sublobata* (Pradheep et al. 2015). Both subspecies are localized in peninsular India with the former being restricted in mid- to high-altitudes of southern Western and Eastern Ghats, and the latter confined to parts of Goa, Maharashtra and adjoining areas. Intermediary forms between cultivated ('Anguina') and wild populations of *T. cucumerina* (often designated as *T. lobata*) are richly distributed in the central and eastern India. In India, snake gourd is commonly known as "padwal," "chichinda," and "serpent gourd." Two fruit colors predominate: light-green with white stripes, and dark-green with pale-green stripes (Fig. 4). Popular open-pollinated cultivars developed through selection from local landraces include APAU Swetha, CO-1, CO-2, CO-4, PLR (SG) 1 PLR (SG) 2, MDU-1, PKM-1, TA-2, TA-19, Baby (TA-23), Konkan Sweta, Manusree, Harithasree, and Kaumudi. Hybrid cultivars released in India include MHSN 1 (Mahyco Seeds), BSS 694 (BeejoSheetal), and Snaky White Short (Ashoka Seeds). These hybrids are becoming popular due to their high yield potential, vigorous and strong vines, and attractive milky-white fruits.

The WorldVeg listed 71 accessions of snake gourds in 2016; 90 % were inactive (Table 1). GRIN listed 37 *T. cucumerina* accessions from eight countries with most from India (25 accessions), but none were available (Table 1).

Sponge Gourd and Ridge Gourd

Sponge gourd (*Luffa cylindrica* Roem., syn. *L. aegyptiaca* Mill) and ridge gourd [*L. acutangula* (L.) Roxb.] are the two cultivated species of the genus *Luffa*. Wild species include *L. graveolens* Roxb. (var. *longistyla*), *L. echinata* Roxb., *L.*

Fig. 4 Snake gourd: fruit of local Indian varieties

tuberosa Roxb., and *L. umbellata* Roem. *Luffa* is considered to be an essentially Old World genus (Seshadri and More 2009). *L. quinquefda* (Hook and Arn) Seem. and *L. astorii* Svans. are wild species confined to the New World (Seshadri and More 2009). Another wild species, *L. saccata* was discovered in the Northern Territory of Australia (Telford et al. 2011). In the absence of convincing evidence, Whitaker and Davis (1962) reported Indo-Burma to be the center of diversity of sponge gourd, and it reached the Mediterranean by the third century CE for use as food (Avital and Paris 2014). Slightly later than that, Byzantine mosaics depicted both immature and mature sponge gourds, and it is presumed they were depicted because they were used as food (immature) and for hygiene (mature) (Avital and Paris 2014).

India has been suggested as the center of origin of ridge gourd and this species is represented by three botanical varieties: var. *acutangula,* which is cultivated in southeastern Asia and other tropical areas but to a lesser extent than sponge gourd; var. *amara* (Roxb.) C.B. Clarke, which is a wild form confined to India; and var. *forsakii* (Harms) Heiser & Schilling, a wild form (Robinson and Decker-Walters 1997). Sponge gourd also is known as towel gourd, smooth loofah, vegetable sponge, and dishcloth gourd. Ridge gourd is also known as angled loofah, ribbed gourd, silk gourd, and Chinese okra. The immature, tender fruits of these two species are consumed as cooked vegetables. The mature, fibrous endocarp can be used as a sponge, the loofah scrubbing sponge, and is popular with consumers in the U.S.A., Japan and Asia.

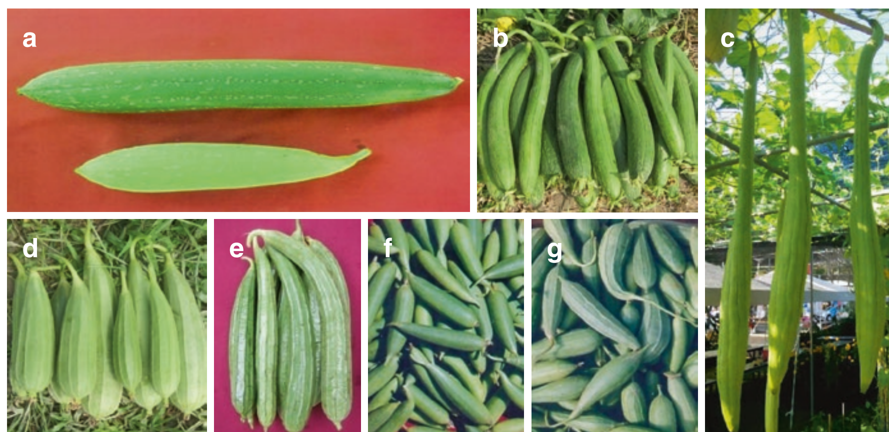

Fig. 5 *Luffa* spp. fruit variation: *L. cylindrica* (**a**–**c**), *L. acutangula* (**d**–**f**), and *L. hermaphrodita* (Satputia) genotype (**g**)

China, India, Korea, Japan and Central America are the major regions of commercial cultivation of *Luffa*. Great variability for fruit size, shape and color is observed in both species of *Luffa* (Fig. 5). Flowers of these two species are monoecious. A variant form of ridge gourd, locally known as "Satputia" in India, is hermaphroditic and bears fruits in clusters of 5–7. It was given a separate taxonomic status as *L. hermaphrodita* (Singh and Bhandari 1963). Gynoecious landraces of ridge gourd have been reported from the Hoogly district of West Bengal, India (Munshi et al. 2010–2011).

Resistance to melon fruit fly (*Bactrocera cucurbitae*) has been observed in Indian ridge gourd cultivars AHRG-29, AHRG-57 and Pusa Nasdar (Haldhar et al. 2015). Sponge gourd cultivar DSG-6, which is resistant to *Tomato leaf curl New Delhi virus* (ToLCNDV) was developed through selection from a landrace that originated from Hoogly district, West Bengal, India (Islam et al. 2011; Munshi et al. 2015). Resistance to this virus is governed by single dominant gene, and two sequence-related amplified polymorphism (SRAP) markers closely linked to the resistance gene have been developed (Islam et al. 2010). 'Arti' is the first begomovirus-resistant ridge gourd hybrid released in India (VNR Seeds).

Popular ridge gourd hybrid cultivars include Naga, Mallika, Rama (East-West Seeds), NS-3 (Namdhari Seeds), Aneeta (Advanta India), MHRG-7, Surekha (Mahyco), Gaurav and Pallavi (Sungro Seeds). These hybrids are widely preferred in India due to their adaptability, prolific fruit setting and best fruit quality (attractive light-green to green color, 25–40 cm long, prominently ridged tender fruits with rich taste). Popular ridge gourd cultivars derived from landraces include Pusa Nasdar, Pusa Nutan, Hisa Kali Tori, Gujarat Anand Ridge Gourd-1, and Pant Tori-1.

Sponge gourd cultivars popular with farmers in India include White Seeded (Century Seeds), Alok (VNR Seeds), Lohit (Tropica Seeds), Nutan and Sonali (Sungro Seeds), NS 441 (Namdhari Seeds), Maya (Bio Seeds), Harita (Mahyco) and NHSG (Nirmal Seeds). These hybrids gained prominence and popularity among

farmers due to their strong and vigorous vines, high yield potential, wide adaptability and nearly cylindrical, 20–30 cm long, tender fruits with attractive dark-green or light-green colors. Popular open-pollinated cultivars developed through selection from landraces of sponge gourd include Pusa Chikni, Pusa Supriya, Pusa Sneha, Azad Tori-2, and Pant Chikni Tori-1. In India, consumers prefer unripe tender fruit of sponge gourds and ridge gourds before it becomes fibrous, irrespective of the size.

The WorldVeg listed 341 accessions of *L. acutangula* in 2016, but 95 % are inactive (Table 1). GRIN listed 67 *L. acutangula* accessions in 2016 from nine countries; 50 of them from India. Fifty-nine of the accessions are currently available (Table 1). The WorldVeg listed 409 accessions of *L. aegyptiaca* in 2016, but 96 % were inactive. There are 78 *L. aegyptiaca* accessions in the GRIN collection from 13 countries; 54 of them are from India. Sixty-four (82 %) of the *L. aegyptiaca* accessions are currently available (Table 1).

Conclusion

These gourd species, except bottle gourd, were domesticated in Asia. Fruits of the wild relatives of these gourds are small, oblong/ovoid/spindle and bitter (Gaikwad et al. 2008; Telford et al. 2011; Pradheep et al. 2015). Selection for large, elongated fruit size and non/less bitterness was practiced during domestication. The selection might have occurred in several independent events in different regions of domestication, as it happened in two separate occasions in the case of evolution of fruit shape from round to elongated in two subspecies of *Cucurbita pepo*: *C. Pepo subsp. Pepo* and *C. pepo* subsp. *Texana* (Paris et al. 2012). These gourds are nutritious and are important sources of livelihood for resource-poor farmers in Asia, and can be grown in various agro-climates. These cucurbits are key components of home and community gardens in the tropics. Landrace-derived cultivars are rapidly being replaced by modern hybrid cultivars, which has already led to a narrowing of their genetic bases. Breeders recycle the genetic material through repeated use of elite hybrids to derive inbred lines for hybrid development. Controlling potyviruses and begomoviruses, and the fungal diseases powdery and downy mildew, are major challenges for cucurbit growers. Development of short-vine and day-neutral cultivars with higher female:male flower ratios is another goal for breeders. Extensive collection, conservation and evaluation of minor cucurbit landraces are necessary to stem genetic erosion and identify sources of resistance to economically important biotic and abiotic stresses.

The germplasm resources of these gourd species are held in an array of genebanks in several countries and may not be readily available for scientific research or to commercial breeders outside of their respective country. Numbers of the WorldVeg and GRIN holdings vary greatly among the species. Their availability ranges from less than 1 % (*T. cucumerina* and *M. charantia*) to 88 % (*L. acutangula*) and many are inactive, ranging from 7 % (*L. acutangula*) to 81 % (*T. cucumerina*). The WorldVeg holdings of these species are larger than GRIN, as might be expected, but their collections suffer from larger percentages of inactive accessions, ranging from 63 % (*M. charantia*) to 97 % (*L. siceraria*).

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The Melon Genome

Josep Casacuberta, Pere Puigdomènech, and Jordi Garcia-Mas

Abstract The availability of next-generation sequencing technologies in the past years has allowed unprecedented access to draft genome sequences for the main crops and plants. These genome sequences offer new possibilities for studying genome evolution, for understanding the biological processes controlling important traits and for improving plant breeding. Among them, the genome of melon (*Cucumis melo* L.), one of the main cucurbit crops, was released in 2012. Since the publication of the reference genome of the double haploid line DHL92 v3.5, the genome assembly and annotation have been improved in new genome versions. The melon genome has been useful for performing several comparative analysis with other cucurbit genomes, for analysing the genome structure and extant variability and for the isolation and characterization of several important genes.

Keywords Genome sequence • Melon • Next-generation sequence • Genome structure • Resequencing

Introduction

Melon (*Cucumis melo* L.) is a diploid species (2n = 2x = 24) that belongs to the Cucurbitaceae family, which includes other main crop species as cucumber, watermelon, pumpkin and squash. Although it was believed that melon originated in Africa, its Asian origin has been recently proposed (Sebastian et al. 2010). Melon is divided in two subspecies *melo* and *agrestis*, which contain 16 botanical groups and include both cultivated and wild accessions (Pitrat 2008).

The melon genome sequence was completed in 2012 in the framework of the MELONOMICS project (http://www.melonomics.net) (Garcia-Mas et al. 2012). The objective of this project was to produce a high quality draft sequence of the

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melon genome after the advent of next-generation sequencing technologies in the late 2000s. The first plant genome sequences were produced using clone-by-clone strategies, as for the Arabidopsis and rice genome projects (Arabidopsis Genome Initiative 2000; International Rice Genome Sequencing Project 2005), and later on using whole genome shotgun, as for poplar (Tuskan et al. 2006), grape (Jaillon et al. 2007) or papaya (Ming et al. 2008). These first plant genome sequences were obtained using Sanger sequencing. Since 2009, when the first whole genome shotgun sequence of a plant was released using exclusively next-generation sequencing (Huang et al. 2009), many plant genomes have been completed and many more are in the pipeline (Michael and Jackson 2013). The availability of the genome sequences of many model plants and crops has boosted plant biology in the last few years, providing new opportunities for both understanding basic biological questions and to help in the process of plant breeding.

Before the availability of the genome sequence, several genetic and genomic resources were obtained in melon, which will be described in other chapters of this book. However it is important to note that some of them were useful for obtaining a high-quality sequence of the melon genome. Genetic maps (Diaz et al. 2011) were used to anchor the genome assembly and build pseudomolecules. BAC-end sequences (Gonzalez et al. 2010) were extremely important for improving the genome scaffolding. Finally, EST and RNA-seq databases (Clepet et al. 2011; Blanca et al. 2011) were used during the gene prediction and annotation step.

In this chapter we describe the main melon genome sequence features and several improvements since its publication in 2012.

Genome Sequence

Genome Assembly into Pseudomolecules

The melon line selected for sequencing the genome was the double haploid line DHL92, which was generated from the cross between the *agrestis* melon type PI 161375 (SC) and the *inodorus* type “Piel de sapo T111” (PS). The use of a double haploid line ensured a better quality of the genome assembly due to its homozygosity. The melon genome was sequenced using a shotgun strategy with 454 single reads complemented with paired-end sequences obtained from 3, 8 and 20 kb libraries. Additionally, 53,203 BAC-end sequences (BES) (Gonzalez et al. 2010) were used to improve the genome scaffolding. The melon genome sequence v3.5 contained 1,594 scaffolds and 29,865 contigs, totalling 375 Mb of sequence with a N50 scaffold size of 4.7 Mb. This represents 82.5 % of the 454 Mb reported genome size (Arumuganathan and Earle 1991). 40 Mb of the assembled sequence corresponded to gaps, and 90 % of the assembly was contained in only 78 scaffolds. The comparison of the genome assembly with the sequence of a set of BACs that were previously sequenced with Sanger confirmed more than 92 % of identity, with mismatches corresponding to gaps in the genome assembly. More than 95 % of unigenes from

the ICuGI database (www.icugi.org) (Clepet et al. 2011) mapped unambiguously to the genome assembly, confirming a good representation of the gene space.

The combined use of 454 sequencing, paired-end reads of up to 20 kb and BES produced a robust assembly with similar statistics to the best published plant genome assemblies to date. However, next-generation sequencing strategies failed to properly assemble repetitive regions, with 14 Mb of contigs sequence not contained in the scaffold assembly (361 Mb) and the unassembled genome fraction mainly containing repetitive reads.

A SNP genetic map was used to build pseudomolecules from the genome assembly. A GoldenGate array containing 768 SNPs (Esteras et al. 2013) was used to genotype the SC x PS double haploid mapping population (Gonzalo et al. 2005). A total of 87 scaffolds containing 87.5 % of the scaffold assembly were assembled in 12 pseudomolecules, of which 71 were oriented. During this procedure, several misassembled scaffolds were detected and corrected.

Genome Annotation

Before the annotation of the gene repertoire of the genome, the transposable element (TE) fraction was annotated using homology and structure-based searches. A conservative approach was used in order to ensure a high quality of the annotation. 19.7 % of the genome assembly was identified as relatively well conserved TEs, corresponding to more than 70,000 copies. The distribution of the TE fraction corresponded to 14.7 % of retrotransposons and 5 % of DNA transposons.

After masking the repetitive fraction of the genome, the genes were predicted using *ab initio* programs, homology to protein databases and transcript support. This procedure allowed predicting 27,427 genes, which were functionally annotated. The genome assembly was also used to predict 1,253 noncoding RNAs (ncRNAs). The melon genome contains 411 predicted disease resistance genes (R-genes), 81 of them from the NBS-LRR type, the rest corresponding to receptor-like kinases. The already known distribution of R-genes in clusters was observed in melon, with some of the clusters mapping in regions where disease resistances have already been mapped (González et al. 2014). A common feature observed in the three cucurbit genomes available (melon, cucumber and watermelon) is the low number of annotated R-genes (<100) when compared with Arabidopsis or grape (212 and 302, respectively).

Genome Structure

Genome Duplications

There is no data supporting recent whole genome duplication (WGD) events in the melon genome since the common paleo-hexaploidization described for eudicots (Jaillon et al. 2007). The same conclusion can be reached from the analysis of the

cucumber and the watermelon genomes. In melon, several analyses were performed that detected a limited fraction of the genome in the form of segmental duplications, which may correspond to the expansion of several gene families.

TE Amplification

An analysis of the complete LTR retrotransposons annotated in the melon genome revealed that most of them were inserted around 2 million years ago, later than the estimated date of divergence between melon an cucumber. The fraction of TEs in the cucumber genome, annotated using the same pipeline than in melon, is much lower than that of melon, suggesting that the difference in genome size between melon and cucumber may be explained, at least in part, by recent bursts of transposition in melon (Garcia-Mas et al. 2012).

The Melon Phylome

A phylogenomic analysis was performed after reconstructing more than 22,000 phylogenetic trees between melon and 22 other dicots, monocots, mosses and algae protein-coding genes. The phylogenetic trees can be accessed at http://phylomedb.org. This is a useful tool that can be used to retrieve orthologues and paralogues in other plant species, and which was also used to help in the functional annotation of the melon genome. The melon genome has been recently included in the plant comparative genomics PLAZA 3.0 resource (Proost et al. 2015), a database that includes 37 plant genomes and allows browsing the annotated genomes, gene families and phylogenetic trees in a comprehensive way.

Comparison Between Melon and Cucumber Genomes

Previous comparisons of melon (2n = 2x = 24) and cucumber (2n = 2x = 14) synteny suggested an ancestral fusion of five melon chromosome pairs in cucumber, with two remaining unaltered chromosomes, and with extensive inter- and intrachromosome rearrangements (Huang et al. 2009; Li et al. 2011). As an example, melon linkage group 1 (LG1) corresponded to cucumber chromosome 7, but with extensive rearrangements and an increase in the total chromosome size (35.8 vs. 19.2 Mb), in line with the overall larger genome size of melon. The recent sequence of the *Cucumis hystrix* genome (2n = 2x = 24), a close relative to cucumber, revealed that the ancestor of cucumber was n = 12 and the cucumber genome was formed through chromosome fusion and extensive rearrangements (Yang et al. 2014).

Recombination Pattern Across the Genome

A region of recombination suppression was found in each melon pseudochromosome, which may correspond to the position of the centromeres and large pericentromeric regions (Sanseverino et al. 2015). The recombination ratio is positively correlated with the gene density and negatively correlated with the TE distribution along all 12 melon chromosomes. This unequal distribution of the recombination frequency may have important consequences, for example when identifying genes by positional cloning.

Recently the karyotype of the PS melon line has been reported and the chromosomes were assigned to the 12 linkage groups using fluorescent in situ hybridization (FISH) with BACs that produced chromosome-specific signals (Argyris et al. 2015b). It was also possible to make inferences on melon chromosome structure by relating the large regions of recombination suppression to centromeres and 45S and 5S heterochromatic regions in silico.

Sequence of the Organellar Genomes

Both the melon chloroplast and mitochondrial genomes were also reported (Rodriguez-Moreno et al. 2011). The chloroplast genome was obtained from the whole-genome sequence reads, it is 156 Kb in size, and includes 132 genes and is 95 % similar to the cucumber chloroplast sequence. The melon mitochondrial genome is 2.74 Mb in size, one of the largest among cucurbits, includes 51 protein-coding genes and a large fraction of repetitive sequences and DNA of nuclear origin. Due to the repetitive nature of the mitochondrial genome, its sequence was obtained after the purification and isolation of mitochondria. A minimum cucurbit mitochondrial genome core of 119 Kb was also reported.

Further Improvements of the Published Genome Version

Since the publication of the melon genome sequence v3.5 in 2012, an improvement of the genome anchoring to chromosomes and pseudomolecules build v3.5.1 has been released (Table 1) (Argyris et al. 2015b). As 98.5 % of the scaffold assembly v3.5 was contained in only 150 scaffolds, and more than a million SNPs were available after resequencing the genome of the parental lines SC and PS (Garcia-Mas et al. 2012), SNPs were selected in the edges of the largest 150 scaffolds and used to build a genetic map containing 580 SNPs with 139 F2 individuals from a cross PS × SC. This "reverse" strategy was designed to map unanchored scaffolds to chromosomes, instead of building a genetic map with random SNPs first and using them to anchor the scaffolds to chromosomes. One hundred forty-one scaffolds

Table 1 Comparison of melon genome assembly versions v3.5 and v3.5.1

Genome version	v3.5	v3.5.1
No of scaffolds	1,599	1,605
Scaffold assembly size (Mb)	361.4	361.3
No anchored scaffolds	87	141
Scaffold assembly anchored (Mb)	316.3	354.8
No oriented scaffolds	71	99
Scaffold assembly oriented (Mb)	291.9	327.0
Reference	Garcia-Mas et al. (2012)	Argyris et al. (2015b)

containing 354.8 Mb of genome assembly (98.2 %) were anchored to the 12 melon chromosomes, with 90.5 % of them oriented. 27.8 Mb of scaffold assembly remained unanchored in pseudomolecule 0. This new assembly represented a substantial improvement of the melon pseudomolecules when compared with v3.5.

Illumina paired-end reads were used to resequence the reference genome line DHL92 in order to close gaps and further refine v3.5 (González et al. 2014). The use of the PGIT toolkit allowed substantially reducing the number of small contigs and slightly decreasing the number of Ns in the genome assembly. However, even after this improvement, the number of gaps is still high due to the use of short reads, and manual curation may be necessary to complete some genomic regions. A further improvement of the assembly has been recently obtained by using whole-genome mapping, an automated optical mapping method (Zhou et al. 2004). Using this technology, the N50 scaffold size has been raised to 15.5 Mb (4.7 Mb in v3.5), and 90 % of the assembly is now contained in 22 scaffolds (78 scaffolds in v3.5) (Garcia-Mas, unpublished data).

This corrected and improved version of the melon genome is being used for an improved annotation. The already reported conservative annotation of TEs is being complemented with a more thorough annotation using very sensitive pipelines, and the gene annotation is being refined using all RNAseq available (Casacuberta, Puigdomènech and Garcia-Mas, unpublished data).

Resequencing of Melon Germplasm

The availability of a reference genome of a species offers an excellent opportunity to study genome diversity at the whole genome level, with the possibility to discover SNP and short indel variation, but also structural variation (SV) and transposon insertion polymorphisms. A first attempt to study melon genetic diversity has been performed resequencing the genomes of seven melon accessions from diverse origin and representing the extant diversity of the species (Sanseverino et al. 2015). The study contained two elite melon lines (PS and Védrantais), three landraces (C-1012, PI 161375 (SC) and PI 124112) and two wild accessions (Ames-24297, previously classified as *Cucumis trigonus* and C-386).

Illumina paired-end sequencing was performed for the seven melon accessions, and reads were mapped to the reference genome. The analysis of the mapped reads allowed

the discovery of 4.4 million SNPs, 719,000 indels and 3,609 SVs. Moreover, the analysis using available as well as a newly designed bioinformatic program (Hénaff et al. 2015) yielded 2,735 transposon insertion polymorphisms. This analysis revealed that the genetic variability was greatly reduced among the elite accessions, which could be related with selection during breeding. The study also revealed that transposons polymorphisms may represent an important part of the variability found in melon. Some of the regions containing large SV corresponded to clusters of R-genes.

Future Prospects

A genome sequence is a useful tool to study the molecular basis of important plant biological processes and to develop approaches for plant breeding. In this sense the melon genome has been shown to be useful in both directions. It has allowed identifying genes and QTLs responsible for important agronomic traits (Argyris et al. 2015a; Cohen et al. 2014; Feder et al. 2015; Tzuri et al. 2015) or studying the basis for genetic variability (González et al. 2014; Sanseverino et al. 2015). A new anchoring of genome scaffolds to the genetic map (Argyris et al. 2015b) has also been published and it may be an important tool for the molecular characterization of genes of interest for breeding.

A reference genome sequence may also be a first step to obtain a complete description of the genome of a given species. On one hand it may be important to obtain genome sequences of the best possible quality. Any genome sequence is always an approximation to the genome of a species. Drafts produced by using next generation sequencing techniques contain a number of gaps and they do not allow the assembly of regions containing highly repetitive sequences. Therefore the use of other approaches such as those generating longer DNA reads (Eid et al. 2009) may be important to complete available genome sequences, even those that are considered of good general quality such as the one from melon. On the other hand just a single genome sequence does not provide a proper insight of the variability of the genome within the populations forming the species. Therefore it would be very important to resequence melon varieties both within breeding collections that would provide tools for understanding the traits important for breeding and within the broad basis of the species that would provide information about domestication or about traits that are important for the species to adapt to specific environments. Whereas in cucumber the resequencing of 115 lines representing the extant variability has been completed (Qi et al. 2013), a similar wide approach is still lacking in melon.

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The Cucumber Genome

Yiqun Weng

Abstract Cucumber, *Cucumis sativus* L. (2n = 2x = 14) is both an economically and biologically important vegetable crop which has been used as a model to study sex expression in plant for a long time. While the genetic and genomics resources in cucumber are limited as compared with field crops, recent advances in technology and instrumentation for sequencing of plant genomes are providing exciting opportunities to expedite cucumber genome research. Among major horticultural crops, cucumber was the first to have a publicly released draft genome. Cucumber has some advantages for genome research due to its relatively small genome size (~367 Mbp), low percentage of repetitive DNA and short life cycle. Since the release of the cucumber genome sequence, significant progress has been made in our understanding of the cucumber genome. In this chapter, I will review recent progress in cucumber draft genome assembly, genetic map development, whole genome features of characterized gene families, and the genome dynamics from evolutionary, domestication and population perspectives.

Keywords *Cucumis sativus* • Whole genome sequencing • Draft genome assembly • Genomics • Genome evolution • Domestication

Introduction

Cucumber (*Cucumis sativus* L.) is both an economically and biologically important vegetable crop. Cucumber has been used as a model to study several biological processes (Weng and Sun 2011). For example, the monoecious cucumber has long been served as a model system for sex determination studies driven by breeding programs for hybrid seed production. Cucumber (also melon and *C. hystrix*) represents a unique model plant for organellar genetics because its three genomes show different modes of transmission: maternal for chloroplast, paternal for mitochondrial, and biparental for nuclear genes (Ward et al. 1981; Havey et al. 1998; Lilly

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and Havey 2001; Shen et al. 2015). The cucumber chloroplast and mitochondrial genomes have been sequenced (Kim et al. 2006; Chung et al. 2007; Plader et al. 2007; Alverson et al. 2011), which is the topic of Chapter 13 of this series. Cucumber and pumpkin/squash are preferred models for phloem physiology because of both the ease of sampling phloem sap and the facile visualization of their large phloem sieve elements (Eschrich et al. 1971; Clark et al. 1997; Zhang et al. 2010). Most species in the Cucurbitaceae family have basic chromosome numbers of 7, 11, 12, 13, or 20. Among the ~52 known species in the genus *Cucumis* (Sebastian et al. 2010), cucumber ($2n=2x=14$) has the lowest chromosome number and its genome size of ~367 is also relatively small (Arumuganathan and Earle 1991), which provides us a good system to study chromosome evolution in cucurbits.

As a minor horticulture crop, as compared with many field crops, most aspects of cucumber research are lagging far behind. Cucumber genetic and genomic resources are rather limited. However, recent advances in technology and instrumentation for sequencing of plant genomes are providing exciting opportunities to expedite cucumber genome research. In fact, among major horticultural crops, cucumber was the first whose genome was sequenced (Huang et al. 2009). Since the release of the cucumber sequence, significant progress has been made in our understanding of the cucumber genome. Here I will review recent progress in cucumber draft genome assembly, genetic map development, whole genome features of characterized gene families, and the genome dynamics from evolutionary, domestication and population perspectives.

Cytological View of the Cucumber Genome

Cytological investigation in cucumber has been carried out for several decades. Early studies focused on establishing a standard karyotype to distinguish different cucumber chromosomes based on morphological features, meiotic behavior, or C-banding of mitotic chromosomes (e.g., Bhaduri and Bose 1947; Trivedi and Roy 1970; Ramachandran and Seshadri 1986; Hoshi et al. 1998; Chen et al. 1999; Koo et al. 2002). Ramachandran and Seshadri (1986) analyzed meiotic chromosome pairing in cucumber pollen mother cells (PMCs) and estimated the mean chiasmata per PMC were 14.84 ± 0.28 (2.12 per bivalent). This gave an estimate of map length for the cucumber genome in the range of 750–1000 cM. However, owing to the small size, poor stainability, and morphological similarity, the chromosomes in cucumber have not been well characterized until molecular cytogenetic tools were developed.

With combined use of C-banding and fluorescence in situ hybridization (FISH) with 45S and 5S rDNA probes, Koo et al. (2002) was able to distinguish 5 out of the 7 cucumber chromosomes. Koo et al. (2005) then isolated two repetitive DNA sequences (CsRP1, CsRP2) as probes in FISH to identify mitotic metaphase and meiotic pachytene chromosomes in cucumber. CsRP1 and CsRP2 are localized mainly in centromeric and/or pericentromeric regions. Simultaneous use of 5S rDNA, CsRP1 and CsRP2 probes allowed establishing a standard karyotype for

Table 1 Cucumber chromosome lengths in terms of physical measurment and assembled DNA sequences in Mbp of each chromosome

Chr	Physical length (μm)[a]	Relative length[b]	Heterochromatin (%)	9930 V1.0 (Mbp)	9930 V2.0 (Mbp)	Gy14 V1.0 (Mbp)
Chr1	107.4	14.9	15.4	26.0	29.1	28.4
Chr2	102.9	14.3	20.6	22.4	23.2	23.5
Chr3	129.1	17.9	5.6	34.1	39.8	40.3
Chr4	102.1	14.2	14.1	22.1	23.4	23.4
Chr5	95.0	13.2	11.4	28.5	28.0	27.5
Chr6	110.0	15.3	6.1	26.8	29.1	30.2
Chr7	73.2	10.2	13.2	17.5	19.2	19.3
Total	719.7	100	n/a	177.4	191.8	192.6
Reference	Koo et al. (2005)	Koo et al. (2005)	Koo et al. (2005)	Huang et al. (2009)	Li et al. (2011a)	Yang et al. (2012)

[a]Pachytene chromosome length
[b]Relative length: 100× (chromosome length/total complement length)

cucumber, which has been used to assign assemblies of the 9930 and Gy14 pseudomolecules to chromosomes. DAPI-stained pachytene chromosomes also revealed heterochromatic regions, which allowed Koo et al. (2005) to measure the pachytene chromosome and heterochromatin sizes. The data are summarized in Table 1.

Repeated DNA sequences evolve quickly which are often lineage-specific and ideal to use in FISH-based karyotyping. Four types of tandemly repeated DNA sequences (Type I, II, III, IV) in the cucumber genome have been characterized, which together accounted for over 90 % of the satellite DNA in the genome (Ganal et al. 1986; Ganal and Hemleben 1988). It was estimated that Type I/II, Type III and Type IV accounted for 10.4 %, 4.0 % and 5.5 % of the cucumber genome respectively (Han et al. 2008). In the genomic sequencing reads, ~0.72 % and 0.48 % belong to CsRP1 and CsRP2 repeats, respectively; whereas 45S rDNA and 5S rDNA sequences accounted for 3.3 % and 0.1 % respectively (Han et al. 2008). The FISH signals of the Type III and 45S rDNA provide useful cytogenetic markers, whose position and fluorescence intensity allow for easy identification of all somatic metaphase chromosomes. Han et al. (2008) developed a karyotype based on FISH signals with Type I–IV repeats and 45S and 5S rDNA probes which were used to integrate genetic and physical maps in assembling the 9930 draft genome (Huang et al. 2009).

During development of the Gy14 cucumber genome assembly, Yang et al. (2012) developed a FISH-based karyotype for cucumber chromosomes using four repetitive DNA probes including the sub-telomeric type IV repeat, the pericentromeric type III repeat, the BAC-E38 repeat (Koo et al. 2010), as well as the chromosome 3-specific pericentromeric CENT3 repeat. After simultaneous application of the four repeated sequence probes, all seven chromosomes showed distinctive labeling patterns, allowing easy recognition of individual chromosomes. More recently, new FISH-based karyotyping methods have been developed with chromosome-specific

fosmid clones, single copy genic DNA or sequence-based oligo probes (Lou et al. 2014; Han et al. 2015), which are making cytological investigation of the cucumber genome more efficient.

Cucumber Draft Genome Assemblies

For the cucurbit research community, the biggest achievement in years may be the sequencing of the cucumber genome. The first cucumber used for sequencing is a northern China fresh market type inbred line '9930'. The 9930 draft genome sequence (Version 1.0, Huang et al. 2009) was assembled using a combination of traditional Sanger and Solexa Next-gen sequencing technologies. Huang et al. (2009) reported 243.5 Mbp of sequence in the 9930 V1.0 assembly. Major statistics of 9930 Version 1.0 and other draft genome assemblies are summarized in Tables 1 and 2. One important finding from the genome assembly was that cucumber did not undergo recent whole genome duplication (WGD) during its evolution (Huang et al. 2009).

The 9930 Version 1.0 draft genome assembly was based on 3.9-fold coverage of Sanger reads and 68.3-fold coverage of Illumina GA reads (average read length 42–53 bp). Due to use of the early NGS technology for sequencing, and the relatively low coverage, the accuracy at nucleotide sequence level was understandably less ideal in 9930 V1.0. The genetic map used for anchoring scaffolds was developed using 77 recombinant inbred lines from a cross between the cultivated cucumber Gy14 and the wild cucumber (*C. sativus* var. *hardwickii*) line PI 183967 (Ren et al. 2009) which only had 581 cM map length in 7 linkage groups. Later studies revealed significant chromosomal structural changes between the cultivated and wild cucumbers (Yang et al. 2012), which was the primary reason of widespread recombination suppression in the population used by Ren et al. (2009). Over one third of the markers on the map were in clusters which made it difficult to order the scaffolds in those regions. Thus, 9930 Version 2.0 was developed by another group to address problems associated with Version 1.0 (Tables 1 and 2) (Li et al. 2011a). During development of Version 2.0, Li et al. (2011a) employed 5.23G additional Illumina GAII sequencing data from 9930 and PI 183967; they also improved the prediction of protein-coding genes with evidence from RNA-Seq reads from 10 tissues of 9930. In 9930 Version 1.0, 26,682 gene models were predicted, whereas 9930 V2.0 contains 3, 434 fewer protein-coding genes, mostly because of the reduced size of the reassembly and the removal of ~2, 000 bacterial genes in 9930 V1.0 (Li et al. 2011a; Table 2). Thus in 9930 Version 2.0, there were about 8, 700 protein-coding gene structures that were corrected including ~5200 genes new to Version 1.0 (Li et al. 2011a). While Version V2.0 is a significant improvement over V1.0, the issue of mis-assemblies of many scaffolds in Version 1.0 was largely unaddressed in Version 2.0, which was found in several genetic mapping studies (e.g., Li et al. 2011b; Miao et al. 2012; Yang et al. 2013; Zhou et al. 2015).

In addition to 9930, the draft genome for the North American pickling cucumber inbred line Gy14 was also developed (Yang et al. 2012; http://www.phytozome.org).

Table 2 Key statistics of cucumber draft genome assemblies of three cucumber genotypes

Genotype	9930 V1.0	9930 V2.0	Gy14 V1.0	B10
Platform	Sanger + Solexa GA	Sanger + Solexa GA	Roche/454 Titanuim	Roche 454
Shotgun	3.9× Sanger + 68.3× Solexa	3.9× Sanger + 68.3× GA + 7.3× GAII + 7× *hardwickii*	24×	8×
3 kb or 20 kb Paired end	n/a		12×	4×
Total genome coverage	72.2×	86.5×	36×	12×
Assembler	hybrid	hybrid	Newbler	Celera or hybrid
Assembly	de novo	de novo	de novo	de novo + 64,022 BES
N50 contig size	19.8 kb	37.9 kb	37.6 kb	27.1 kb
N50 scaffold size	1140 kb	488.2 kb	993 kb	232.40 kb
Total # scaffolds	47.837	12.845	4.219	4.173
Total sequences in assembly	243.5 Mbp	197.3 Mbp	203.1 Mbp	224.5 Mbp
Annotated genes	26.682	23.248	21.491	26.587
References	Huang et al. (2009)	Li et al. (2011a)	Yang et al. (2012); http://phytozome.org	Wóycicki et al. (2011)

Main statistics of the Gy14 assembly Version V1.0 are presented in Tables 1 and 2. The Gy14 genome was sequenced with the Roche/454 technology. Anchoring of scaffolds was based on a genetic map with 735 SSR markers with a population derived from the cross between Gy14 and 9930. Integration of genetic and physical maps resulted in a chromosome-level draft genome assembly composed of 193 Mbp (Table 2). The JGI annotation of the Gy14 assembly suggested 21,491 protein-coding genes in this assembly (http://www.phytozome.org/).

Draft genome sequences for two additional cucumbers have also been reported. The first is the European pickling type inbred line 'B10' (Wóycicki et al. 2011) (statistical data in Table 2). Qi et al. (2013) carried out *de novo* sequencing and assembly of the wild cucumber accession PI 183967 with a total length of 204.8 Mb. The quality of the assemblies in both lines is not known. Both Gy14 V1.0 and 9330 V2.0 assemblies only cover ~55 % of the estimated 367 Mbp cucumber genome. Clearly a more complete, better annotated, high quality cucumber draft genome assembly is still needed to serve as a community standard.

Whole Genome Features of Important Gene Families

Many genes or gene families are present in the genome in different locations and copy numbers. The availability of draft genome allows a genome-wide view of the structure, organization and evolution of those important genes and gene families in the cucumber genome. For example, in the 9930 draft genome, Huang et al. (2009) identified 61 nucleotide-binding (NB)-containing resistance (R) gene homologs, which was 67 in the Gy14 assembly (Yang et al. 2013). Genome-wide features of a number of gene families, especially transcription factor gene families have been characterized in the cucumber genome, which are summarized in Table 3.

Cucumber Genome Sequence-Guided Genetic Map Development

Cultivated cucumber has a very narrow genetic base. Early genetic mapping efforts in cucumber were hampered by the low level of polymorphism. The whole genome sequence provides a platform for large-scale development of highly polymorphic molecular markers for many marker-based applications such as genetic mapping, gene or QTL cloning, genetic diversity analysis and genome-wide association studies. Based on the Sanger sequences of the 9930 genome, Ren et al. (2009) developed over 2000 SSR markers which have been widely used in subsequent genetic mapping studies in cucumber. Cavagnaro et al. (2010) conducted whole genome survey of microsatellite sequences in the Gy14 draft genome. From 112,073 perfect SSRs detected in the Gy14 genome, primer sequences for more than 83,000 SSRs were designed. These SSR markers have been proven very useful in various applications especially in fine genetic mapping of genes in target regions. Due to the decreasing cost of genome sequencing, more and more cucumber lines are being re-sequenced, and almost unlimited number of SNPs could be identified and employed for genetic mapping through high throughput SNP genotyping or more directly, genotyping by sequencing (GBS). Therefore, in the last serval years, a number of high-density cucumber genetic maps have been published using various marker genotyping platforms. Table 4 summarizes genetic maps developed since the release of the cucumber draft genome in 2009, which are all valuable resources for the cucumber research community. However, a high-density genetic map with high resolution from a large segregating population is still needed.

The Cucumber Genome Under Domestication

Cucumber is native to the Southern Asia continent. Three botanical varieties of *C. sativus* have been recognized including cultivated cucumber *C. sativus* L. var. *sativus*, the wild cucumber *C. sativus* L. var. *hardwickii*, and the semi-wild Xishuangbanna

Table 3 Genome-wide characterized important gene families in the cucumber genome

Gene family	Function	# members	Chr locations	Reference
bZIP	bZIP transcription factors	64	All 7 chromosomes	Baloglu et al. (2014)
CDPK	Calcium-dependent protein kinase	19	All 7 chromosomes	Xu et al. (2015a)
Dof	Dof (DNA-binding with one finger) transcription factor	36	Chr 1-6	Wen et al. (2016)
HD ZIP I	Homeodomain leucine zipper (HD-Zip) Class I proteins	13	Chr1, 3, 4, 5, 6	Liu et al. (2013)
HD ZIP II	Homeodomain leucine zipper (HD-Zip) Class II proteins	11	Chr1, 3–7	Fu et al. (2013)
HD ZIP III	Homeodomain leucine zipper (HD-Zip) Class III proteins	5	Chr1, 3, 6, 7	Fu et al. (2013)
HD ZIP IV	Homeodomain leucine zipper (HD-Zip) Class IV proteins	11	Chr1, 2, 3, 6, 7	Fu et al. (2013)
LecRK	L-type lectin receptor kinase	25	All 7 chromosomes	Wu et al. (2014)
LOX	Lipoxygenase (LOX) family	23	Unknow	Huang et al. (2009)
LRR-RLK	Leucine-rich repeat-receptor-like kinases	192	All 7 chromosomes	Wang et al. (2014)
LRR-RLP	Leucine-rich repeat-receptor-like proteins	42	All 7 chromosomes	Wang et al. (2014)
MADS-box	MADS-box transcription factors	43	All 7 chromosomes	Hu and Liu (2012)
MAPK	Mitogen-activated protein kinase	79	All 7 chromosomes	Wang et al. (2015)
MLO	MLO type R gene	13	Chr1-6	Schouten et al. (2014)
NBS-LRR	Resistance gene homologs of R genes	61	All 7 chromosomes	Huang et al. (2009)
NBS-LRR	Resistance gene homologs of R genes	57	All 7 chromosomes	Wan et al. (2013)
NBS-LRR	Resistance gene homologs of R genes	67	All 7 chromosomes	Yang et al. (2013)
PAL	Phenylalanine ammonia-lyase	7	Chr 4, 6	Shang et al. (2012)
PG	Polygalacturonase (PG)	53	All 7 chromosomes	Yu et al. (2014)
R2R3MYB	R2R3MYB transcription factors	55	All 7 chromosomes	Li et al. (2012)
WRKY	Transcriptional regulators	55	All 7 chromosomes	Ling et al. (2011)
YUC	YUCCA (YUC) proteins	10	Chr1,2,3,6,7	Yan et al. (2016)

Table 4 Cucumber genetic maps developed since release to cucumber draft genome assemblies

Year	Map	Population	Marker type	# Loci mapped	Map length (cM)	Notes	Reference
2009	Gy14 × PI 183967 RIL	71 RILs	SSR	995	572.9	Used for anchoring scaffolds	Ren et al. (2009)
2010	G421 × H19 RIL	46 RILs	SSR, SCAR	176	400.7	mapped 2 genes (*de*, *ll*)	Weng et al. (2010)
2011	9110Gt × 9930 RIL	148 RILs	SSR	248	711.5	mapped 7 genes	Miao et al. (2012)
2011	PI 249561 × PI 308915 F2	46 F2	SSR	187	527.5	mapped *cp* locus	Li et al. (2011b)
2012	Gy14 × 9930 F2	91 F2	SSR	783	706.7	Used for anchoring scaffolds	Yang et al. (2012)
2012	Consensus map	Consensus map	SSR, SCAR	1.369	700.5	Integrated from 2 maps	Zhang et al. (2012)
2013	Consensus map	Consensus map	SSR	1.681	730.0	Intergrated from 4 maps	Yang et al. (2013)
2013	WI2757 × True Lemon F2	132 F2	SSR	240	610.0	QTL mapping of PM	He et al. (2013)
2014	WI7167 × WI7200 F2	138 F2	SSR	225	775.2	QTL mapping	Qu et al. (2014)
2014	CC3 × NC76 F2	148 F2	SLAF	1.800	890.8	GBS	Wei et al. (2014)
2015	GY14 × 9930 RIL	143 RILs	SNPs	11.156	598.7	SNP array	Rubinstein et al. (2015)
2015	SWCC8 × CC3 RIL	124 RILs	SSR	269	705.9	QTL mapping	Bo et al. (2015)
2015	9110Gt × 9930 RIL	150 RILs	SNPs	116.710	1384.4	Sequence based	Zhou et al. (2015)
2015	D8 × Jin5-508 F2	102 F2	SLAF	1.892	845.9	GBS	Xu et al. (2015b)

SSR simple sequence repeats, *SLAF* specific length amplified fragment sequencing, *GBS* genotyping by sequencing

cucumber, *C. sativus* L. var. *xishuangbannanesis*. It is of interest to understand chromosome differentiation events during domestication among the three taxa. Yang et al. (2012) conducted comparative FISH analysis of pachytene chromosomes between wild and cultivated cucumbers and found significant differences in the amount and distribution of heterochromatin, as well as chromosomal rearrangements between the two taxa. In particular, 6 inversions, 5 paracentric and one pericentric, were revealed in Chromosomes 4, 5 and 7. Yang et al. (2012) compared the order of fosmid loci in Chromosome 7 of cultivated and wild cucumbers, and its syntenic melon Chromosome I and suggested that the paracentric inversion in cultivated cucumber chromosome 7 occurred during domestication of cucumber. The work by Yang et al. (2012) also supported the subspecies status of these two cucumber taxa, and *C. sativus* var. *hardwickii* as the progenitor of cultivated cucumber.

The Xishuangbanna cucumber (XIS) is a semi-wild landrace that is endemic to the tropical southwest China and surrounding regions with some unique traits such as tolerance to low light, large fruit size and heavy fruit weight, as well as orange flesh color in mature fruits that are very useful for cucumber breeding. Bo et al. (2015) conducted QTL mapping of domestication-related traits with recombinant inbred lines derived from the cross of the XIS cucumber with a cultivated cucumber inbred line. Comparative analysis of orders of common marker loci or marker-anchored draft genome scaffolds among the wild, semi-wild, and cultivated cucumber genetic maps revealed that the XIS cucumber shares the major chromosomal rearrangements in chromosomes 4, 5 and 7 between the wild and cultivated cucumbers suggesting the origin of the XIS cucumber through diversification selection after cucumber domestication.

The Cucumber Genome During *Cucumis* Chromosome Evolution

In the genus *Cucumis*, cucumber is the only species with $2n=2x=14$ chromosomes. The majority of the remaining species, including melon (*C. melo*) and the sister species of cucumber, *C. hystrix*, have $2n=2x=24$ chromosomes. How the 7 cucumber chromosomes were evolved from an ancestor with 12 chromosomes during evolution is a fascinating question. Based on melon marker–cucumber genome sequence alignment, Huang et al. (2009) speculated that five of the cucumber's 7 chromosomes arose from fusions of 10 ancestral chromosomes after divergence from melon. Li et al. (2011c) conducted comparative genetic mapping with common markers between cucumber and melon, and found that cucumber Chromosome 7 was syntenic to melon Chromosome I along its whole length. Except for a possible inversion, cucumber Chromosome 7 has largely remained intact in the past 9 million years since its divergence from melon. Meanwhile, cucumber Chromosomes 2 and 6 each contained genomic regions that were syntenic with melon chromosomes III + V + XI and III + VIII + XI, respectively. Likewise, cucumber Chromosomes 1, 3, 4, and 5 each was syntenic with genomic regions of two melon chromosomes previously designated as II + XII, IV + VI, VII + VIII, and IX + X, respectively (Li et al. 2011c).

Yang et al. (2014) investigated the synteny among cucumber, *C. hystrix*, and melon chromosomes using integrated and complementary approaches. Fourteen inversions and a *C. hystrix* lineage-specific reciprocal inversion between *C. hystrix* and melon were identified. Based on the location and orientation of 53 *C. hystrix* syntenic blocks on the 7 cucumber chromosomes, Yang et al. (2014) inferred at least 59 chromosome rearrangements that led to the 7 cucumber chromosomes, including five fusions, four translocations, and 50 inversions. Yang et al. (2014) proposed a hypothesis to explain the mechanisms of dysploid chromosome reduction from n = 12 to n = 7. In this hypothesis, the 12 ancestral chromosomes (AK1-AK12) similar to melon and *C. hystrix* had undergone strikingly different evolutionary fates, with cucumber chromosome C1 resulting from the insertion of chromosome AK12 into the centromeric region of translocated AK2/AK8, cucumber C3 originating from a Robertsonian-like translocation between AK4 and AK6, and C5 from the fusion of AK9 and AK10. Chromosomes C2, C4 and C6 were the result of complex reshuffling of syntenic blocks from 3 (AK3, AK5, and AK11), 3 (AK5, AK7, and AK8) and 5 (AK2, AK3, AK5, AK8 and AK11) ancestral chromosomes, respectively, through 33 fusion, translocation and inversion events. Cucumber C7 stayed largely intact during the entire evolution of *Cucumis* (Yang et al. 2014).

Han et al. (2015) developed an oligonucleotide chromosome-painting technique to differentiate cucumber and *C. hystrix* mitotic or meiotic chromosomes in the interspecific hybrids between cucumber and *C. hystrix*. Using the chromosome 7 long arm-specific oligo probe set in FISH, they found that most parings between cucumber chromosome 7 and *C. hystrix* chromosome H1 at the pachytene stage were bona fide homoeologous pairings, but most of these pairings did not result in chiasma formation at meiotic metaphase I. However, the bona fide homoeologous pairings may serve as the foundation for the unexplained short exchanged interstitial segments (introgression) derived from interspecific hybrids. *C. hystrix* is the closest and the only sexually compatible relative of cucumber that diverged from the cucumber lineage ~5 million years ago (Sebastian et al. 2010). The work of Han et al. (2015) provides a theoretical foundation for *C. hystrix* introgression line development in cucumber genetic background.

Genetic Diversity in Cucumber Natural Populations

The knowledge of genetic variations in germplasm collections is essential for their conservation and efficient use. The abundant molecular markers from mining the sequenced genomes allow a comprehensive evaluation of genetic diversity in cucumber collections. Lv et al. (2012) fingerprinted 3345 accessions from worldwide cucumber collections with 23 highly polymorphic SSR markers. Marker-based clustering identified three distinct populations (I, II and III) of the 3345 accessions which largely corresponded to three geographic regions. Population 1 corresponds to germplasm from China (except for the unique semi-wild Xishuangbanna cucumber) and East Asia; Population 2 to Europe, America, and Central and West Asia,

and Population 3 to India and Xishuangbanna. Admixtures were also detected, reflecting hybridization and migration events between the populations. The Indian population is highly heterogeneous implying that the Indian cucumbers maintain a large proportion of the genetic diversity and only a small fraction was introduced to the rest of the world. Based on marker data, a core collection was defined consisting of 118 accessions and capturing over 78 % of the SSR alleles. Qi et al. (2013) further explored ~3.6 million nucleotide variants generated by deep resequencing of 115 of the 118 cucumber lines in this core collection. The 115 cucumber lines can be divided into 4 geographic groups: the Indian group, the Xishuangbanna group, the Eurasian group, and the East Asian group. The basal nature of the Indian group compared to the other three groups is supported by its significantly higher nucleotide diversity π and by its large numbers of private variants that account for 39.0 % and 46.7 % of the total SNPs and indels, respectively. Estimated nucleotide diversity ($\pi \times 10^{-3}$) for the four groups was 4.48, 1.06, 1.85 and 1.03, respectively. The respective linkage disequilibrium (LD) decay (to r^2 of 0.2) with physical distance between SNPs occurred at only 3.2 kb in the Indian group, which was 140.5 kb, 55.2 kb, and 56.4 kb for the Xishuangbanna group, the Eurasian group, and the East Asian group, respectively. Zhang et al. (2015) analyzed structural variations (SVs) among the 115 re-sequenced lines and their possible functional impacts. They characterized 26,788 SVs based on resequencing data from 115 accessions in the core collection and found that the largest proportion of cucumber SVs was formed through nonhomologous end-joining rearrangements, and the occurrence of SVs is closely associated with regions of high nucleotide diversity. These SVs affect the coding regions of 1676 genes, some of which are associated with cucumber domestication.

Future Perspectives

The cucumber genome sequences have provided exciting opportunities to address a number of fundamental issues as well as advance traditional cucumber breeding with applied genomics tools, which is evidenced by the exponential growth of publications in cucumber in recent years. Cucumber offers some advantages for genomic research due to its economic importance, small genome size with relatively low percentage of repetitive DNA, short life cycle (50–90 days) and its unique position in the phylogenetic tree of the Cucurbitaceae family. However, to maximize the potential of the genomics resources, more research tools need to be developed before we are able to reap the fruit from genomics research. The quality of current cucumber draft genome assembly is far from satisfactory in both assembly quality and coverage. A high quality draft genome with more accurate and more complete annotations is needed to serve as the reference for the cucumber research community. Mutants are important to understand gene functions which are very limited in cucumber. We need community standards for gene ontology, gene and QTL nomenclature. A reproducible and efficient genetic transformation system is indispensable for functional genomics research which is lacking in cucumber.

Development of these tools will allow leveraging the cucumber genomic resources to address biologically important issues such as sex expression, phloem biology, organellar genome transmission, genome evolution in Cucurbitaceae species. From molecular breeding perspective, there is still a large gap between the quickly emerging genome sequences and their practical use in cucumber breeding. Very few genes or QTL have been cloned, not to mention their functions in the context of interactions with genetic backgrounds and environments. We need high-resolution genetic maps and high-throughput genotyping systems. We also need better understanding of the population structure and genetic diversity of natural populations through genome-wide association studies. Finally, a publicly accessible platform to integrate and host the genetic and genomic resources is also critical for efficient use of such information for the community.

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The Watermelon Genome

Xu Yong and Shaogui Guo

Abstract Watermelon grows throughout the world, and is one of the most important cucurbit crops. The draft genome sequence of the East-Asia watermelon cultivar '97103' was published in 2013. The genome sequence allowed the prediction of 23,440 protein coding genes. Comparative genomics analysis showed that the 11 watermelon chromosomes are derived from a seven-chromosome paleohexaploid eudicot ancestor. Twenty watermelon accessions representing three different *C. lanatus* subspecies were re-sequenced, which produced numerous haplotypes and revealed the extent of genetic diversity and population structure of watermelon germplasm. Preferentially selected genomic regions were identified and several disease resistance genes were found to be lost during domestication. Integrative genomic and transcriptomic analyses identified genes critical to valuable fruit quality traits. The draft watermelon genome sequence represents an important resource for plant research and crop genetic improvement, and also support further evolutionary and comparative genomics studies of the Cucurbitaceae.

Keywords Watermelon • Genome sequence • Next-generation sequence • Genome structure • Resequencing

Introduction

Watermelon belongs to the xerophytic genus *Citrullus* Schrad. ex Eckl. et Zeyh. of the botanical family Cucurbitaceae. It's widely accepted that the possible center of origin of *Citrullus* is southern Africa (Erickson et al. 2005). Based on nuclear and plastid data analysis, Chomicki and Renner (2015) suggested that sweet watermelon originated from West Africa. Evidence from archaeological artefacts, iconography and literature indicate that northeastern Africa may be the centre of origin of the dessert watermelon, where watermelons were domesticated for water and food over 4000

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years ago, and that sweet dessert watermelons emerged in Mediterranean lands approximately 2000 years ago (Paris 2015). More evidence, such as wild watermelon genome data, are needed to clarify this controversy. There are three subspecies in *C. lanatus*, including: *C. lanatus* subsp. *lanatus* which represents a group of ancient cultigens that naturally thrive in southern Africa called "Tsamma" or "Citron" watermelon; *C. lanatus* subsp. *mucosospermus* which has large edible seeds with fleshy pericarp representing the "Egusi" watermelon group (Fursa 1972); and *C. lanatus* subsp. *vulgaris* which represents the modern cultivated watermelon with sweet (dessert) flesh (Jeffrey 2001). Recently, the species of watermelon were examined based on nuclear and plastid data (Chomicki and Renner 2015). It was reported that the type specimen of the name *Citrullus lanatus*, which was prepared by a Linnaean collector in South Africa in 1773, is not the species now thought of as watermelon, but a representative of another species that is sister to *C. ecirrhosus*. It is now thought that there are seven species in the genus *Citrullus*, because of the disintegration of the previously accepted *Citrullus lanatus* species. However, Renner et al (2014) support use of the name *Citrullus lanatus* as it has been widely accepted, and broadly used in scientific papers and publications relating to the watermelon.

Watermelon (*Citrullus lanatus*) is among the top five most consumed fresh fruits, accounting for 7 % of the world area devoted to vegetable production. The annual world watermelon production is about 90 million tons (http://faostat.fao.org). Although comprised mainly of water (often over 90 %), the large edible watermelon fruits also contain important nutritional compounds including sugars, lycopene and cardiovascular health-promoting amino acids, such as citrulline, arginine, and glutathione (Hayashi et al. 2005; Perkins-Veazie et al. 2006; Collins et al. 2007). The diversity of fruit shape, size, color, texture, flavour, and nutrient composition in modern watermelon varieties is very high. However, the genetic base of watermelon is narrow due to years of cultivation and selection targeting yield and desirable fruit qualities (Levi et al. 2001), resulting in a major bottleneck to watermelon improvement.

Several genetic and genomic resources were developed prior to availability of the genome sequence. A high resolution genetic map was constructed, consisting of 11 linkage groups including 698 simple sequence repeat (SSR), 219 insertion-deletion (InDel) and 36 structural variation (SV) markers, and spanning 800 cM, with a mean marker interval of 0.8 cM (Ren et al. 2012). Comprehensive transcriptome profiles for watermelon fruit development also were generated, representing a valuable increase in our knowledge base of watermelon fruit biology and providing a rich source of candidates for future functional analysis (Wechter et al. 2008; Guo et al. 2011). A bacterial artificial chromosome (BAC) library was constructed for watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Naka var. *lanatus*) with an average insert-size of 106 kb, providing 21 haploid genome equivalents (Joobeur et al. 2006). These resources provided a solid foundation for the decryption of the watermelon genome.

In this chapter we describe current genomic resources, including a high-quality genome sequence of the East-Asia watermelon cultivar 97103 ($2n = 2x = 22$), and re-sequencing of 20 watermelon accessions representing the genetic diversity of *C. lanatus* (Guo et al. 2013). These data allow for inferences about structure and

evolution of the watermelon genome, genetic diversity and structure of watermelon populations, and can assist in expanding watermelon genetic diversity and accelerating biological discovery and crop improvement.

Genome Sequence

Genome Assembly and Chromosome Anchoring

The Chinese elite watermelon inbred line 97103 was sequenced using Illumina technology, providing a total of 46.18 Gb of high-quality genomic sequence, representing 108.6-fold coverage of the entire watermelon genome (Guo et al. 2013). The watermelon genome size has been estimated to be ~425 Mb based on 17-mer depth distribution analysis of the sequenced reads and flow cytometry analysis (Guo et al. 2013). *De novo* assembly of the Illumina reads generated a final assembly of 353.5 Mb, representing 83.2 % of the watermelon genome (Guo et al. 2013). The current assembly consists of 1793 scaffolds (≥500 bp) with N50 lengths of 26.38 kb and 2.38 Mb for contigs and scaffolds, respectively (Guo et al. 2013). Quality of the assembled watermelon genome was evaluated using approximately one million ESTs, four completely sequenced BACs and paired-end sequences of BAC clones. The genome assembly covered 97.8 % and 97.6 % of two BACs (GenBank accessions: JN402338 and JN402339) that were located in gene rich euchromatin regions, respectively; whereas it only covered 90.2 % and 64.2 % of two BACs (GenBank accessions: JX027061 and JX027062) that were located in centromere highly repetitive regions, respectively. Out of the 302 BAC pair-ends aligned to the same scaffold, none were aligned inconsistently with the genome assembly. Compared to several other recently published plant genomes sequenced using NGS technologies, the watermelon genome assembly is of high quality.

The assembled scaffolds were anchored using a high density genetic linkage map comprised of 953 markers (698 SSRs, 219 InDels and 36 SVs), spanning 800 cM with a mean marker interval of 0.8 cM. All together, 234 scaffolds totaling 330 Mb, accounting for 93.5 % of the assembled 353 Mb genome of 97103 were anchored to the 11 watermelon chromosomes. Of these 126 and 94 scaffolds, accounting for 70 % and 65 % of the assembled genome, were ordered and oriented, respectively. The rest of scaffolds were assembled to Chr0.

Unassembled Reads and Transposon Annotation

The unassembled component (17.4 % of the total reads) of the *de novo* genome assembly was largely composed of repeat sequences. Sequences associated with centromeres, telomeres and rDNA were identified based on substantial read depth, sequence similarities to centromeres, telomeres and rDNA clusters, and fluorescence

in situ hybridization (FISH). Unassembled reads distributed on watermelon chromosomes showed patterns characteristic of transposable elements (TEs).

Transposable elements (TEs) are major components of eukaryotic genomes. A total of 159.8 Mb (45.2 %) of the assembled watermelon genome were identified as TE repeats. Among them, those annotated within known repeat families represented 68.3 %. The long terminal repeat (LTR) retrotransposons, mainly LTR/Gypsy and LTR/Copia, are predominant; 920 full-length LTR retrotransposons representing 7.8 Mb were identified. LTR retrotransposons in watermelon accumulated much faster than in cucumber over the past 4.5 million years (Huang et al. 2009), suggesting that the differential LTR retrotransposon accumulation could result in their different genome sizes.

Gene Annotation

Comparison of predicted watermelon genes to SwissProt, TrEMBL and Arabidopsis protein databases using NCBI BLASTP (E-value ≤ 1e-4), and protein databases including Pfam, PRINTS, PROSITE, ProDom, and SMART, allowed for identification of putative functional domains and assignment of Gene Ontology (GO) terms. Functions of predicted watermelon genes were assigned using the AHRD pipeline (Automated assignment of Human Readable Descriptions) The resultant 23,440 high-confidence protein-coding genes predicted in the watermelon genome is similar to the number of genes predicted in the cucumber genome (Huang et al. 2009). The percentage of predicted genes with known homologs or functional classification is approximately 85 %.

The watermelon protein coding genes displayed a clear enrichment pattern within sub-telomeric regions, which is in accordance with previously reported plant genomes. In contrast, the TE-related genome fraction was preferentially located in the centromeric and pericentromeric regions. The repeat sequences are highly abundant on the short arms of chromosomes 4, 8 and 11. One 5S and two 45S rDNA clusters were observed on the short arm of chromosomes 4 and 8 of the 97103 genome. Investigation of the rDNA patterns in genomes of 20 representative watermelon accessions via FISH analysis showed that the number and location of 5S and 45S rDNA sites were identical between the genomes of 97103, ten representative modern cultivated (*C. lanatus* subsp. *vulgaris*) and six semi-wild watermelon (*C. lanatus* subsp. *mucosospermus*) accessions. However, one 45S and two 5S rDNA sites were observed in the genomes of the four wild watermelon (C. *lanatus* subsp. *lanatus*) accessions, with the additional 5S rDNA on the short arm of chromosome 11. These divergent rDNA patterns reflect the processes of chromosome fusion and fission during the evolution of *C. lanatus* species. The phylogenetic relationship of these three watermelon subspecies was further confirmed by the rDNA analysis. Moreover, this provides additional evidence to support *C. lanatus* subsp. *mucosospermus* as the recent ancestor of *C. lanatus* subsp. *vulgaris*.

Non-coding RNAs

tRNA genes were identified by tRNAscan-SE with default parameters. The C/D box snoRNAs were identified by Snoscan. Other ncRNAs, including miRNAs, snRNAs, and H/ACA box snoRNAs were identified using INFERNAL software by searching against the Rfam database with default parameters. In total, 123 rRNA, 789 tRNA, 335 snRNA and 141 miRNA genes were identified.

Disease Resistance Genes

Numerous diseases cause significant losses in watermelon production. Therefore, improvement of disease resistance is an ongoing watermelon breeding objective. Search for three major classes of resistance genes, nucleotide-binding site and leucine-rich repeat (NBS-LRR) genes, lipoxygenase (LOX) genes, and receptor-like gene families led to identification of 44 NBS-LRR genes, 26 LOX genes, and 197 receptor-like genes. The 44 NBS-LRR genes, included 18 TIR-NBS-LRR and 26 CC-NBS-LRR encoding genes. No sequence exchanges were detected between different watermelon NBS-LRR homologs, suggesting their independent evolution process, similar to those of Type II R genes in *Arabidopsis* and lettuce (Kuang et al. 2004), and reflect the low diversity of NBS-LRR genes in watermelon. Comparison of the number of NBS-LRR genes in the watermelon genome with cucumber (Huang et al. 2009), papaya (Ming et al. 2008), maize (Schnable et al. 2009), rice (Yu et al. 2002) and apple (Velasco et al. 2010) showed that that the number of NBS-LRR genes in watermelon genome is similar to that in cucumber and papaya, but fewer than that in rice, maize and apple. In contrast, the watermelon LOX family appears to have undergone an expansion. Nineteen of the 26 members are arranged in two tandem gene arrays. This may represent a possible complementary mechanism to cope with pathogen invasion, similar to what has been reported in the cucumber LOX gene family (Huang et al. 2009). Of the 197 receptor-like genes identified in the watermelon genome, 35 RLP lack a kinase domain and 162 have an intracellular kinase domain in addition to the extracellular LRR and transmembrane domains. Cluster distribution of many of these resistance genes on chromosomes suggested tandem duplications as their evolutionary basis.

It has been proposed that continuous cultivation and selection for desirable fruit qualities caused the lack of disease resistances in modern watermelon cultivars (Levi et al. 2001; Harris et al. 2009). De novo assemblies of unmapped reads pooled from modern cultivated (*C. lanatus* subsp. *vulgaris*) and semi-wild/wild (*C. lanatus* subsp. *mucosospermus* and *C. lanatus* subsp. *lanatus*) accessions, identified 11 and 69 genes homologous to known plant proteins from the cultivated and semi-wild/wild groups, respectively. It is noteworthy that the 69 novel genes identified from the semi-wild/wild group contain many disease-related genes including 6 TIR-LRR-NBS, one PR-1 and three lipoxygenases. In contrast, none of the 11 genes identified in the cultivated group were related with disease resistance. Additionally,

all the 44 NBS-LRR genes of the 97103 genome were also found in the semi-wild/wild accessions. These findings support the speculation that many disease resistance genes have been lost during watermelon domestication.

Genome Structure

Genome-wide duplication is common in angiosperms, representing an important molecular mechanism shaping modern plant karyotypes. In the watermelon genome, seven major triplications were identified, corresponding to 302 paralogous relationships which covered 29 % of the genome. These ancestral triplicates corresponded to the eudicots shared paleohexaploidization event (referenced as γ) (Jaillon et al. 2007), dating back to 76–130 million years ago. This would occur far before the cucurbit genome speciation event about 15–23 mya. The syntenic relationships between watermelon, cucumber, melon (Deleu et al. 2009) and grape (Jaillon et al. 2007) were analyzed to access the nature of evolutionary events leading to modern cucurbit genome structures. Grape, the closest relative to the eudicot ancestor structured in seven protochromosomes, was chosen as the reference (Salse 2012). A total of 3543 orthologous relationships were identified, covering 60 % of the watermelon genome. The independent analyses of duplications within the four eudicot genomes of watermelon, cucumber, melon and grape, and their syntenies were integrated to characterize the seven paleotriplications in watermelon based on the definition of seven ancestral chromosomal groups in eudicots (Abrouk et al. 2010). The evolutionary scenario of watermelon chromosome structure evolution was proposed based on the ancestral hexaploidization (γ) reported for the eudicots. The eleven watermelon chromosomes evolved from the seven-chromosome eudicot ancestors, through the 21 paleohexaploid intermediates. We suggest that 81 fissions and 91 fusions were involved in the process from the 21-chromosome eudicot intermediate ancestors to the modern 11-chromosome structure of watermelon (Fig. 1).

Further Improvements of the Published Genome Version

The watermelon genome project anchored a total of 234 scaffolds covering approximately 330 Mb (93.5 % of the assembled genome) to the 11 watermelon chromosomes. However, only 94 scaffolds accounting for 65 % of the assembled genome were oriented, because of previous SSR marker loci limitation. In order to fine anchor and orient the watermelon genome for mapping genes, a 96 RILs (recombinant inbred line) segregating population derived from a cross of the high sugar content watermelon (*C. lanatus* subsp. *vulgaris*) elite Chinese line 97103 and the low sugar content PI 296341-FR (*C. lanatus* subsp. *lanatus*) were resequenced (unpublished data). In total, 289 Gb of high-quality genomic sequence were generated using Illumina sequencing technology for the 96 RIL population representing

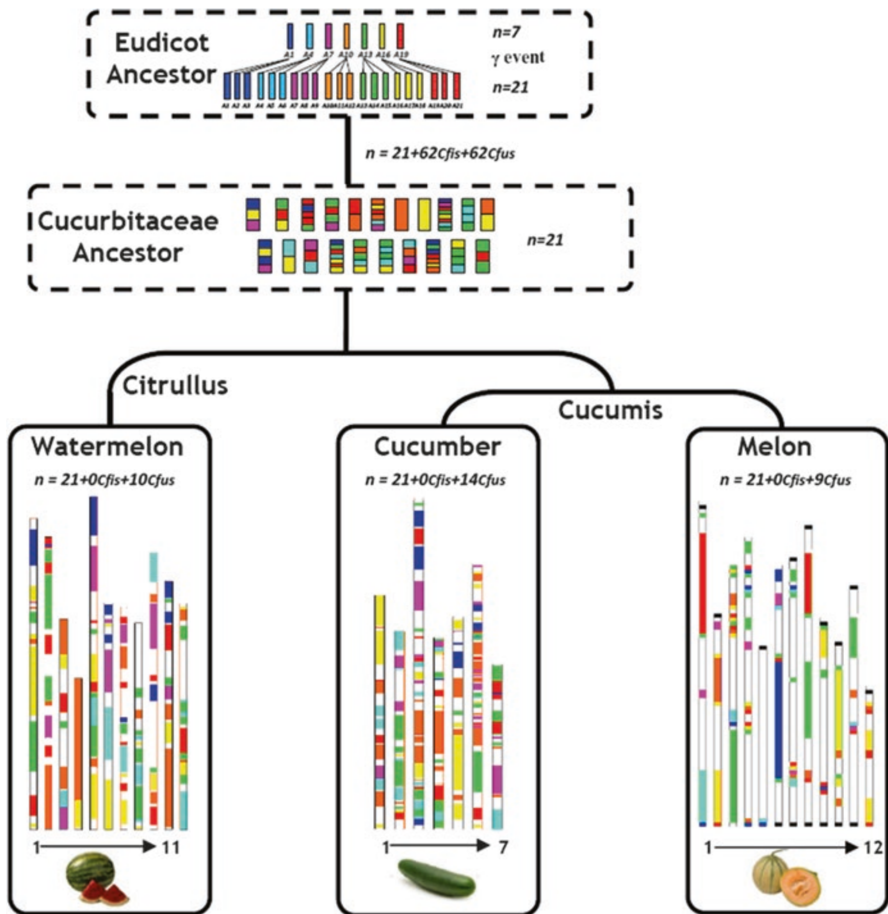

Fig. 1 Evolution of the watermelon genome from the common eudicot genome ancestors of seven chromosomes and the derived paleohexaploid *n* = *21* ancestor intermediate

5.2-fold coverage of the entire watermelon genome of each line, which has an estimated 74 % coverage of genome of RILs. Each line was aligned to the watermelon reference genome, which covered 947,400 high quality SNP loci for map construction. An ultrahigh-density SNP map was constructed using a bin mapping strategy by lumping the co segregating SNP markers between two contiguous block borders as a bin. The skeleton bin map was constructed with a total of 2492 recombination bins on chromosome 1–11 for the RILs. The average physical length of the recombination bins was 138.1 kb, ranging from 3.0 kb to 270 kb. A total of 386 scaffolds covering approximately 344 Mb were anchored to the 11 watermelon chromosomes. In total, 314 scaffolds (332.6 Mb) accounting for 93.6 % of the assembled genome were oriented, which represents a big improvement compared to the previous assembly in which only 65 % of scaffolds were oriented (unpublished data).

Resequencing of Germplasm

Twenty representative watermelon accessions, selected based on fingerprint analysis of the worldwide collection of 1156 watermelon accessions, were used for genome resequencing, including 10 representative cultivated *C. lanatus* subsp. *vulgaris* accessions, six semi-wild *C. lanatus* subsp. *mucosospermus* accessions and four wild *C. lanatus* subsp. *lanatus* accessions (Guo et al. 2013). Mapping of the reads to the genome of 97103 identified 6,784,860 candidate SNPs and 965,006 small indels among the 20 resequenced lines and 97103. The major variations were identified between *C. lanatus* subsp. *lanatus* and the other two subspecies. In contrast, the genome variation within the cultivated watermelon accessions is relatively low, especially for the *C. lanatus* subsp. *vulgaris* America ecotype. The SNP and indel variations were confirmed by Sanger sequencing and the accuracies were 99.3 % and 98 %, respectively. This genome wide variation dataset of representative watermelon accessions provides a valuable genomic diversity resource for further biological discovery and germplasm improvement and utilization (Guo et al. 2013).

The higher genetic diversity of wild watermelon accessions, indicated that they can be utilized as an additional genetic opportunity for watermelon improvement. The population structure and relationships among the watermelon accessions were also investigated through construction of a neighbor-joining tree and principle component analysis (PCA) and FRAPPE analysis. *C. lanatus* subsp. *vulgaris* was found to be close to *C. lanatus* subsp. *mucosospermus* and a new subgroup in the *C. lanatus* subsp. *mucosospermus* group was identified. Moreover, there were some admixtures between *C. lanatus* subsp. *vulgaris* and *C. lanatus* subsp. *mucosospermus*. This new subgroup showed some characteristics of the cultivated watermelon, such as pink flesh color, soft flesh texture and moderate sugar content. These results further supported the rDNA FISH evidenced evolutionary scenario of *C. lanatus* subsp. *mucosospermus* to *C. lanatus* subsp. *vulgaris*.

The genome regions with highest differences between *C. lanatus* subsp. *mucosospermus* and *C. lanatus* subsp. *vulgaris* were scanned. Given that modern watermelon cultivars are thought to have been domesticated from *C. lanatus* subsp. *mucosospermus*, these high divergence regions should represent potential selective sweeps during watermelon domestication. A total of 108 regions of 7.78 Mb in size containing 741 candidate genes were identified. Several biological processes significantly enriched in candidate genes related to important selected traits were identified, such as carbohydrate metabolism, sugar mediated signalling, regulation of carbohydrate utilization, response to sucrose stimulus, cellular response to nitrogen starvation, regulation of nitrogen compound metabolism, and growth.

It is worth mentioning that certain non-centromeric genomic regions, especially the large region on chromosome 3 from ~3.4 M to ~5.6 M, have particularly high divergence only within *C. lanatus* subsp. *mucosospermus* accessions. Analysis of genes in the high-diversity region on chromosome 3 showed significant enrichment for the gene categories related to recognition of pollen and pollen-pistil interaction. Additionally, a large cluster of 12 tandemly arrayed S-locus protein kinase genes

involved in reproductive barriers (Nasrallah and Nasrallah 1993) were identified in this high divergence region. It is suggested that these high divergence regions were highly associated with genes related to reproductive barriers. A similar pattern in three different rice crosses/populations has been reported (Harushima et al. 2002). Considering that *C. lanatus* subsp. *mucosospermus* has been found to be the recent progenitor of modern cultivated watermelon, we suggest that watermelon domestication could be a possible force responsible for the rapid evolution of reproductive barriers, as reported in rice (Harushima et al. 2002), based on the high nucleotide divergence of reproductive barrier genes within *C. lanatus* subsp. *mucosospermus*. In addition, plant responses to abiotic/biotic stress related genes were also significantly enriched in this high-diversity region, along with genes related to several known selected traits, such as carbohydrate metabolism, fruit flavour, and seed oil content. Further sequencing of 112 watermelon accessions, including both elite breeding collections and the broad basis of current available watermelon species, *Citrullus lanatus, Citrullus ecirrhosus, Citrullus colocynthis, Citrullus rehmii* and *Citrullus naudinianus* identified a total of 24,752,660 SNP (unpublished data). These will provide abundant genomic datasets to explore traits important for breeding, domestication, and adaptation to specific environments.

Utility of the Genome

The draft watermelon genome sequence represents an important resource for plant research and crop genetic improvement, and will also support further evolutionary and comparative genomics studies of the Cucurbitaceae. Several genetic and genomic findings of importance to understanding developmental biology, disease resistance, and genome evolution already have been accomplished based on the watermelon genome resource as described below.

A comprehensive floral transcriptome sequence comparison of a male fertile line and its near-isogenic male sterile line revealed essential genes responsible for stamen development, including pollen development and pollen tube elongation, and allowed their functional classification (Rhee et al. 2015). The watermelon gene *CitACS4*, which is expressed specifically in carpel primordia, was identified; reduced *CitACS4* activity may hamper the programmed cell death in stamen primordia, leading to the formation of hermaphroditic flowers (Ji et al. 2016). Comparison of the transcriptome profiles of fruit tissues of cultivated watermelon 97103 and wild watermelon PI 296341-FR identified critical genes potentially involved in controlling fruit quality traits including fruit sugar content, carotenoid metabolism, and flesh texture (Guo et al. 2015). Analysis of expression of 11 WOX genes in watermelon tissues, brought new evidence for WOX genes acting as conserved factors during watermelon development and suggested that distinct expression profiles during shoot initiation might lead to different shoot regeneration abilities (Zhang et al. 2015). Genome-wide transcript analysis of scions from watermelon grafted onto bottle gourd and squash

rootstocks found 787 and 3485 genes differentially expressed, respectively, providing insights into the molecular aspects of gene regulation in grafted watermelon (Liu et al. 2015). Analysis of the *Citrullus colocynthis* transcriptome during water deficit stress detected 5038 full-length cDNAs, with 2545 genes showing significant changes during drought stress. Up regulation of many transcription factors, stress signalling factors, detoxification genes, and genes involved in phytohormone signalling and citrulline metabolism occurred under the water deficit conditions (Wang et al. 2014).

With respect to disease resistance, 15 ClMPK and six ClMKK genes from watermelon and their phylogenetic relationships, expression patterns and protein-protein interactions and functions in disease resistance were analyzed. ClMPK1, ClMPK4-2 and ClMPK7 positively regulated, while ClMPK6 and ClMKK2-2 negatively regulated resistance to *B. cinerea* when transiently expressed in *N. benthamiana,* and ClMPK7 was observed to function as a regulator of HR-like cell death through modulating the generation of H_2O_2 (Song et al. 2015). Genetic analysis and chromosome mapping of resistance to *Fusarium oxysporum* f. sp. *niveum* (FON) race 1 and race 2 in watermelon detected one major QTL on chromosome 1 for FON race 1 resistance with a LOD of 13.2 that explained 48.1 % of the phenotypic variation. Two QTLs of FON race 2 resistance on chromosomes 9 and 10 were also discovered based on the high-density integrated genetic map constructed. The nearest molecular markers should be useful for marker-assisted selection of FON race 1 and race 2 resistance (Ren et al. 2015).

A high-resolution watermelon genetic map of 1096 cM was constructed, consisting of a set of 10,480 single nucleotide polymorphism (SNP) markers generated by GBS. A strong selective sweep on chromosome 3 was identified, including important genes that might have had a role in sweet watermelon domestication (Reddy et al. 2014), and a genome-wide scan of selective sweeps and association mapping of fruit traits found evidence of convergent evolution for the presence of diverse ecotypes with special reference to American and European ecotypes (Reddy et al. 2015). Analysis of changes in gene expression and the occurrence of alternative splicing (AS) in diploid and tetraploid sweet watermelon found 5362 and 1288 genes that were up- and down-regulated, respectively, in tetraploid as compared with diploid watermelon. It has also been confirmed that 22 genes underwent AS events across tissues, indicating possibilities of generating different protein isoforms with altered functions of important transcription factors and transporters (Saminathan et al. 2015).

This extensive set of examples in just a few years demonstrate great value of the assembled watermelon genome and genomic resources. Futhermore, genome resequencing of representative watermelon accessions has provided a large source of haplotype data with great potential for genome manipulation, trait discovery and allele mining. Insights on genetic diversity and population structure of watermelon accessions as well as chromosome regions and genes under human selection will shape future efforts on watermelon genetic research and breeding.

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Genetics and Genomics of *Cucurbita* spp.

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Abstract *Cucurbita pepo* is one of the main crops of the Cucurbitaceae family. Despite its agricultural and biological importance, genomic research on this species has started later and progresses slowerthan in other cucurbits. Here we review the latest genetic and genomic tools developed for *C. pepo*. A whole-genome shotgun strategy based on pair-end and mate-pair Illumina sequencing,has generated a whole genome draft of 263 Mb into 26,005 scaffolds and 32,754 contigs (contig N50:110 kb and scaffold N50:1.8 Mb). The genome sequence has been annotated using the transcriptome (73,239 unigene clusters, with an average length of 1050 bp) and anchored using a high density genetic map based on a recombinant inbred line (RIL) population (with 6763 SNPs distributed across 21 linkage groups, with a total length of 2635 cM). Further RNA-seq resequencing and GBS genotyping efforts in *C.pepo*have been conducted to better represent the whole species variation, including the three subspecies (*pepo*, *ovifera* and *fraterna*). Similar information has been generated in related cultivated (*C. moschata, C. maxima, C. argyrosperma*, *C. ficifolia*) and wild species of the *Cucurbita* genus, to provide a valuable insight into the *Cucurbita* genetic variation. These studies have generated large SNPs collections usefulfor different breeding purposes. All this sequence information along with high throughput reverse genetic tools, such as the first *C.pepo* TILLING population, and with new mapping populations suitable for complex trait genetic dissection, are allowing the identification of candidate genes underlying the variation of key traits for *C. pepo* breeding.

Keywords *Cucurbita* • Genome • Transcriptome • SNPs • Genetic maps • TILLING

Introduction

Pumpkin, squash and gourd species (*Cucurbita* spp.) belong to the Cucurbitaceae family, which also includes other main crops such as cucumber (*Cucumis sativus* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai), and melon

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(*Cucumis melo* L.). It is generally accepted that the *Cucurbita* genus is of American origin, from where the domesticated species spread worldwide (Robinson and Decker-Walters 1997; Schaefer et al. 2009). Each cultivated species is thought to have been domesticated independently from the others in distinct regions of North, Central and South America, all in the pre-Columbian era (Sanjur et al. 2002; Smith 1997; Esteras et al. 2012a).

The *Cucurbita* genus includes several important vegetable and fruit crops, with 24.7 million tons produced worldwide in 2013 and nearly 1.8 million ha cultivated (FAOSTAT 2016). They are particularly important in Asian, American and Mediterranean countries. China, India, Russia, Iran, USA, Ukraine, Mexico, Egypt, Spain and Italy are the main producers, all with more than 0.5 million tons per year, and Mexico and Spain are the main exporters worldwide.

Species in the *Cucurbita* genus are all diploid ($2n = 2x = 40$) (Esteras et al. 2012a). *Cucurbita pepo* L. is the most economically important species. It was domesticated at least twice in Southern USA or Northern Mexico, resulting in cultivated germplasm of the two subspecies, *pepo* and *ovifera* (L.) Decker (also known as subsp. *texana* (Scheele) Filov). A third subspecies, *fraterna* (L.H. Bailey) Lira, Andres and Nee, is also known, including only wild populations growing in northeastern Mexico (Andres 1987; Decker-Walters 1990; Paris 2015).

Cucurbita pepo is also the most variable species. Apart from its diverse origin, it has undergone great diversification in Europe and Asia. Eight edible fruited cultivar-groups are distinguished by their evolutionary histories, fruit shapes and types of uses as immature/mature fruits (summer/winter squash), four in subsp. *pepo* (Pumpkin, Vegetable Marrow, Cocozelle, Zucchini) and four in subsp. *ovifera* (Acorn, Scallop, Straightneck, and Croockneck) (Ferriol et al. 2003a; Paris et al. 2003). Zucchini is the group with the most recent origin and the highest economic value, appreciated by its uniformly elongated cylindrical fruits, used when immature as vegetables. This group was selected in Europe, like the other two summer squashes, Cocozelle and Vegetable Marrow, also with elongated fruits. The oldest Pumpkin group, domesticated in the Americas, generally has round fruits, and has been used as winter squashes for eating, decoration, seed consumption, and extraction of seed oil. The cultivar groups of the subsp. *ovifera* were also selected in America in pre-Columbian times, being appreciated today as winter (Acorn) and summer squashes (Crookneck, Straightneck and Scallop) in the United States, some with unique fruit shapes, such as necked, turbinate and discoidal, and high culinary properties (Paris 2000, 2015).

Apart from *C. pepo*, the genus includes four species of growing economic value. Two of them, *C. moschata* Duchesne and *C. maxima* Duchesne, are mainly used for their mature fruits, appreciated by the high quality of the fruit flesh, a rich source of nutritional compounds. Their centers of origin are the lowlands of northern South America (*C. moschata*) and southern South America (*C. maxima*). Both species spread worldwide experiencing a great diversification in Europe, Asia and Africa. Nowadays they are extensively cultivated, and used as food staples in many developing countries where a wide range of morphotypes can be found as landraces (Ferriol et al. 2003b, 2004; Robinson and Decker-Walters 1997; Ferriol and Pico

2008). The monetary value of these species is increasing, as they are preferred rootstocks for watermelon and other cucurbits due to their vigorous roots and tolerance to biotic and abiotic soil stress (Lee et al. 2010).

The two other cultivated species, *C. argyrosperma* and *C. ficifolia*, have a narrower distribution and specific uses. Mature fruits of *C. argyrosperma* C. Huber, from southern Mexico, have less flesh quality and are most frequently used as feed for livestock, and their flowers, young stems and immature fruits are eaten as vegetables. In addition, the consumption of seeds is common in this species. *C. ficifolia* Bouché, from northern or central South American highlands, is less variable and used for jam production and as rootstock tolerant to salt and cold stresses (Ferriol et al. 2007).

Wild *Cucurbita* taxa are also widespread from the USA to Argentina. These species shared wild features that have been lost during the domestication process, such as highly branched vines, small seeds and small round fruits (<10 cm diameter) with smoothskin andthin, fibrous bitter flesh (Sanjur et al. 2002; Smith 2006; Lira et al. 2009).

Despite their agricultural and biological importance, genetic and genomic research on this genus has been limited, compared with the advances in other cucurbits (reviewed in previous chapters). Part of the vast phenotypic diversity found in the cultivated species has been studied. The published gene lists (Paris and Brown 2005; Paris and Kabelka 2009; Paris and Padley 2014, last update of the Cucurbit Genetics Cooperative genelist, http://cuke.hort.ncsu.edu/cgc/) describe simple genetic controls for the variation of traits related to seed coat (naked seeds), growing habit (vine-bushy), leaf and tendril morphology and color (leaf indentation and silverleaf), stem color (light to dark stem), sex type (androecious-monoecious-gynoecious), fruit shape (from discoidal or spherical to very elongated and variable number of cavities), fruit rind texture (lignified or not, smooth, ribbed, furrowed, warted or wrinkled), flesh and rind colors (white, yellow, orange, green, black), color patterns and flesh properties (bitterness). Also monogenic/oligogenic controls have been reported for the resistance to pathogens including *Phytophtora capsisci*, *Podosphaera xanthii*, *Cucumber vein mosaic virus, Zucchini yellow mosaic virus, Papaya ring spot virus, Watermelon mosaic virus* and *Squash leaf curl virus*. Most of the resistance traits have been reported in accessions of the cultivated *C. moschata* or in the wild *C. ecuadorensis*, *C. lundelliana* and *C. okeechobeensis*. Only a few of these traits have been mapped, due to the lack of appropriate genetic maps. The first genetic maps were of low density and included only dominant markers (Brown and Myers 2002; Zraidi et al. 2007). More recently, SSR-based maps allowed further mapping of important agronomical traits (Gong et al. 2008a, b). Despite these mapping efforts, little is known about the genes and mechanisms that underlie these attributes. Different types of molecular markers have been also applied to genetic diversity studies (Ferriol et al. 2003a, b, 2004; Paris et al. 2003; Gong et al. 2012). All this information has been used in breeding programs to introgress disease resistance and to enhance fruit quality. However, the notable absence of genomic tools in the *Cucurbita* genus has seriously hindered an efficient molecular breeding in these species.

It has not been until the development of Next Generation Sequencing technologies (NGS), when genomic tools have started to be generated for non-model species efficiently and cost-effectively. The use of NGS in the *Cucurbita* genus has started later and progresses lower compared with the other important cucurbits, cucumber, watermelon and melon,for which whole genome sequences are already available (Huang et al. 2009; Garcia-Mas et al. 2012; Guo et al. 2013). After the development of the first *C. pepo* transcriptome in 2011, the first SNP-based genetic map that is suitable for complex trait genetic dissection, and the first TILLING platform (Blanca et al. 2011a; Esteras et al. 2012b; Vicente-Dólera et al. 2014), newgenome/transcriptome sequencing/resequencing and mapping initiatives have been undertaken in *C.pepo* and other cultivated species including *C. moschata* and *C. maxima* (Blanca et al. 2015).

The newly generated information and tools are reviewed in the current chapter. These genomic tools provide a valuable insight into the *Cucurbita* genetic variation and can be used by the *Cucurbita* breeders. New transcriptomes provide an invaluable tool for biological research. The collections of high quality molecular markers and the new mapping populations are the basis for genetic linkage studies of complex traitsand the efficient management of QTLs in breeding programs. The genome sequences arean excellent toolfor understanding the genome structure and evolution of the species, enabling the phylogenic comparison with the other main cucurbits. All together these tools will be essential to speed up the process of breeding new and better adapted squash varieties.

The Sequencing of the *Cucurbita pepo* Genome

The inbreeding line MU-CU-16, belonging to the Zucchini morphotype of the subsp.*pepo* of *Cucurbita pepo*, was chosen to obtain the whole genome sequence. This is the main summer squash in European markets. It is early flowering, with parthenocarpic uniform cylindrical dark green fruits and is highly productive.

A whole-genome shotgun strategy based on Illumina sequencing, using a combination of 2 pair-end and 2 mate-pair (3 Kb and 7 Kb) libraries, was employed. The first sequencing assay produced 441 and 173 million paired-end and mate-pair reads, respectively. The mate-pair chimeric reads were analyzed by trimming the chimeric section. Also, the mitochondrial and chloroplastic reads were detected. The chloroplast and mitochondria reads represented around 25 % of the total reads. A preliminary assembly was then performed with a set of selected clean reads. Assembly was done with SOAPdenovo2 (Li et al. 2010). Assembly was also improved by splitting the scaffolds generated by SOAPdenovo2 using REAPR (Hunt et al. 2013), a tool that precisely identifies errors in genome assemblies without the need for a reference sequence, and scaffolding again with SSPACE, a tool for scaffolding pre-assembled contigs that was reported to show higher N50 value compared with common *de novo* assemblers, like SOAPdenovo2 (Boetzer et al. 2011). The assembly was improved with Gapcloser (Li et al. 2010), yielding an assembly of 247 Mb with contig and scaffold N50 of 57.8 Kb and 0.24 Mb, respectively.

Table 1 Metrics of the last version of the *C. pepo* genome assembly (cpepo_genome_v3.2 in https://cucurbigene.upv.es/)

Assembly	Measure
GC (%)	36.52
No. of contigs (≥0 bp)	32,754
No. of contigs (≥500 bpin)	13,896
No. of large contigs (≥1000 bp)	8217
Bases in contigs (≥0 bp)	247,816,249
Bases in contigs (≥500 bpin)	242,141,895
Largest contigs (bp)	639,487
N50 contig size (bp)	110,136
N75 contig size (bp)	49,377
L50 contig number	606
L75 contig number	1407
No. of scaffolds (≥0 bp)	26,025
No. of scaffolds (≥500 bpin)	7994
No. of large scaffolds (≥1000 bp)	3709
Bases in scaffolds (≥0 bp)	263,500,453
Bases in scaffolds (≥500 bpin)	258,108,973
Largest contig (bp)	6,123,784
N50 scaffold size (bp)	1,749,822
N75 scaffold size (bp)	453,344
L50 scaffold number	42
L75 scaffold number	112

To further improve the assembly, two additional mate-pair libraries (10Kb and 20 Kb) were constructed and sequenced. One hundred forty-five million mate-pair reads were obtained. Assembly was done using the same methodology as reported in the preliminary assembly (SOAPdenovo2 with pair-ends, break scaffolds, scaffolding with SSPACE with 3, 7, 10 and 20 Kb, and Gapcloser). This final assembly of 263 Mb into 26,005 scaffolds and 32,754 contigs resulted in a more complete version of the whole genome draft (contig N50 of 110 Kb and scaffold N50 of 1.8 Mb) (Table 1).

The *C. pepo* genome assembly can be considered of good quality compared with other cucurbit genomes (Huang et al. 2009; Garcia-Mas et al. 2012; Guo et al. 2013). The difference between the estimated and the assembled genome size could be due to unassembled regions of repetitive DNA, similar to what has been found in genomes obtained with NGS.

The *C. pepo* Transcriptome

Several transcriptomes of *C. pepo* have been produced. The first version of the *C. pepo* transcriptome was developed in 2011 using the 454 GS FLX Titanium technology (Blanca et al. 2011a, b). Normalized cDNA libraries from several tissues (root, leaves and flowers) were generated using two varieties with contrasting phenotypes for plant,

flowering and fruit traits, representing the two *C. pepo* main subspecies. One was the same Zucchini genotype (subsp. *pepo*) used for whole genome sequencing, and the second was the inbreeding lineUPV-196 of the Scallop morphotype (subsp. *ovifera*) with late flowering, semi-determinate, and white, flattened fruits with scalloped margins. The two genotypes also represent different domestication processes, with Scallop types already being selected by native Americans, whereas elongated Zucchini forms being selected in Italy after the arrival in Europe (Paris 2000). A total of 512,751 expressed sequence tags (ESTs) were obtained and *de novo* assembled, generating a collection of 49,610 *Cucurbita* unigenes with an average length of 626 bp. Sixty percent of these unigenes were functionally annotated, and about 34 % were detected to have known orthologs in Arabidopsis or melon, including genes potentially involved in disease resistance, flowering and fruit quality.

This transcriptome was significantly improved with Illumina Solexa technology by sequencing cDNA coming from several new tissues, in order to get additional unigenes involved in flowering, and fruit development and shelf life. Apical shoots from plants in the male and female phase of development, flower buds collected at two early stages of flower development when sex determination occurs (Martínez et al. 2013), and pre-harvest and post-harvest fruits subjected to different treatments (ethylene, MCP and cold) were used for RNA isolation. Equivalent amounts of RNA from each tissue were mixed into two pools, one per cultivar (Zucchini MU-CU-16 and Scallop UPV196), and used to construct two independent cDNA libraries. One Illumina lane (100 bp-PE) was performed on each of the two libraries. Raw reads were processed using the ngs_crumbs software (http://bioinf.comav.upv.es/ngs_crumbs) to eliminate adapter sequences, low quality bases and sequences of less than 40 bp. This analysis gave rise to nearly 47 million of processed sequences per library (total residues 6,957,952,969 and 6,606,419,950 in MU-CU-16 and UPV-196 respectively), comprising a total of 13 Gb of sequences.

The new Illumina reads of *C. pepo* were used for *de novo* assembly, along with the previously generated 512,751 *C. pepo* 454 reads (Blanca et al. 2011a, b) using the Trinity assembler (Grabherr et al. 2011). This new assembly yielded a total of 108,062 transcribed clusters. After Trinity assembly, CAP3 (Huang and Madan 1999) was used to eliminate redundancies and also low complexity transcripts were filtered. Trinity subcomponents were split using blast. Two transcripts with overlapping regions shorter than 100 bp were considered to belong to different unigene clusters. Additionally, transcripts expressed less than 1 % of the most expressed transcript in each Trinitysubcomponent were also filtered using RSEM (http://deweylab.biostat.wisc.edu/rsem/).Finally, Trinity grouped in 73,239 unigene clusters. The assembled transcripts had an average length of 1050 bp, comprising approximately 113 Mb in total (*C. pepo* v3; https://cucurbigene.upv.es/). The transcript number assembled in this new version of the transcriptome was nearly twice of that assembled in the first version. In addition this assembly rendered unigenes with an average length almost twice of that reported previously in *C. pepo* (1,050 bp vs. 626 bp) (Blanca et al. 2011a, b), which is in accordance with the higher number of sequences and the wider range of tissues.

The functional annotation was performed by sequence comparison with public databases. All transcripts were sequentially compared using blast (cut-off e-value of 1e-20) with the sequences in three major public protein databases, prioritizing manually

annotated and reviewed databases (Swiss-Prot, Arabidopsis proteinsand UniRef90). Also, a bi-directional blast search comparison was performed in order to obtain a set of putative orthologs with two protein databases (cucumber_v2i.pep and arabidopsis_pep tair 10). Additionally, we performed a functional classification of the unigenes following the Gene Ontology (GO) scheme with Blast2GO (Conesa et al. 2005). ORFs were predicted in the unigenes with the aid of the ESTScan software (Iseli et al. 1999).

Both transcriptomes were compared by blast and nearly all (49,065 out of 49,610) of the unigenes of the first version have a significant BLAST (max_evalue: 1e-20) with unigenes of the new version, but 30 % of the unigenes of the new transcriptome were not sequenced in the first version. A total of 16,557 of these new unigenes could be annotated and had functions related mostly to flower and fruit biological processes. A comparative GO Slim analysis of the two transcriptomes versions is shown in Fig. 1 showing that some GO terms that were not represented or underrepresented in the old transcripts were assigned to some of the new unigenes. Also other were over represented in the new unigenes set. Both transcriptomes are available in https://cucurbigene.upv.es/ (v1 and v3). These transcriptomes were used to annotate the genome.

These transcriptomes represent major advances in *C. pepo* genomics, revealing the repertoire of genes expressed throughout Summer Squash plant, flower and fruit development. Recently, a fruit and seed transcriptome of a *C. pepo* Acorn squash cultivar was generated (Wyatt et al. 2015). This transcriptome is expected to contribute to enhancing the efficiency of breeding for high fruit and seed quality in Winter Squash. Around 140 million of high-quality paired-end Illumina reads were assembled into 55,949 unigenes. Most of them (85 %) had homology with previously identified genes and over 62 % could be functionally annotated and included key candidate genes associated with carotenoid and carbohydrate metabolism (Wyatt et al. 2015).

Some of the genes annotated in these transcriptomes have been functionally characterized (Martínez et al. 2013, 2014). Tools for functional characterization are also being developed, such as high throughput reverse genetic tools. The first TILLING (Targeting Induced Local Lesions IN Genomes) resource for this species has been recently produced (Vicente-Dólera et al. 2014), using the same Zucchini accession employed for whole genome sequencing. This population has an overall mutation density of 1/133 Kb and has been used to demonstrate the genotype/phenotype correlation of some mutations in selected genes. The Zucchini TILLING population provides new mutations, complementary to the natural ones, and can be used to address the major challenge of linking sequence information to biological function and identify novel variations for Zucchini breeding.

Transcriptome Resequencing and Genotyping by Sequencing in *C. pepo*

C. pepo is a highly variable species, and molecular markers are needed to study its variability. The previously described *Cucurbita* unigenes were screened for the presence of microsatellites, yielding a total of 1935 potential SSRs in 1822 unigenes

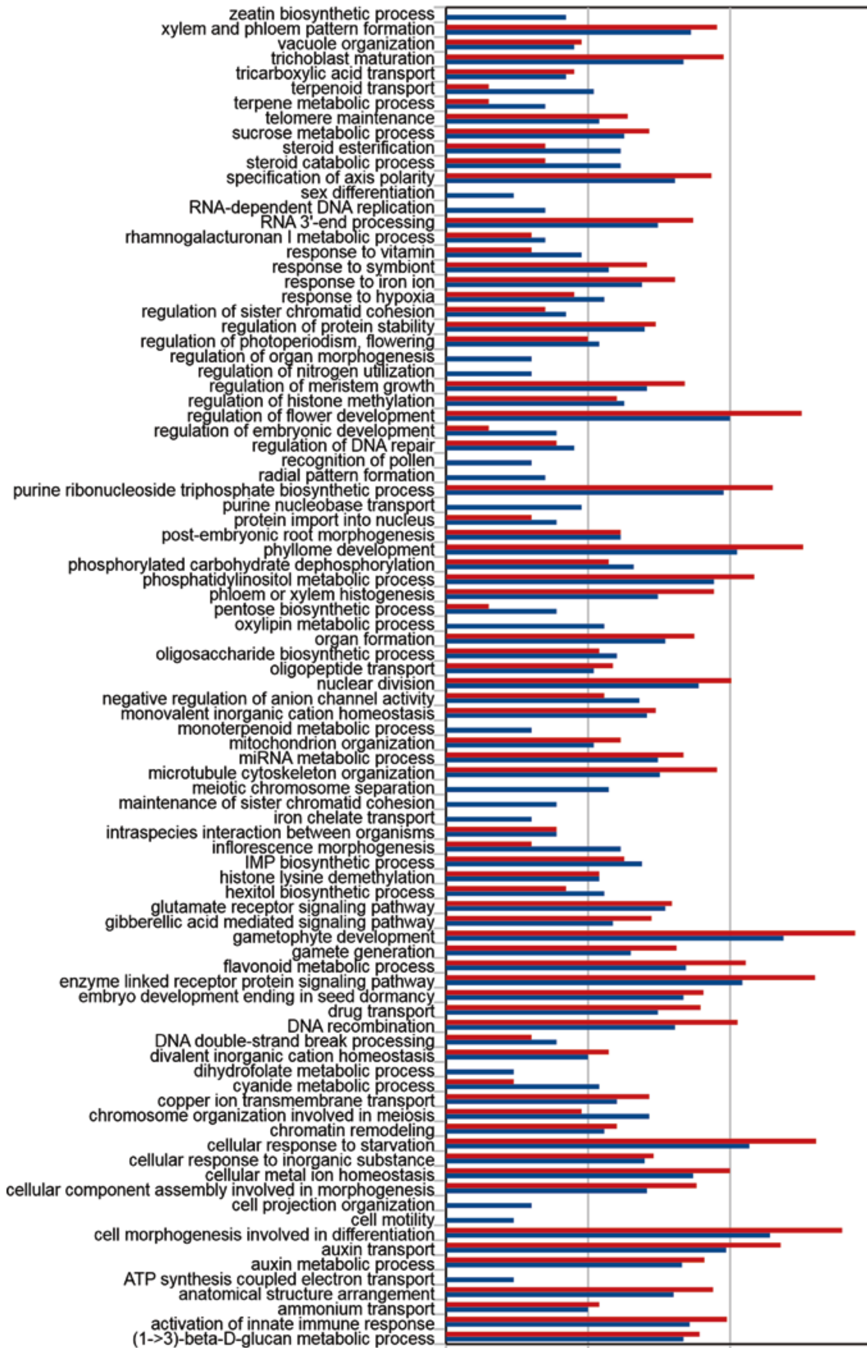

Fig. 1 Number of *Cucurbita* unigenes in each functional category. *Cucurbita* unigenes of v1 (*red*) and v3 (*blue*) were classified into different functional groups based on a set of GO slims in the biological process category

(Blanca et al. 2011a). Also, large SNPs collections were generated. Since the transcriptomes above described were done using two different cultivars belonging to two subspecies, Zucchini and Scallop, they provided several SNPs collections, polymorphic between these genotypes. Ngs_backbone (Blanca et al. 2011b) was used to detect the SNPs in the first version of the transcriptome by mapping the 454 processed reads against the unigene assembly using BWA (Burrows-Wheeler Aligner) (Li and Durbin 2009). Stringent quality criteria were used for distinguish sequence variations from sequencing errors and mutations introduced during the cDNA synthesis step. A total of 19,980 SNPs distributed in 8147 unigenes were detected. Different filters were applied to facilitate the management of the variants and their implementation in high throughput genotyping platforms. These filters allow discarding SNPs located in highly variable regions, close to other SNPs, to introns or to the sequence ends, or those within or between the genotypes. Using these filters, we selected 9043 high confidence SNPs between Zucchini and Scallop, of which 3538 SNPs met criteria for use with high throughput genotyping platforms (Blanca et al. 2011a).

The transcriptome v3 was also screened for SNPs. Freebayes (Bayesian haplotype-based polymorphism discovery and genotyping. http://arxiv.org/abs/1207.3907) was used to detect SNPs. Illumina reads were mapped against the unigene assembly using BOWTIE2 (http://bowtie-bio.sourceforge.net/bowtie2/index.shtml). We only kept SNPs meeting stringent quality criteria: minimum number of alleles 2 and at least 3 reads supporting each allele. Reads with mapping quality below 30 were not considered for SNPs calling. The higher number of unigenes of this new version of the transcriptome provided a collection of a total of 244,771 SNPs, 12 times of those in the first collection. A summary of the detected SNPs is shown in Table 2. All these SNPs are available at https://cucurbigene.upv.es/.

The SNPs detected in the transcriptomes represented the variation between the two sequenced genotypes. To represent the whole species variation, two additional resequencing assays were conducted. First, one cDNA library was generated from a pool of RNA extracted from leaf tissues of 12*C. pepo* genotypes, representing the variability within *C. pepo* subsp. *pepo* (Ferriol et al. 2003a). These genotypes included landraces of the four main morphotypes: the more ancient and variable Pumpkin and Vegetable Marrow, and the more modern and less variable Cocozelle and Zucchini (Styrian pumpkin, a hulless oil seed variety, four Central and South

Table 2 SNPs detected in the last version of the *C. pepo* transcriptome (cpepo_transcriptome_v3 in https://cucurbigene.upv.es/)

	Total SNPs	SNPs with MAF>0.1	SNPs that meet criteria for Golden gate platforms
SNPs within Zucchini MU-16	72,380	69,782	32,271
SNPs within Scallop UPV-96	69,649	68,059	28,980
SNPs between MU-16-UPV-196	102,742	100,574	50,047

Total number, SNPs with minor allele frequency (MAF)>0.1, and those that meet criteria to be genotyped with high-throughput genotyping platforms are shown

Fig. 2 Diversity in fruit traits of the genotypes used in the cDNA pool of: (**a**) *C. pepo* subsp. *pepo* (From *left* to *right* and *top* to *bottom*, Pumpkin: Styrian Pumpkin, CATIE9394, CATIE18887, CATIE11368, PI 171628, AS-CU-3, ECU-25; Vegetable marrow: AFR-CU-12, CM-CU-21, V-CU-21; Cocozelle: V-CU-112; and Zucchini: E-CU-27). (**b**) *C. moschata*. Genotypes representing different fruit morphotypes (Ferriol et al. 2004) and origins are included (From *left* to *right* and *top* to *bottom*: PI 604506, GUA1, CA-CU-30, Nigerian Local, PI 482527, PI 165033, MENINA, ECU-1, AN-45, CA-CU-7, CUB-2, PI 369346, PI 550689, B-CU-39)

American, and one Turkish Pumpkin landraces, five Spanish landraces belonging to the four morphotypes, and one selected Vegetable Marrow from Morocco) (Fig. 2a). This library was sequenced with 100 bp single end Illumina. A total of 26,023,710 raw reads were generated and produced 25,557,203 processed reads, totalling 942 Mb. Var Scan (Variant detection in massively parallel sequencing data) was used to detect SNPs. A total of 45,205 SNPs were detected (35,101 with MAF >0.1) of which 10,841 met criteria to be detected in genotyping arrays. We compared this new set of SNPs with those generated from the Zucchini-Scallop transcriptome and identified 14,150 that were variable in this pool of subsp. *pepo*genotypes, but were not variable in the previous Zucchini-Scallop transcriptome.

Table 3 Illumina sequences generated by GBS in a collection representative of the subspecific/morphotype variation of *C. pepo*

Subspecies/morphotype	Number of sequences	Mapped SNPs within each taxon
subsp. *pepo* morphotype Pumpkin	16,806,051	7941
subsp. *pepo* morphotype Vegetable Marrow	14,061,610	7147
subsp. *pepo* morphotype Cocozelle	10,710,827	6352
subsp. *pepo* morphotype Zucchini	2,660,835	1588
subsp. *ovifera*	4,158,029	4764
subsp. *fraterna*	3,089,804	3970

SNPs mapped in the RILs map and located in the genome, which are variable within each subgroup, are indicated

More recently, genotyping-by-sequencing (GBS) (Elshire et al. 2011) was used to better represent the whole species variation, including the three subspecies of the species (*pepo, ovifera* and *fraterna*). We used GBS to genotype a collection of 37 accessions including the four main morphotypes of the subsp. *pepo*, but also the four main morphotypes of the subsp.*ovifera* (Scallop, Acorn, Straightneck and Croockneck) and two Mexican representatives of the subspecies *fraterna*, representing the wild ancestor of the species. More than 51 million of sequences were produced, ranging from 1 to 2 million per genotype. This assay also included a RIL mapping population (described below). All genomic reads were mapped against the last version of the *C. pepo* genome for SNP mining. Then a set of selected SNPs (7941), polymorphic within and between the three subspecies, were mapped and located in the genome. These SNPs can be used for mapping purposes in crosses of accessions belonging to these subspecies (Table 3). Since the GBS has been performed in individual samples, it has provided the genotype of each accession for all the identified markers.

Transcriptome Resequencing in the *Cucurbita* Genus

Apart from *C. pepo*, the *Cucurbita* genus includes some important cultivated species. *Cucurbita moschata*, the most rustic among the cultivated *Cucurbita*is a source of genetic variations for breeding *C. pepo* and an important vegetable crop cultivated worldwide. Its transcriptome was sequenced by RNA-Seq using the Illumina Hiseq 2000. The more than 52 million reads were assembled in to 62,480 unigenes,a 76.7 % of the unigenes were annotated. This transcriptome was also used to generate a collection of 7814 SSRs (Wu et al. 2014). SNP collections have been also generated in this species, by sequencing with 100 bp single end Illumina a cDNA library generated from a pool of RNA extracted from leaf tissue of a set of 20 genotypes representing the wide variability found in this species (2 commercial varieties, 10 primitive South and Central American landraces, two commercial American varieties, 3 Spanish landraces, and selected accessions from Turkey, Nigeria,

Zimbabwe, and Portugal) (Fig. 2b). The more than 27 million of reads (totalling 1Gb) were mapped against the *C.pepo* transcriptome and 120,503 SNPs were detected (33,888 with MAF >0.1), and 14,282 met criteria to be implemented in a high throughput genotyping platform.

The *Cucurbita* genus includes other three cultivated species, apart from *C.pepo* and *C.moschata* and several wild species. To gain insight into the variation of the whole genus, 96 *Cucurbita* accessions, representing the variation of the five cultivated species (*C. pepo*, *C. maxima*, *C. moschata*, *C. argyrosperma*, and *C. ficifolia*) and most of the wild types of the genus (*C. cordata C. ecuadoriensis*, *C. okeechobeensis*, *C. foetidissima*, *C. lundelliana*, and *C. pedatifolia*) were sequenced using RNA-Seq (Table 4). A total of 814,262,466 sequences were mapped against the *C. pepo* transcriptome and produced a large collection of SNPs within and between the species of the genus. A large collection of SNPs were polymorphic in all the cultivated /wild species of the genus and can be used in genus–wide variability studies. Table 4 shows the number of variants identified within each species and those of each species common to the most economically important species *C. pepo*. For example, 264,386 and 118,548 SNPs were identified in *C. moschata* and *C. maxima*, respectively, of which 65,217 and 24,809 were also variable in *C. pepo* and can be used in wide studies of variability within the genus.

Apart from cDNA sequencing, high-throughput sequencing of small RNAs, involved in post-transcriptional regulation of gene expression, has been conducted in *C. moschata*, *C. maxima* and *C. pepo*. This sequencing has allowed the identification of conserved and novel specific miRNAs. Some miRNAs are differentially expressed in different tissues and species in response to salt stress (Jagadeeswaran et al. 2012; Xie et al. 2015). The study of miRNAs expression in grafted plants has demonstrated that miRNAs may play significant roles in mediating physiological processes of grafted seedlings by regulating the expression of target genes (Li et al. 2014).

Anchoring the Genome

The latest version of the genome (cpepo_genome_v3.2 in https://cucurbigene.upv.es/) was anchored using a genetic map based on a recombinant inbred line (RIL) population. The RIL population was developed through single seed descent from an F_2, derived from across between Zucchini and Scallop, same genotypes that were used to generate the transcriptome. This F_2 was used to generate the first SNP-based genetic map in *Cucurbita* (Esteras et al. 2012b). Only RAPDs, AFLPs and SSRs based maps were available previously (Lee et al. 1995; Brown and Myers 2002; Zraidi et al. 2007; Gong et al. 2008a, b). This first SNP-based map included 304 SNPs and 11 SSRs, distributed into 22 major linkage groups with an average distance among markers of 6.06 cM (Esteras et al. 2012b). The SNP markers were selected from those generated in the first version of the Zucchini-Scallop transcriptome (Blanca et al. 2011a).

Table 4 Illumina sequences generated by RNA-Seq in a collection of representative genotypes in the *Cucurbita* genus

Cultivated species	Nº. sequences	Nº. SNPs	Wild species	Nº. sequences	Nº. SNPs
C. pepo	231828993	321,733	*C. pedatifolia*	44020540	65,713/7428
C. moschata	193635910	264,386/65,217	*C. lundelliana*	36462326	56,639/13,418
C. maxima	136460231	118,584/24,809	*C. foetidissima*	28174614	47,631/6610
C. argyrosperma	58109224	68,085/17,392	*C. ecuadorensis*	23634912	18, 062/4811
C. ficifolia	22720500	6361/2185	*C. okeechobensis*	18976304	11,652/4662
			C. cordata	10811722	3671/1031

Total number of SNPs with minor allele frequency (MAF) > 0.1 within each subgroup and between each taxon and *C. pepo* are indicated

Table 5 Summary of the genetic map generated with the GBS of the RIL population that has been used to anchor the last version of the *C. pepo* genome

Linkage group	N° SNPs	Genetic distance (cM)	Average spacing (cM)	Maximum spacing (cM)	Number scaffolds	Number nucleotides (% of total genome)
1	652	209.3	0.3	11.6	19	21,302,769 (7.95)
2	465	146.2	0.3	6.6	16	14,361,414 (5.36)
3	428	160.1	0.4	9.0	12	13,761,414 (5.14)
4	412	137.6	0.3	8.2	8	10,858,678 (4.05)
5	399	127.0	0.3	8.2	5	10,667,745 (3.98)
6	354	124.4	0.4	9.0	11	10,134,556 (3.78)
7	345	145.8	0.4	16.8	4	10,056,303 (3.75)
8	328	114.1	0.3	12.6	8	9,911,322 (3.70)
9	324	140.3	0.4	24.7	8	9,828,092 (3.67)
10	311	120.6	0.4	9.0	10	9,823,969 (3.67)
11	298	100.7	0.3	13.6	8	9,820,194 (3.67)
12	292	122.5	0.4	9.0	7	9,347,089 (3.49)
13	283	111.8	0.4	6.6	11	8,951,933 (3.34)
14	277	120.5	0.4	13.6	4	8,813,444 (3.29)
15	267	151.3	0.6	36.5	14	8,682,934 (3.24)
16	262	102.4	0.4	11.6	9	8,672,504 (3.24)
17	239	138.9	0.6	24.7	10	8,327,454 (3.11)
18	229	74.5	0.3	5.9	2	8,239,682 (3.08)
19	215	124.2	0.6	11.6	5	8,114,804 (3.03)
20	198	91.5	0.5	6.6	7	7,958,368 (2.97)
21	185	71.6	0.4	9.8	3	4,746,772 (1.77)
overall	6763	2635.3	0.4	36.5	181	21,238,1440 (79.27)

The F_2 population was selfed during eight generations to produce the F8 RIL population with 130 individuals. This RIL population was genotyped by GBS, and more than 60,000 SNPs were identified segregating on it. These SNPs were filtered to discard those that were not biallelic or with quality <90, missing data more than 30 %, minor allele frequency <10 %,or heterozygosity >70 %. Using R packages R/qtl and ASMap (Broman et al. 2003; Taylor and Buttler 2015) a map was constructed that consisted of 6763 SNPs distributed across 21 linkage groups. The individual linkage groups had between 185 and 652 markers and a total length of 2635 cM (results summarized in Table 5). The average distance between successive markers was 0.4 cM, indicating a sufficient density for QTL mapping. Only 3 linkage groups (9, 15 and 17) had intervals between adjacent markers larger than 20 cM.

This map was used to anchor the last version of the genome v.3.2. A total of 181 scaffolds were anchored in the linkage map, with linkage groups having between 2 and 19 scaffolds. These scaffolds covered 212 Mb and represented nearly 80 % of the current version of the Zucchini genome assembly. We detected 6 scaffolds that

mapped to two genomic locations due to misassemblies. These scaffolds were manually corrected.

The homozygous lines of the RIL population were also phenotyped in different environments and the map was used for QTL analysis. Major QTLs were found for growth habit, leaf morphology, early flowering, fruit size and shape and flesh color. The anchoring of genetic and physical maps allowed the identification of candidate genes underlying these major QTLs, such as orthologs of the tomato *OVATE* controlling fruit shape and genes encoding enzymes in the carotenoid metabolism pathway that determines flesh colour.

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Comparative Genomics of the Cucurbitaceae

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Abstract The genome size for watermelon (*Citrullus lanatus*), melon (*Cucumis melo*), cucumber (*Cuucmis sativus*) and pumpkin (*Cucurbita pepo*) is 425, 454, 367 and 502 Mbp, respectively, and considered medium size as compared with most other crops. Whole-genome duplication is common in angiosperm plants. Research has revealed a paleohexaploidy (ϒ) event in the common ancestor of eudicots after the divergence of monocotyledons and dicotyledons. While analysis of published whole-genome sequences of cucumber, melon and watermelon showed traces of these ancient duplication events, there was not evidence of more recent whole-genome duplications in these species. Analysis of the syntenic relationships among watermelon, cucumber, melon and grape has identified 3543 orthologous relationships covering the watermelon, melon, and cucumber genomes. Comparison of melon and cucumber genomes synteny to detect shorter regions of rearrangements confirmed previously reported ancestral fusions of five melon chromosome pairs in cucumber, and several inter- and intra-chromosome rearrangements between the two species. Sequenced genomes of cucumber, melon and watermelon revealed a comparable range of genes from 23 k to 27 k protein coding genes with an average of four to six exons per gene. Current progress in gene mapping technologies such

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as optical mapping, which produces maps of large individual DNA molecules, can improve cucurbit comparative genomics to detect large structural variations and DNA rearrangements across the species.

Keywords Chromosomal rearrangement • Cucumber • Genome annotation • Melon • Synteny • Watermelon • Whole genome duplication

Introduction

The family Cucurbitaceae contains 118 genera and 825 species (Jeffrey 1980). Members are morphologically similar, which implies strong synteny at the molecular level. Cucurbits, which are herbaceous annuals, are often prostrate or climbing by means of tendrils. Stems are typically 5-angled, characterized anatomically by bicollateral vascular bundles often arranged in two concentric rings. Leaves are alternate, exstipulate, simple or occasionally palmately compound, palmately veined and usually lobed (Bates et al. 1990; Whitaker et al. 1976). A notable feature is unisexual flowers with determinate inflorescence. Calyx are symsepalous with five lobes and corolla sympetalous, usually composed of five lobes, and flowers consisting of one to five stamens (usually 3: 2 double stamens and 1 single stamen), anthers, and dehiscing longitudinally (Bates and Robinson 1995). The gynoecium is an inferior ovary with three carpels. Fruits are fleshy, often large, containing several to hundreds of seeds; the exocarp is soft leathery to hard and lignified with phytoliths. Fruit types range from a gourd-like berry or pepo, frequently containing bitter purgative cucurbitacins (bitter-tasting substances) (Nee 1990).

Cucurbitaceae or the cucurbit family is monophyletic because of morphological and biochemical distinctness and represents economically important species, particularly those with edible and medicinal fruits. The family Cucurbitaceae includes domesticated species for food: *Citrullus lanatus* (watermelon), *Cucumis sativus* (cucumber), *Cucumis melo* (melon), *Cucurbita* (five species of squash and pumpkin), *Cucumis anguria* (bur gherkin), *Momordica charantia* (bitter melon), *Sechium edule* (chayote), *Luffa* (two species of loofah), *Lagenaria siceraria* (bottle gourd), *Benincasa hispida* (wax gourd), *Trichosanthes* (two species of snake gourd), *Telfairia* (two species of oyster nut), *Sicana odorifera* (casabanana), *Coccinia grandis* (ivy gourd), *Praecitrullus fistulosus* (tinda), *Cyclanthera pedata* (slipper gourd), and *Cucumeropsis mannii* (white-seeded melon) (Bates and Robinson 1990). The most important cucurbit crops worldwide are cucumber, watermelon, melon, squash and pumpkin (McCreight 2016). In the United States, per-capita civilian utilization (farm weight) of cucurbits (watermelon 6.5 kg, cantaloupe 5.0 kg, honeydew melons 1.0 kg, cucumbers 3.0 kg) is 20% of the total vegetable consumption in the United States, contributing a farm value of $500 million (Statistics of Vegetables and Melons).

Cucumber (*C. sativus* L.; $2n = 2x = 14$) originated in Asia and is currently a major vegetable crop in countries in Asia, Europe, and North America (Nagele and Wehner 2016). By using molecular markers, several researchers have concluded that the genetic base within each market class of cultivated cucumber is very narrow (Perl-Treves et al. 1985; Knerr et al. 1989; Knerr and Staub 1992; Qi et al. 2013; Lv et al. 2012; Pandey et al. 2013). Resequencing analyses identified four geographic groups: Indian; cultivated lines from Eurasian, Europe, and the United States; cultivated types from East Asia; and Xishuanghanna, comprised largely of landraces cultivated in tropical southwestern China (Qi et al. 2013). The Indian materials, which contained the most genomic diversity, may provide novel genetic resources.

Melon, a diploid plant species (*C. melo* L.; $2n = 2x = 24$) is an important fruit crop, with 26 million tons of melons produced worldwide in 2009 (http://faostat.fao.org) (Garcia-Mas et al. 2012). Melons comprise a diverse group of fresh dessert fruits that include the orange-flesh cantaloupes, green-flesh honeydew, and mixed melons (Casaba, Crenshaw, Persian, Santa Claus, Juan Canari) (Pitrat et al. 2000; Robinson and Decker-Walters 1997). Botanical diversity in melon cultivated morphotypes is of interest because of the specific biological properties and offers a unique opportunity to perform basic research for understanding various biological properties such as fruit quality, disease resistance and sex expression (Sebastian et al. 2010; Nimmakayala et al. 2016).

Watermelon ($2n = 2x = 22$) (Shimotsuma 1963) belongs to the genus *Citrullus* Schrad. Ex Eckl. et Zeyh. Its seven species thrive in dry regions throughout Africa and Asia and in semi-desert regions from the Atlantic Islands eastwards to Afghanistan and Pakistan (Jeffrey 1967). *Citrullus lanatus* Matsum. and Nakai, the common sweet watermelon, is indigenous to north Africa (Wasylikowa and Van Der Veen 2004; Chomicki and Renner 2014; Paris 2015) and may be derived from the 'egusi' melon *C. mucosospermus* Fursa (Chomicki and Renner 2014). In contrast, the citron or tsamma melon (*C. amarus* Schrad.) is native to southern Africa. Genetic diversity within the *Citrullus* species provide a resources of genes conferring resistance to a numerous fungal, oomycete and viral disases, as well as resistances to nematodes and several insect pests (Levi et al. 2016).

The genus *Cucurbita* ($2n = 2x = 40$) is native to the Americas and found in the wild from the United States to Argentina (Gong et al. 2013). Five species of *Cucurbita* known as pumpkins and squash have been cultivated for millennia in the Americas, mostly for their edible fruits (Gong et al. 2013). *Cucurbita* is one of 95 genera of the gourd family Cucurbitaceae (Schaefer and Renner 2011). The five cultivated species have different native ranges and climactic adaptations (de Oliveira et al. 2016). They were distributed during cultivation differently, usually allopatrically, throughout all but the coldest parts of the Americas in pre-Columbian times, from North America to South America and from coastal lowland regions to interior highland regions (Zheng et al. 2013). Archaeological remains of *C. pepo* and *C. argyrosperma* have been found at sites in North America, and *C. moschata*, *C. maxima*, and *C. ficifolia* were found in South America (Whitaker and Cutler 1965; Fritz 1994; Lira and Montes 1994; Kong et al. 2014; Paris et al. 2015).

The completion of reference genome sequences for many important crops and the ability to perform resequencing related genomes is revolutionizing crop plant comparative genomics, including for the Cucurbitaceae, for which draft sequences are currently available for cucumber, melon and watermelon (Huang et al. 2009; Guo et al. 2013; Garcia-Mas et al. 2012). These genomes provide critical resources for comparative plant genomics. Examination of similarities and divergences among the genomes of various taxa belonging to crucial nodes of phylogenies can uncover the functional regions of genome, structural variants, inversions and translocations among the genomes (Caicedo and Purugganan 2005; Chaney et al. 2016; Gerats and Vandenbussche 2005; Morrell et al. 2012). The recent increase in genomic data is also revealing an unexpected perspective of gene loss as a pervasive source of genetic variation that can cause adaptive phenotypic diversity (Albalat and Canestro 2016). Recent advances in low-cost mapping tools such as improved optics, informatics tools for optical mapping and creative innovations to resolve structural variants have made genome-mapping technology more widely available (Chaney et al. 2016) and can be used for cucurbit comparative genome studies in future.

The genome size of watermelon, melon, cucumber and pumpkin is 425, 454, 367 and 502 Mbp, respectively (Arumuganathan and Earle 1991), and considered small as compared with other crops such as wheat (15,966 Mbp), tomato (907 Mbp), cotton (2500 Mbp), onion (15,290 Mbp), pepper (3420 Mbp) and corn (2716 Mbp). Use of the melon, watermelon and cucumber genome sequences has allowed for an extensive phylogenic comparison of cucurbit species (Huang et al. 2009; Guo et al. 2013; Garcia-Mas et al. 2012). The genome sequences and genetic maps are excellent tools for understanding the genome structure and evolution of various species with different chromosome number (melon, $2n = 2x = 24$; cucumber, $2n = 2x = 14$; watermelon $2n = 2x = 22$ and pumpkin, $2n = 2x = 40$) as will be described in the following sections.

Syntenic Relationships Among the Cucurbit Genomes

While several synteny maps are available many important plant families such as Solanaceae and grasses (refs), syntenic relationships among Cucurbitaceae remain to be resolved. However, numerous recent studies have provided insight into their relationships. Yang et al. (2012) investigated genetic differentiation between *C. sativus* var. *sativus* and the wild *C. sativus* var. *hardwickii* by comparative fluorescence *in situ* hybridization analysis of pachytene chromosomes with selected markers from the genetic map and draft genome assembly. This study revealed significant differences in the amount and distribution of heterochromatin, as well as chromosomal rearrangements, between the two taxa. In particular, six inversions, five paracentric and one pericentric, were revealed in chromosomes 4, 5 and 7. Comparison of the order of fosmid loci of selected markers on chromosome 7 of cultivated and wild cucumbers and the syntenic melon chromosome 1 suggested that the paracentric inversion in this chromosome occurred during domestication of cucumber.

These results supported the sub-species status of these two cucumber taxa and suggest that *C. sativus* var. *hardwickii* is the progenitor of cultivated cucumber.

After sequencing the cucumber genome, Huang et al. (2009) proposed that five cucumber chromosomes arose from a fusion of ten ancestral chromosomes after divergence from *C. melo*. The authors reported that 348/522 (66.7%) melon genetic markers and 136/232 (58.6%) watermelon genetic markers were aligned on the cucumber chromosomes. The comparison revealed cucumber chromosome 7 corresponds to melon chromosome 1 and watermelon group 7. Li et al. (2011a) constructed a consensus melon linkage map derived from two previous genetic maps with the largest number of cross-species cucumber molecular markers and identified that melon chromosome 1 was syntenic with cucumber chromosome 7. Furthermore, melon chromosomes 2 and 12 were syntenic with cucumber chromosome 1, melon chromosomes 4 and 6 with cucumber chromosome 3, and melon chromosomes 9 and 10 with cucumber chromosome 5. Similarly, the 3 melon chromosomes 3, 8, and 11 contained blocks that were syntenic with 2 cucumber chromosomes, 2 + 6, 4 + 6, and 2 + 6, respectively. This study further concluded that the arrangement of melon syntenic blocks across the seven cucumber chromosomes indicates that cucumber chromosome evolution is more complex than simple chromosome fusions. For instance, cucumber chromosome 7 was homologous to melon chromosome 1 along its entire length. Cucumber chromosomes 2 and 6 each contained 3 syntenic blocks detected in melon chromosomes 5 + 11 + 3, and 3 + 11 + 13, respectively, and the remaining 4 cucumber chromosomes (1, 3, 4, and 5) were syntenic with 2 melon chromosomes but differed in patterns of arrangement of melon syntenic blocks. Cucumber chromosome 1 was syntenic with melon chromosomes 2 and 12, whereas cucumber chromosome 5 was syntenic with melon chromosomes 9 and 10. In both cases, the syntenic blocks from the 2 melon chromosomes were arranged alternatively along each cucumber chromosome. In contrast, the syntenic blocks residing in melon chromosomes 6 and 4 were in a side-by-side alignment in cucumber chromosome 3. Finally, cucumber chromosome 4 housed syntenic blocks of melon chromosomes 7 and 8. Taken together, these syntenic patterns were suggestive of a complex history of chromosomal structure changes during cucumber evolution.

Garcia-Mas et al. (2012) compared an alignment of melon and cucumber genomes synteny to detect shorter regions of rearrangements that were not previously noted, to confirm most of the previously reported ancestral fusions of five melon chromosome pairs in cucumber and several inter- and intra-chromosome rearrangements. This study confirmed findings of Li et al. (2011a) that melon LGI corresponded to cucumber chromosome 7, with higher resolution of several inversions and an increase in the total chromosome size (35.8 vs. 19.2 Mb). Likewise, this study noted that melon LGIV and LGVI were 30.4 and 29.8 Mb, whereas their putative fusion in cucumber was chromosome 3 (39.7 Mb). The first distal 8.5 and 5 Mb of melon LGIV and cucumber chromosome 3, respectively, are highly collinear and melon shows a progressive increase in size toward the centromere because of transposon amplification. Garcia-Mas et al. (2012) identified 19,377 one-to-one ortholog pairs between melon and cucumber, yielding 497 orthologous syntenic

blocks. Further refinement of the physical maps and sequencing of other *Cucumis* species may shed additional light on the genome structure of the ancestor of cucumber and melon.

Guo et al. (2013) analyzed the syntenic relationships between watermelon, cucumber, melon and grape to identify 3543 orthologous relationships covering 60% of the watermelon genome. This study further resolved complicated syntenic patterns using detailed chromosome-to-chromosome relationships within the Cucurbitaceae family and identified orthologous chromosomes between watermelon, cucumber and melon. The insights of high degree of complexity of chromosomal evolution and rearrangement by using chromosome-to-chromosome orthologous relationships unveiled genomic relationships of these three important crop species of the Cucurbitaceae family. Integration of independent analyses of duplications within, and syntenies among, the four eudicot genomes (watermelon, cucumber, melon and grape) led to the precise characterization in watermelon of the seven paleotriplications identified recently as the basis for defining seven ancestral chromosomal groups in eudicots (Abrouk et al. 2010). With the ancestral hexaploidization (γ) reported for the eudicots, Guo et al. (2013) proposed an evolutionary scenario that has shaped the 11 watermelon chromosomes from the 7-chromosome eudicot ancestors through the 21 paleohexaploid intermediates. The authors suggested that the transition from the 21-chromosome eudicot intermediate ancestors involved 81 fissions and 91 fusions to reach the modern 11-chromosome structure of watermelon, represented as a mosaic of 102 ancestral blocks in the watermelon genome.

Genome Duplications

Ancient whole-genome duplications (WGDs), also referred to as paleopolyploidizations, have been reported in most evolutionary lineages. Vanneste et al. (2014) performed a Bayesian evolutionary analysis of 38 full genome sequences and three transcriptome assemblies to note clustering of angiosperm paleopolyploidizations around the Cretaceous–Paleogene (K–Pg) extinction event, about 66 million years ago. This study further demonstrated a strongly nonrandom pattern of genome duplications over time, with many WGDs clustering around the K–Pg boundary. With the increase in number of available plant genomes described, the observation of WGD events will help in understanding their evolution. In cucurbits, the description of the genome sequence of additional species will help determine whether the lack of a recent WGD is unique to this lineage (Huang et al. 2009; Guo et al. 2013; Garcia-Mas et al. 2012). Traces of duplications observed in cucumber, melon and watermelon may correspond to the ancestral paleo-hexaploidization that occurred after the divergence of monocots and dicots, with subsequent genome rearrangements and genome size reduction. Transposable elements have accumulated to a greater extent in melon than cucumber, with peak activity about 2 Mya, which suggests that the larger genome size of melon, probably to a large extent, may be due to transposon amplification. However, loss of chromosome fragments during

chromosome fusion in cucumber may also explain the larger melon genome. Melon and cucumber diverged only around 10 Mya and represent an interesting evolution relating to differences in genome size and chromosome number (450 vs. 367 Mb and $x = 12$ vs. $x = 7$).

WGD is common in angiosperm plants and produces a tremendous source of raw material for gene genesis. Previous research has revealed a paleohexaploidy (ϒ) event in the common ancestor of *Arabidopsis thaliana* and grapevine after the divergence of monocotyledons and dicotyledons (Jaillon et al. 2007; Bowers et al. 2003). Subsequently, two WGDs (α and β) occurred in Arabidopsis and one (*p*) in poplar, with no recent WGD in grapevine or papaya (Tuskan et al. 2006). Rice underwent an ancient WGD (Yu et al. 2005). A collinear gene-order analysis of the cucumber genome revealed no recent WGD and only a few segmental duplication events (Huang et al. 2009). A distance-transversion rate at fourfold degenerate sites (4DTv method) was used to analyze paralogous gene pairs between syntenic blocks for Arabidopsis and cucumber (Huang et al. 2009). Two peaks (~0.06 and ~0.25) in Arabidopsis support the two recent WGDs. Cucumber showed ancient duplication events (peak at ~0.60) but not a recent WGD. This lack of recurrent WGD in the small cucumber genome provides an important complement to the grapevine and papaya genomes to study ancestral forms and arrangements of plant genes. Duplication analysis of entire phylomes has been used to confirm ancient WGD events that represent duplication peaks in the corresponding evolutionary periods (Huang et al. 2009). Melon results were consistent with the absence of WGD in the lineages leading to *C. melo* (Garcia-Mas et al. 2012). In the watermelon genome, Guo et al. (2013) identified seven major triplications that corresponded to 302 paralogous relationships covering 29% of the genome. This event would confirm a speciation event in the ancestral cucurbit genome 15–23 Mya.

Gene Prediction and Annotation

Huang et al. (2009) sequenced the whole genome of cucumber (9930V1.0) to identify 26,682 genes with a mean coding sequence size of 1046 bp and a mean of 4.39 exons per gene. Gene model prediction in this study was supported by three gene prediction methods, of which 25% had both ab initio prediction and homology-based evidence, and 7.4% had ab initio prediction supported by transcriptome datasets. In addition, 292 rRNA fragments and 699 tRNA, 238 small nucleolar RNA, 192 small nuclear RNA and 171 microRNA genes were revealed. The cucumber genes represent 15,669 families; 4362 are unique to cucumber, with 3784 single-gene families. Li et al. (2011b) improved annotation of protein coding genes from extensive RNAseq for ten different tissues of 9930 to identify 3434 lesser genes after removal of bacterial genes and corrected protein-coding structures for eight, 700 genes and identified ~5200 new genes to Version 2.0. The annotation of the melon genome predicted 27,427 genes with 34,843 predicted transcripts encoding 32,487 predicted polypeptides (Garcia-Mas et al. 2012). The average gene size for melon is 2776 bp, with 5.85 exons per gene, similar to

Arabidopsis, and a density of 7.3 genes per 100 Kb. A total of 16,120 genes (58.7%) had exons supported by ESTs, totaling 18,948 genes (69.1%) supported by transcript and/or a protein alignment. A total of 1253 noncoding RNA genes were identified in the melon genome. Of the 140 potential microRNA loci identified, 122 corresponded to 35 known plant miRNA families. In watermelon, among 23,440 high-confidence protein-coding genes, 85% had transcriptome support. In addition, 123 ribosomal RNA, 789 transfer RNA, 335 small nuclear RNA and 141 miRNA genes were located in the watermelon cultivated genome, which is comparable to the other sequenced genomes of cucurbits.

Transposon Annotation

By using homology and structure-based searches, Garcia-Mas et al. (2012) identified 323 transposable element representatives in the melon genome belonging to known superfamilies. With these sequences used for melon genome analysis, 73,787 copies of various superfamilies were found to occupy 19.7% of the genome space. Use of the same annotation pipeline to compare retrotransposons in the Gy14 cucumber genome revealed that retrotransposons represented 1.5% of the genome, significantly less than in melon, which suggests that retrotransposon activity was greater and more recent in the melon than cucumber lineage. Garcia-Mas et al. (2012) further compared CACTA, MULE and PIF/Harbinger, the three most represented superfamilies, to show 10× more amplification in melon for CACTA, 47× for MULE, and 3.8× for PIF, thereby confirming a divergence of 10.1 Mya between melon and cucumber. Guo et al. (2013) identified 159.8 Mb (45.2%) of the assembled watermelon genome as transposable element repeats; 68.3% could be annotated with known repeat families. Transposable element divergence rates peaked at 32%. The authors further identified 920 (7.8 Mb) full-length LTR retrotransposons in the watermelon genome. Over the past 4.5 million years, LTR retrotransposons accumulated much faster in watermelon than cucumber, so the overall difference in their genome sizes may reflect the differential LTR retrotransposon accumulation (Guo et al. 2013).

Disease Resistance Genes

Only 61 nucleotide binding site (NBS)-containing resistance (NBS-R) genes have been identified in cucumber, similar to papaya (55), but only a fraction of that found in Arabidopsis (200), poplar (398) and rice (600) (Huang et al. 2009). Distribution of NBS genes on cucumber chromosomes is non-random, with only five genes located on chromosomes 1, 6 and 7 and 20 on chromosome 2. Three-quarters of the NBS genes are located within 11 clusters, which indicates that they evolved by tandem duplication, similar to other known plant genomes. A total of 411 putative disease resistance genes were identified in the melon genome (Garcia-Mas et al.

2012); 81 represented NBS, leucine-rich repeat (LRR) and Toll-interleukin receptor (TIR) domains that were non-randomly distributed, and 45% of the NBS-LRR genes were grouped within nine clusters similar to cucumber. In watermelon, 44 NBS-LRR-TIR genes (18 TIRs and 26 coiled-coil NBS-LRR–encoding genes) were identified (Guo et al. 2013). In cucumber, 23 lipoxygenase (LOX) pathway genes were identified; such genes have an important role in defense and pest resistance by generating short-chain aldehydes and alcohols (Huang et al. 2009). In watermelon, 45 members belonging to the LOX gene family were arranged in two tandem arrays. Among the 197 receptor-like genes in the watermelon genome, 35 encode receptor-like proteins lacking a kinase domain in addition to the extracellular LRR and transmembrane domains (Guo et al. 2013). In melon, 290 transmembrane receptors, 161 receptor-like kinases (RLKs), 19 kinases containing an additional anti-fungal protein ginkbilobin-2 domain, and 110 receptor-like proteins genes were also documented (Garcia-Mas et al. 2012).

Conclusions

Use of genome sequences is becoming a strategic tool for gene expression and genome-wide association studies to accelerate plant breeding and basic biological research. Comparative cucurbit genome analysis involves examining the similarities and unique differences to shed light on the underlying genome evolution and identify economically important traits. Defining syntenic blocks by comparative mapping has shown that numerous alterations in diverse genomes contributed to genetic diversity among plants. Over time, chromosomes are broken, reassembled, partially or wholly duplicated, and even eliminated, ultimately resulting in reproductive isolation and speciation (Koenig and Weigel 2015; Hall et al. 2002). For example, comparative genome analysis to understand conserved syntenic blocks in cucurbit genomes holds promise for clarifying the selection pressures driving genetic changes. Modern genome mapping strategies such as optical mapping, which uses microscopic imaging to produce ordered restriction enzyme recognition site maps from a single linearized DNA molecule, allows for detecting DNA with resolution of 1 Kb to several mega base pairs (Chaney et al. 2016). Genomic alterations are an important source of genetic and phenotypic diversity. For example, structural variations that include insertions, deletions, duplications, inversions and translocations resolved with optical mapping strategies have been associated with stress tolerance, resistance, increase in yields, reproductive morphology, adaptation and speciation (Chaney et al. 2016). Such investigations will elucidate alterations at the level of the whole genome, for diversifying cultivars with narrow genetic backgrounds. Comparative maps of all the other cereals have been useful to bridge information from one species to the other, of immense use for breeding, ecology and molecular biology. The development of whole-genome sequence drafts has provided a foundation for widening a narrow genetic background, marker-assisted selection and to understand intricate genome rearrangements to study genetics and breed improved

varieties in less-important crops. Furthermore, by using the reference maps of various cucurbits that are anchored with several other crop genomes, we can identify major genes affecting agronomic characters found in different species.

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Organellar Genomes of the Cucurbits

Michael J. Havey

Abstract The cucurbit organellar DNAs possess distinctive characteristics of practical and theoretical significance. Whereas the cucurbit chloroplast DNAs are similar in size, structure, and transmission to most Angiosperms, their mitochondrial DNAs show enormous size differences. Plants in the genus *Cucumis* have some of the largest mitochondrial DNAs among all plants, due in part to accumulation of repetitive DNAs and inter-genomic transfers. Recombination among these repetitive motifs produces structurally diverse mitochondrial DNAs associated with paternally transmitted mosaic phenotypes and altered gene expression. The mitochondrial DNAs of *Cucumis* species are paternally transmitted, which is relatively rare among Angiosperms. The unique characteristics of the *Cucumis* organelles are interesting not only from an evolutionary point-of-view, but also may allow for characterization of beneficial organellar-nuclear interactions, generation of mitochondrial mutants, transformation of the mitochondrial DNA, and knock-downs of mitochondrial-gene expression.

Keywords DNA • Mitochondrion • Mutation • Plastid • Transformation • Transmission genetics

The Organellar Genomes

The plant cytoplasm carries two organelles, the plastid and mitochondrion, each with their own DNA. The organellar DNAs encode for proteins important for photosynthesis and respiration, as well as a subset of the transfer and ribosomal RNAs necessary for protein synthesis (Unseld et al. 1997; Mackenzie and McIntosh 1999; Notsu et al. 2002; Handa 2003; Ogihara et al. 2005; Sugiyama et al. 2005; Allen et al. 2007). In addition to organellar-encoded products, hundreds of nuclear-encoded proteins are required for functional organelles (Emanuelsson et al. 2000; Giegé et al. 2005). The intimate interaction between the organelles and nucleus is

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imperative for overall plant performance (Kihira 1982), stress tolerances (Millar et al. 2003; Atkin and Macherel 2009; Vanlerberghe et al. 2009; Gill and Tuteja 2010), and programmed cell death (Gottlieb 2000; Gechev et al. 2006; Reape and McCabe 2008).

Sizes and Structures of the Cucurbit Organellar Genomes

Plastids

Restriction-enzyme analyses of the plastid DNAs of the major cucurbits demonstrated that their sizes and structures are similar to most other angiosperms with large and small single-copy regions separated by two inverted repeats (Palmer 1982; Perl-Treves and Galun 1985; Lim et al. 1990). The sequences and annotations of the plastid DNAs from cucumber (Kim et al. 2006; Chung et al. 2007; Pląder et al. 2007) and melon (Rodriguez-Moreno et al. 2011) have been published. The reported sizes of the cucumber plastid DNA vary from 155,293 (Pląder et al. 2007) to 155,527 basepairs (bp) (Kim et al. 2006), likely due to small differences in intergenic regions. The melon plastid DNA is slightly larger than cucumber at 156,017 bp (Rodriguez-Moreno et al. 2011). GC contents were similar for cucumber (40 %) and melon (45 %). Although different numbers of coding regions were reported for the plastid DNAs of cucumber versus melon, sequence differences were only about 5 % primarily due to short indels and single nucleotide polymorphisms (Kim et al. 2006; Rodriguez-Moreno et al. 2011).

Variations in plastid sequences have been used to estimate maternal phylogenies within the Cucurbitaceae, which correlated well with floral and pollen morphologies (Kocyan et al. 2007). Schaefer et al. (2009) extracted DNA from herbarium specimens of cucurbit species, successfully amplified and sequenced regions from the plastid DNAs, and constructed a maternal phylogeny supporting long-distance transoceanic dispersal of cucurbits probably by floating fruit. Plastid-sequence variation, together with mitochondrial and nuclear sequences, revealed phylogenies indicating that monoecy may have evolved from a progenitor dioecious state numerous times in the Cucurbitaceae. Variation for fragment lengths and sequences in the genus *Cucumis* revealed wild species more closely related to cultivated species (Chung et al. 2006; Sebastian et al. 2010), information important for use of these species for genetic improvement of cucumber and melon.

Mitochondria

The cucurbit mitochondrial DNAs show several-fold size differences (Ward et al. 1981). Watermelon possesses a relatively small mitochondrial genome of 379 kilobases (kb), and squash has a mitochondrial genome 2.5 times bigger at 983 kb (Alverson et al. 2010). Cucumber and melon possess huge mitochondrial genomes of

1,685 kb (Alverson et al. 2011) and approximately 2,460 kb [calculation based on estimate by Ward et al. (1981) of 1,600 megadaltons divided by 650 daltons per basepair], respectively. Sequencing revealed a larger mitochondrial DNA of melon than estimated by Ward et al. (1981), and assembled into five scaffolds covering 2,740 kb and corresponding to approximately 95 % of the genome (Rodriguez-Moreno et al. 2011). The significant size differences among cucurbit mitochondrial DNAs cannot be attributed to increased mitochondrial volume (Bendich and Gauriloff 1984); accumulation of conserved repetitive DNAs, microsatellites, or transposable elements (Ward et al. 1981; Rodriguez-Moreno et al. 2011); or increased numbers of coding regions (Alverson et al. 2010, 2011; Rodriguez-Moreno et al. 2011). In fact, only about 119 kb of highly conserved sequences were shared among the mitochondrial DNAs of melon, squash, and watermelon (Rodriguez-Moreno et al. 2011). Sequencing has revealed that accumulation of short repetitive motifs and DNA transfers from the chloroplast contributed to expansion of mitochondrial-DNA sizes in cucumber and melon (Lilly and Havey 2001; Bartoszewski et al. 2004a; Alverson et al. 2011; Rodriguez-Moreno et al. 2011). For melon, DNA transfer from the nucleus to the mitochondrion accounted for 1.14 megabases of sequence, or 42 % of its mitochondrial DNA. This large-scale transfer of nuclear DNA into the melon mitochondrion is the largest so far reported and significantly contributed to genome-size expansion.

Organellar Transmission

The vast majority of plants show maternal transmission of their organelles (Gillham 1978; Harris and Ingram 1991); however examples of paternal (Boynton et al. 1987; Neale and Sederoff 1989; Havey et al. 1998) or biparental (Medgyesy et al. 1986; Smith 1989; Mason et al. 1994; Erickson et al. 1989; Erickson and Kemble 1990) transmission of the organelles have been documented. A unique characteristic of organellar transmission among the cucurbits is paternal transmission of the *Cucumis* mitochondrial DNAs (Matsuura 1995; Havey 1997; Havey et al. 1998; Shen et al. 2013), versus maternal transmission of the squash and watermelon mitochondrial DNAs (Havey et al. 1998). The cucurbit plastids appear to be maternally transmitted, as evidenced by maternal transmission of chlorophyll deficiencies, presumably due to mutations in the chloroplast genome, in squash (*Cucurbita maxima* Duch.; Hutchins and Youngner 1952) and melon (Ray and McCreight 1996). Corriveau and Coleman (1988) used a DNA fluorochrome and epifluorescence microscopy to demonstrate physical exclusion of chloroplast DNA from the male gametophyte of cucumber, indirect evidence for maternal transmission.

Paternal transmission of the mitochondrial DNA is likely a derived state and possibly restricted to the genus *Cucumis*, although this exceptional transmission mode has been conclusively demonstrated only for cucumber, melon, and *C. hystrix* (Havey et al. 1998; Shen et al. 2013). Shen et al. (2015a) studied the prevalence of organellar DNAs in the developing male gametophyte of cucumber and presented evidence that the mitochondrial DNA is protected from nuclease(s) during microsporogenesis, resulting in retention and delivery via the sperm cells to progenies. One question commonly asked

when I speak or write about the paternal transmission of *Cucumis* mitochondria is what evolutionary advantage would be provided by paternal transmission of an organelle, since its occurrence is so rare? One hypothesis that I can offer is that as the size of the mitochondrial DNA increases due to accumulation of repetitive DNAs in direct and reverse orientations, the probability of pairing among these repeats increases allowing for intra- and inter-molecular recombination to shift the order of genes and/or produce sub-genomic molecules (sublimons). These recombination events can affect the transcription of mitochondrial genes by removing or altering the positions of up-stream promoter region(s) (Bartoszewski et al. 2004b), as well as affecting relative copy numbers because sublimons without origins of replication could become rarer [a process known as substoichometric shifting (Woloszynska 2010)] and potentially lost. These events would negatively affect mitochondrial function. With strict maternal transmission of the mitochondrial DNA, it may be more difficult to select against or eliminate deleterious sublimons from the population of mitochondrial DNAs transferred to progenies via the egg cell. In the case of paternal transmission, presumably relatively few mitochondria are delivered via the male gametophyte to the zygote and subsequently to progenies. This may provide a bottleneck restricting the number of mitochondria present in the sperm cells, representing a method to select for superior-performing, or against poor-performing, mitochondria. However if this hypothesis were true, one would expect that other plants with enormous mitochondrial DNAs would tend to show paternal transmission. At least in the case of *Silene conica*, the size of its mitochondrial DNA is estimated at 11,318 kb and transmission is maternal (Sloan et al. 2012); however there is evidence for occasional paternal or bi-parental transmission of the mitochondrial DNAs in the genus *Silene* (McCauley et al. 2005; Pearl et al. 2009; Bentley et al. 2010). Banana (*Musa acuminata*) also shows paternal transmission of its mitochondria (Fauré et al. 1994); however I could find no estimates of the mitochondrial DNA sizes for *Musa*. It will be interesting to determine the transmission modes and sizes of mitochondrial DNAs throughout the genus *Cucumis*, and establish if any relationship exists between size and transmission. An approach would be to first estimate the sizes of the organellar DNAs across *Cucumis* species, for example by differential centrifugation (Ward et al. 1981) or building contigs among large genomic clones (Bartoszewski et al. 2009), followed by next-generation sequencing and assembly to the cucumber and melon organellar DNAs to identify polymorphisms between plants of the same species. Then reciprocal hybrids can be genotyped for these polymorphic markers to establish transmission of the organellar DNAs. Another important line of research would be to determine if any cucurbits outside of the genus *Cucumis* show paternal transmission of the mitochondrial DNA.

Organellar Phenotypes

Maternal transmission of chlorophyll deficiencies, presumably due to mutations in the plastid DNA, has been demonstrated for *Cucurbita maxima* (Hutchins and Youngner 1952) and melon (Ray and McCreight 1996). A maternally transmitted tolerance to

cold temperature has been reported from the pickling cucumber ‘Chipper’ (Chung et al. 2003). Sequencing of the plastid DNAs from cold-tolerant and susceptible cucumbers revealed three single nucleotide polymorphisms, one of which resulted in a single amino-acid change in the atpB protein (Chung et al. 2007). It remains unclear how this single amino-acid change would condition cold tolerance; however because sequencing was completed using amplicons from the polymerase chain reaction, it is possible that rarer sublimons exist among the plastid DNAs from cold-tolerant cucumber that could condition or elicit this abiotic-stress tolerance. To the knowledge of the author, no other maternally transmitted phenotypes have been documented in the cucurbits.

A paternal effect on chilling tolerance has been reported in cucumber (Ali et al. 2014). Although reciprocal crosses between chilling-tolerant (CH1) and susceptible (CH4) cucumbers resulted in identical phenotypes in F_1 families, the F_2 family from CH4 × CH1 was more tolerant to chilling than the F_2 family from CH1 x CH4 indicating a parent-of-origin effect on this trait which could be due to a mitochondrial effect or preferential expression of paternal nuclear allele(s).

The mosaic (MSC) mutants of cucumber are paternally transmitted phenotypes showing chlorotic to necrotic regions on leaves, reduced vigor and fertility, and enhanced expression of stress-response genes (Malepszy et al. 1996; Bartoszewski et al. 2007; Juszczuk et al. 2007; Szal et al. 2009). The MSC mutants were first observed among progenies from plants regenerated from cell cultures established using the highly inbred line ‘B’ selected from the Polish pickling cultivar ‘Borszczagowski’ (Fig. 1; Malepszy et al. 1996; Ładyżyński et al. 2002; Bartoszewski et al. 2004b, 2007). Paternal transmission of the MSC phenotypes (Malepszy et al. 1996) immediately focused attention on their mitochondrial DNAs. The MSC mutants possess mitochondrial DNAs with structural polymorphisms and deleted regions relative to wild-type inbred B (Lilly et al. 2001; Del Valle-Echevarria et al. 2015), and independently derived MSC mutants possess different mitochondrial polymorphisms indicating that they do not trace back to a single sublimon in B (Lilly et al. 2001; Bartoszewski et al. 2004b; Del Valle-Echevarria et al. 2015). Passage of cucumber through cell cultures may allow mitochondria carrying deleterious lesion(s) to sort by their reducing negative effects. Another possibility is that passage through cell culture may enhance recombination among repetitive DNAs to produce structural rearrangements or deletions affecting the expression of mitochondrial genes. Sequencing of the mitochondrial DNAs from three independently derived MSC lines (3, 12, and 16) revealed that all lines differed for regions that were missing or of significantly lower copy number relative to inbred B. MSC3 possessed significantly fewer copies of the polycistronic region coding for exons 4 and 5 of NADH dehydrogenase subunit 5 and ATPase subunit 4, while MSC 12 and 16 possessed an under-representation of the ribosomal protein S7 (rps7) coding region (Del Valle-Echevarria et al. 2015). Although sharing an under-representation of the same rps7 coding region, the mitochondrial DNAs of MSC12 and 16 differ both structurally (Bartoszewski et al. 2004b) and for sizes of overall missing or under-represented regions (Del Valle-Echevarria et al. 2015).

MSC plants are heteroplasmic for both mutant and wild-type mitochondrial genomes, with the former predominating, and relatively rare wild-type progenies result from sorting of the mitochondrial genome in MSC pollen (Lilly et al. 2001).

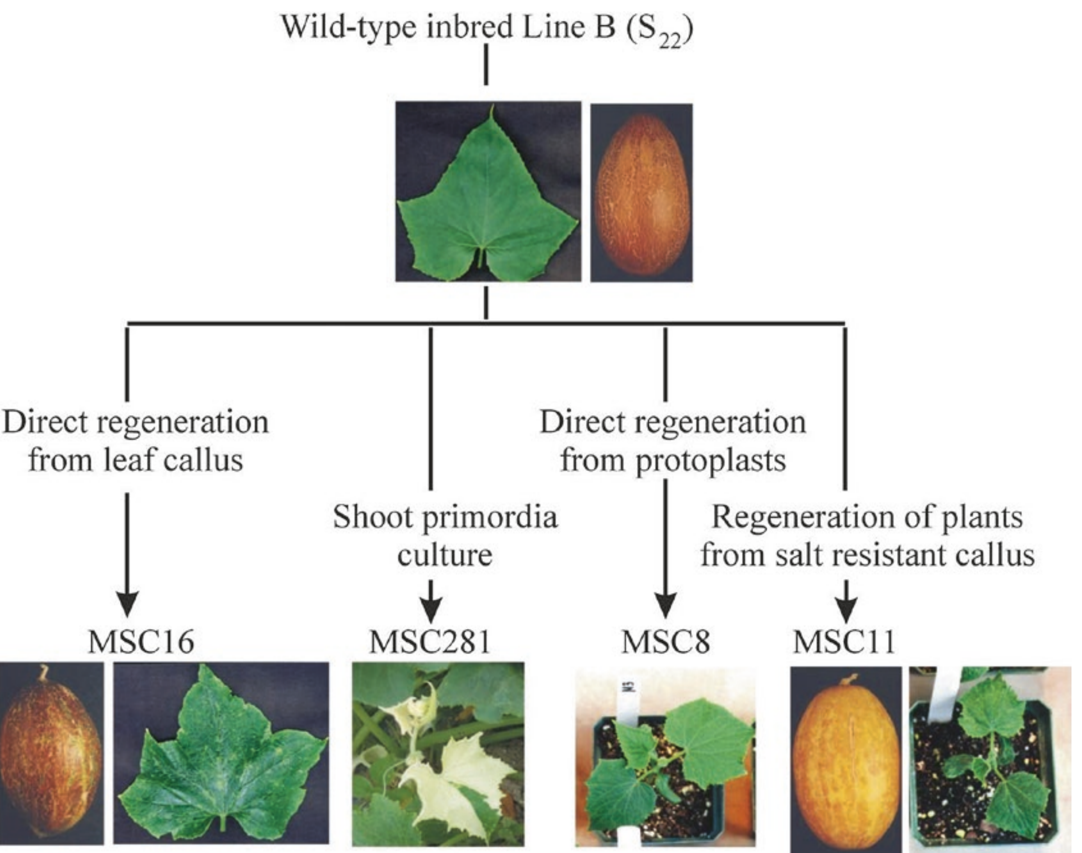

Fig. 1 Phenotypes of mosaic (MSC) lines identified among regenerated plants after passage of the wild-type inbred line B through various cell cultures

We demonstrated that testcrosses with MSC as the male parent to wild-type plants produce rare wild-type progenies at or below 1 % (Malepszy et al. 1996; Lilly et al. 2001). At the end of meiosis, the mitochondria in cucumber microspores are cup-shaped. By the time free microspores are produced, the mitochondria are few and gigantic (Abreu et al. 1982). These huge mitochondria are only observed in uninucleate microspores and may result from organelle elimination or fusion. After the mitotic division that produces bi-cellular microspores, the mitochondria divide and resume normal shape, size, and numbers (Abreu et al. 1982). The formation of relatively few, huge mitochondria during microsporogenesis may create a bottleneck and reduce the diversity among mitochondrial genomes transferred to progenies via the male gametophyte, contributing to the sorting of mitochondrial genomes revealed during transmission studies (Lilly et al. 2001; Bartoszewski et al. 2004b).

Uniparental Effects

Differential transmission of the *Cucumis* organellar DNAs allows for the separation of chloroplast and mitochondrial effects by reciprocal crossing. Because the vast majority of plants show maternal transmission of both the plastid and mitochondrial DNAs, one would expect that significant cross-talk between the organelles and nucleus would result in beneficial interactions. However the large size and frequent recombinations within and among the mitochondrial DNAs can produce structurally polymorphic molecules among plants with a population (Havey et al. 1998; Lilly et al. 2001; Bartoszewski et al. 2004b; Alverson et al. 2011; Rodriguez-Moreno et al. 2011). Plants in the genus *Cucumis* provide a unique system to identify and characterize chloroplast and mitochondrial effects on phenotypes by exploiting differential transmission of the organelles. Shen et al. (2015b) crossed among doubled haploid (DH) lines from divergent cucumber populations to produce a complete set of reciprocal hybrids. Significant differences were detected for plant growth between reciprocal hybrids possessing identical nuclear genotypes, revealing the potential of beneficial organellar effects on plant performance. The significantly better performance of a DH as the male parent could be due to beneficial mitochondrial interactions with the nucleus. Superior performance as the female parent may reveal better interacting chloroplasts. These results indicate that different organellar types may exist within *Cucumis* species, and inbreds possessing specific organelles may perform better as the male or female to produce more vigorous hybrids.

Future Research

Evolution of Organellar Transmission in the Cucurbits

Paternal transmission of the *Cucumis* mitochondrial DNA is likely a derived state because of the prevalence of maternal transmission in other Cucurbits and most other plants (Havey et al. 1998). A systematic evaluation of mitochondrial-DNA

transmission in other *Cucumis* species and related genera should reveal the commonness of this unique transmission mode, or if occasional or strict maternal transmission occurs in this genus. If the latter were true, interspecific crossing and segregation analyses may reveal the genetic basis of differential mitochondrial transmission and ultimately lead to identification of causal gene(s). This approach may be more fruitful than trying to alter organelle transmission by mutagenesis of plants showing strict maternal transmission of both organelles. Occasional biparental transmission of one or both organellar genomes may also exist, and would be useful to determine any nuclear role and its inheritance. A deeper understanding of any genetic control of organellar transmission would allow identification and introgression of superior plastids or mitochondria to enhance plant performance.

Mitochondrial "Knock-Downs"

The MSC mutants offer a unique opportunity to develop "knock-downs" of mitochondrial genes. Presently there is no efficient way to mutate mitochondrial genes (Jacobs 2001). Passage of cucumber (and presumably melon) through cell culture, regeneration of plants, and screening of progenies may provide a mechanism to identify and select plants with under-representation of mitochondrial genes. I recommend the development of doubled-haploid (DH) lines from diverse populations of cucumber and melon, in order to guarantee a uniform nuclear genotype and sample a wide range of putative mitochondrial DNA diversity. These DHs could be passed through various cell cultures, and regenerated plants evaluated for paternally transmitted phenotypes. Complete absence of a mitochondrial gene would likely be lethal; however substoichometric shifting could result in under-representation of specific coding regions (Del Valle-Echevarria et al. 2015) and produce distinct phenotypes, as observed in cucumber (Fig. 1). After confirming paternal transmission of the phenotype, sequencing of the mitochondrial DNAs can be completed to reveal genes carried on regions significantly under-represented relative to the wildtype parental DH line. These mitochondrial "knock-downs" would be a useful tool to study their effects on plant growth and development, mitochondrial function, and nuclear responses.

Mitochondrial Transformation

There is presently no method to efficiently introduce foreign DNA into the mitochondrion. Although mitochondrial transformation has been reported in *Chlamydomonas* (Randolph-Anderson et al. 1993) and yeast (Butow et al. 1996), it has never been successfully developed for a higher plant. Microprojectile bombardment is used to transform the chloroplast genomes of *Chlamydomonas* (Boynton et al. 1987; Kindle et al. 1991), tobacco (Svab and Maliga 1993; Zoubenko et al.

1994), tomato (Ruf et al. 2001), and Brassica (Cheng et al. 2010). The lack of a robust technique to transform the mitochondrial DNA restricts assessment of mitochondrial effects on overall performance, stress tolerances, and programmed cell death, as well as retrograde (mitochondrion-to-nucleus) signaling and associated nuclear responses. Challenges for transformation of the mitochondrial DNA include efficient introduction of foreign DNA into a mitochondrion, recombination to incorporate the transgene into the mitochondrial DNA, the absence of selectable markers, and the relatively large numbers of mitochondria per cell and mitochondrial genomes per mitochondrion. Cucumber has potential as a model plant for mitochondrial transformation (Havey et al. 2002) because of two unique attributes: uninucleate microspores of cucumber possess relatively few, huge mitochondria (Abreu et al. 1982) and mitochondria show paternal transmission (Havey 1997). These two attributes may allow for delivery of transformed mitochondria to progenies via the male gametophyte. A potential approach would be to isolate cucumber microspores at the uninucleate stage, biolistically introduce a DNA construct conditioning antibiotic resistance into the huge mitochondria of uninucleate microspores, mature microspores to functional pollen (Alwen et al. 1990), and finally deliver transformed mitochondria to progenies via the male gametophyte.

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Databases and Bioinformatics for Cucurbit Species

Yang Bai, Zhonghua Zhang, and Zhangjun Fei

Abstract Cucurbitaceae is a very large and diverse plant family, comprising several economically important crops such as cucumber (*Cucumis sativus*), melon (*Cucumis melo*), watermelon (*Citrullus lanatus*) and squash/pumpkin (*Cucurbita spp.*). As the rise of genomic research, the genomes of the first three major cucurbits have been sequenced and well annotated, while squash and pumpkin have prepublication genome sequences available online. Genetic and transcriptomic research in cucurbit crops have also increased exponentially in the last two decades. Web-based databases have been developed to store, manage and provide access to the vast amount of genetic and genomic data. In this chapter, we describe most-current cucurbit databases and several other databases useful for cucurbit genomic research. Most importantly, the family-wide cucurbit genomics database (CuGenDB, http://www.icugi.org) is a comprehensive up-to-date repository of genetic, genomic and related resources for all four major cucurbits. CuGenDB provides browsing, searching and downloading services for the genomes of cucumber and watermelon, ESTs and genetic maps for all four cucurbits, and associated data mining tools. In future, the cucurbit databases will not only store more genomes and associated resources, but also provide users better services, such as fast data updates, easy data access, and powerful tools for sequence visualization, retrieval and analysis.

Keywords Cucurbit • Watermelon • Melon • Cucumber • Pumpkin • Genomics • Database

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Introduction

The family Cucurbitaceae (cucurbit) comprises approximately 118 genera and 825 species mainly distributed in temperate and tropical regions around the world (Robinson, Decker-Walters 1997). Major economically important cucurbit crops include cucumber (*Cucumis sativus* L.), melon (*Cucumis melo* L.), watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] and squash / pumpkin (*Cucurbita spp.*). Cucurbit research has been greatly advanced by genetic and genomic studies and breeding efforts during the last decade. The whole genome sequence of cucumber was reported in 2009 (Huang et al. 2009), followed by the genomes of melon in 2012 (Garcia-Mas et al. 2012) and watermelon in 2013 (Guo et al. 2013). More genome sequences of cucurbit species such as pumpkin, bottle gourd and wax gourd will be available in the near future. In cucumber, melon and watermelon, whole genome resequencing studies for different cultivated and wild varieties have revealed evolutionary history and provided millions of variants for genomic-enabled breeding (Guo et al. 2013; Qi et al. 2013; Sanseverino et al. 2015). In addition, high-density genetic maps have been developed for cucumber, melon, watermelon and squash, providing an unprecedented amount of SSR and SNP markers (Ren et al. 2009; Miao et al. 2011; Zhang et al. 2012; Sun et al. 2013; Zhou et al. 2015; Wei et al. 2014; Yang et al. 2013; Ren et al. 2014; Harel-Beja et al. 2010; Xu et al. 2014; Jiang et al. 2015; Esteras et al. 2012b; Diaz et al. 2011). Furthermore, a large amount of transcriptomic data have been generated by different RNA-Seq studies in cucumber (Zhao et al. 2015; Zhang et al. 2014; Jiang et al. 2015; Guo et al. 2010; Ando et al. 2012; Martinez et al. 2011; Wu et al. 2010; Chen et al. 2014a; Li et al. 2011), melon (Blanca et al. 2012; Gao et al. 2015; Omid et al. 2007; Kim et al. 2016; Zhang et al. 2016; Gonzalez-Ibeas et al. 2007) and watermelon (Guo et al. 2011; Fan et al. 2014; Guo et al. 2015; Grassi et al. 2013; Rhee et al. 2015). Accompanying genome assemblies, all these markers, maps, mRNAs, gene predictions, and cross-species homologies can serve as annotations for data analysis and interpretation. As the amount of genome and annotation data increases exponentially, how to maintain, present and distribute these data becomes a major challenge for researchers. Several cucurbit genome and annotation databases have been or are being developed for efficient data storage and retrieval. In this chapter, the contents and functions of these databases and related genomic tools are described.

Genomic Databases for Cucurbits

To facilitate the usage and application of genomic resources in cucurbits, a family-wide cucurbit genomics database (CuGenDB, http://www.icugi.org) and several species-specific databases have been developed to integrate genomic sequences and annotation data (Table 1). Genome sequences of three major cucurbit crops, cucumber, melon and watermelon, haven been published (Huang et al. 2009; Garcia-Mas et al. 2012; Guo et al. 2013), while squash and pumpkin genomes have been generated but not officially published yet. Squash mainly has resources of transcriptomes

Table 1 Databases for cucumber, melon, watermelon and squash/pumpkin

Species	Database name	Website
Cucumber (*Cucumis sativus*)	Cucumber Genome Database	http://cucumber.genomics.org.cn
	CuGenDB	http://www.icugi.org
Melon (*Cucumis melo*)	MELONOMICS MeloGene	https://melonomics.net/ https://melogene.upv.es/
Watermleon (*Citrullus lanatus*)	Watermelon Genome Database	http://www.iwgi.org
	CuGenDB	http://www.icugi.org
Squash and pumpkin (*Cucurbita spp.*)	CucurbiGen	https://cucurbigene.upv.es
	CuGenDB	http://www.icugi.org

and genetic maps (Esteras et al. 2012a; Blanca et al. 2011a), as well as an online draft version of zucchini genome at CucurbiGen (https://cucurbigene.upv.es). Pumpkin currently has small RNAs (sRNAs) available at CuGenDB, which is used in an evolutionary study of microRNAs in vascular plants (Chavez Montes et al. 2014). In January 2016, a pre-publication pumpkin (*C. maxima*) genome sequence was released at CuGenDB, together with a high-density genetic map and SNP markers used for anchoring the genome sequences and identifying dwarf vine QTLs in pumpkin (Zhang et al. 2015).

Species-Specific Databases

Cucumber Genome Database

The cucumber genomic resources including genome sequences (version1), genetic maps and EST sequences were initially stored in the Cucumber Genome Database (http://cucumber.genomics.org.cn), which is developed by the Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences and Beijing Genomics Institute. The data can be searched, browsed and downloaded. Later, these data and further updates have been migrated into CuGenDB, the cucurbit genome database, which serves as the main platform for cucumber genomic resources.

Melon Genome Databases (See Chap. 8)

Melon genome sequences and annotations are available at a database called MELONOMICS (https://melonomics.net). Genetic maps and markers used to anchor and order the melon genome can also be accessed at MELONOMICS (see Chap. 8). The melon transcriptome of 49,741 unigenes and a large transcriptomic SNP collection (239,521) are available at another database MeloGene (https://melogene.upv.es).

Melonomics

MELONOMICS is an integrated database that provides access to melon genome sequences and genetic maps. MELONOMICS supports genome sequence browsing, BLAST searching, genetic map viewing and downloading of various melon genomic data. In the genome browser, users can search a genomic region either on contigs or scaffolds and view different tracks of data in that region, such as annotations of genes, mRNAs and proteins, coverage of ESTs and RNA-Seq reads, non-coding RNAs, transposons, markers and restriction sites. The resulting tracks can be customized and saved in snapshots, and sequences of interest can be retrieved in FASTA format. Notably, alternative splicing events could be easily viewed with one gene transcribed into several mRNAs. The BLAST function allows users to search nucleotide or protein sequences against melon databases of genome, protein, transcripts and two sets of transcript unigenes, as well as retrieving sequences of resulting homologs. Furthermore, there is also a comprehensive search function for fast retrieval of melon nucleotide and protein sequences, and the associated annotations.

The genetic maps and marker details for anchoring the melon genome sequences could be accessed in MELONOMICS. The ICuGI genetic maps with 870 SSR and SNP markers were compiled prior to the release of the melon genome and are maintained in CuGenDB (Diaz et al. 2011). Recent efforts anchored 836 of ICuGI markers onto the melon genome, yielding a genetic map with a total of 1850 SSR and SNP markers (Diaz et al. 2011). This new genetic map, together with QTLs from the ICuGI consensus map, can be downloaded from MELONOMICS. A physical map (Gonzalez et al. 2010), including marker and BAC-end sequence positions on contigs and/or scaffolds, can also be downloaded from MELONOMICS. Gene ontology (GO) term statistics are available in MELONOMICS, but more comprehensive and up-to-date transcriptome data in melon are maintained in another database MeloGene.

MeloGene

MeloGene provides access to a melon transcriptome assembly, including unigene annotations and SNP markers. Sequences used to assemble this transcriptome were generated from several different tissues and conditions (Gonzalez-Ibeas et al. 2007; Blanca et al. 2011b), and later from 67 different genotypes representing the extant variation of the species (Blanca et al. 2012). This assembled transcriptome accelerates melon genetic and genomic studies. For example, a golden gate genotyping array of 768 SNPs has been developed based on these transcriptome resources to study genetic variation and population structure in 74 melon accessions (Esteras et al. 2013). Similar to the melon genome database MELONOMICS, MeloGene offers transcriptome browsing, key word searching, BLAST and downloading functions.

Watermelon Genome Database (See Chap. 10)

The watermelon genome sequences were initially deposited in both Watermelon Genome Database (http://www.iwgi.org) and CuGenDB. Now the watermelon genome and related resources are only maintained in CuGenDB.

Squash and Pumpkin Genome Databases

The genus of *Cucurbita* mainly includes squashes, gourds and pumpkins, some of which are the earliest domesticated plant species (Robinson, Decker-Walters 1997). The zucchini type of squashes (*C. pepo*) are vegetables of high values. Pumpkins (*C. pepo*, *C. maxima* and *C. moschata*) are good nutritional sources of vitamins and fat and serve as food staples worldwide.

Squash (*Cucurbita pepo*) (See Chap. 11)

CucurbiGen (https://cucurbigene.upv.es) is a squash genome database, which currently mainly presents squash transcriptome and genetic map datasets (Blanca et al. 2011a; Esteras et al. 2012b), as well as the pre-publication zucchini genome. The organization and functions in CucurbiGen are largely similar to melon databases MELONOMICS and MeloGene, which were constructed by the same Spanish group.

In the database, two versions of the pre-publication zucchini genome sequences (v3.1 and v3.2) can be downloaded, but without annotation information. There are also two versions of squash transcriptomes available for browsing, BLAST, key word searching and GO classification. The version one transcriptome, available for downloading, was assembled in 2010 by sequencing two lines (one zucchini MU16 and one scallop UPV196), each in three tissues. The version two transcriptome, unpublished and not available for downloading, was assembled in 2014 with additional Illumina pair-end reads from the same lines of zucchini MU16 and scallop UPV196. Genetic maps constructed from a recombinant inbred line (RIL) population of MU16 × UPV196 are also available with display options. SNP markers on the maps are also shown in the transcriptome browsers.

Pumpkin (*Cucurbita spp.*)

A pre-publication pumpkin (*C. maxima*) genome, together with genetic maps and SNP markers (Zhang et al. 2015), have been available for download in CuGenDB since January 2016.

General Cucurbit Database

In addition to the above species-specific databases, the Cucurbit Genomics Database (CuGenDB, http://www.icugi.org), developed at Boyce Thompson Institute (Ithaca, NY, USA), integrates genomic resources of four major cucurbit crops into one user-friendly database, supports various analytical tools and provides mailing list service and up-to-date research news. CuGenDB includes all resources of genomes, transcriptomes, annotations, genetic maps and markers for cucumber and watermelon, and partial genomic resources for melon and squash/pumpkin. The home page of CuGenDB is well-designed for users to efficiently locate any data set or information by listing data links in a "Quick Start" panel and highlighting important news (Fig. 1). Each of the major sections in CuGenDB is described below, including genome, EST, sRNA, map, tool, download and community.

Genome Sequences

Currently CuGenDB contains two versions of cucumber 'Chinese long' genomes (Huang et al. 2009; Li et al. 2011) and one version of watermelon 97,103 genome (Guo et al. 2013). For each crop, there are an introduction webpage, a genome browser and searching tools for single and batch queries. The batch query function allows users to quickly retrieve sequences and annotations by providing a list of gene IDs of interest. Annotations based on homologues from four databases including GenBank, Swiss-Prot, TrEMBL and TAIR, are available. This tool is very useful for downstream functional analyses involving a large set of genes, such as annotating differentially expressed genes from RNA-Seq experiments.

ESTs

Expressed sequence tag (EST) is a sub-sequence of a cDNA. CuGenDB maintains ESTs, unigenes and markers for all four major cucurbit crops. EST sequences of cucumber (Ando et al. 2012; Guo et al. 2010), melon (Clepet et al. 2011; Gonzalez-Ibeas et al. 2007), watermelon (Guo et al. 2011; Levi et al. 2006) and squash (Blanca et al. 2011a) have been assembled into 93,903, 24,444, 75,068 and 136,038 unigenes, respectively. As in the genome section, these unigenes and annotations can be efficiently searched by key words and retrieved in batch. More importantly, the SNPs and SSRs within these unigenes have been well-organized and maintained in great details in CuGenDB, providing an invaluable resource for cucurbit breeders. For each SNP, the alignments across different accessions can be visualized; and for each SSR, the sequences and potential PCR primers are provided.

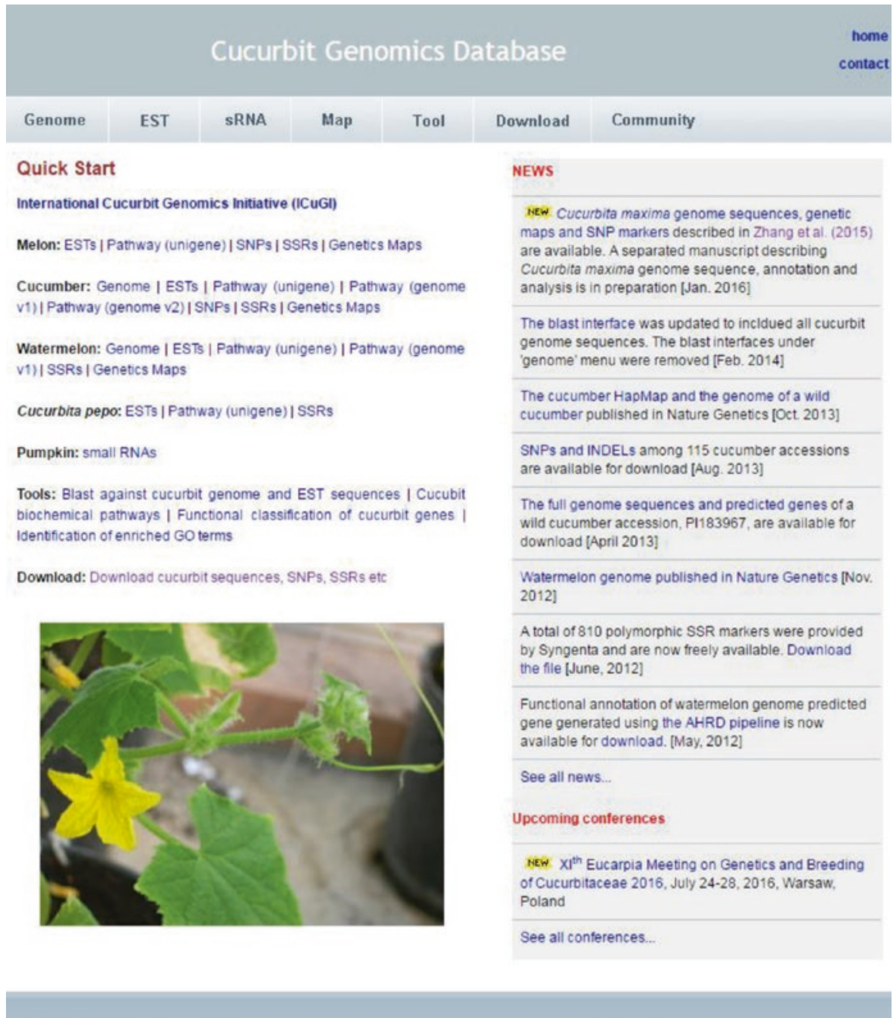

Fig. 1 Homepage of Cucurbit Genomic Database (CuGenDB)

Small RNAs

Currently only pumpkin sRNAs are available in CuGenDB. The ~9.4 million pumpkin sRNAs (4.1 million unique) were downloaded from the database of Comparative Sequencing of Plant Small RNAs (Chavez Montes et al. 2014) (http://smallrna.danforthcenter.org/). MiRNA candidates are identified from the unique sRNAs by comparing to miRNA databases and predicting by structure folding programs. The miRNA targets in cucumber, melon and watermelon unigenes are predicted using the target prediction tool implemented in the

database. For each miRNA, the sequence, predicted target genes, expression levels, alignment to known miRNAs and precursors (if known) are summarized in one web-page.

Genetic Maps

A total of 4, 15 and 2 genetic maps are included in CuGenDB for cucumber (Bradeen et al. 2001; Fazio et al. 2003; Park et al. 2000), melon (Oliver et al. 2001; Gonzalo et al. 2005; Danin-Poleg et al. 2002; Wang et al. 1997; Silberstein et al. 2003; Baudracco-Arnas, Pitrat 1996) and watermelon (Levi et al. 2001; Levi et al. 2002), respectively. The maps can be not only visualized, but also redrawn according to users' requests. Users can redefine the start, end, map size, map direction and type of markers. By clicking each marker on the map, users can access all information of that marker. In addition, users can search markers by their types in different crops and maps. For example, 2180 melon SSR markers with corresponding genetic positions can be found by searching "SSR" in the map section.

Tools

A set of data analysis tools, including BLAST, pathways, gene classification and GO term enrichment analysis, are implemented in CuGenDB for searching and annotating cucurbit genes.

The BLAST search tool allows users to pick blast programs, to define search parameters and to choose search database from a comprehensive collection of cucurbit genomic resources. For users interested in finding orthologous genes within the cucurbit family, this BLAST tool has all the databases in need in one place.

The pathway tool provides predicted biochemical pathways from EST unigenes and genome predicted genes for all four major cucurbits. For example, 377 pathways are predicted in the cucumber genome version 2. These pathways are displayed on the basis of a classification hierarchy for metabolic pathways, e.g. the pathway 'Biosynthesis' contains 13 sub-classes such as 'Amines and Polyamines Biosynthesis' and 'Amino Acid Biosynthesis'. For each pathway, the candidate genes involved are shown and linked with the genome sequences. For example, in cucumber, UDP-D-glucose can be catalyzed into flavonol-3-*o*-b-D-glucoside or quercetin-3-glucoside or kaempferol-3-glucosid and three genes (Csa3M200710.1, Csa4M415930.1 and Csa6M045050.1) are predicted to be responsible for the formation of these three flavonoids.

The gene classification tool can classify a list of genes into different GO slim terms (http://geneontology.org/page/go-slim-and-subset-guide). The gene datasets include EST-assembled unigenes in all four major cucurbits and genome predicted genes in cucumber and watermelon. The output includes a GO term classification table and a bar plot of gene numbers in each GO term, as well as links to detailed gene information.

The GO enrichment analysis tool uses the GO annotations of reference genes to analyze a query gene list, looking for over-represented GO terms. The reference

gene sets include genome predicted genes for cucumber and watermelon and EST-assembled unigenes for all four major cucurbit crops. After a reference dataset is selected, users can customize ontology types, *p*-value correction method and cutoff *p*-values for the enrichment analysis. In addition to a list of enriched GO terms, the analysis also generates GO directed acyclic graphs (DAG) to illustrate the relationships between the enriched GO terms. This is a useful tool especially for the analysis of differentially expressed gene sets generated by RNA-Seq experiments.

Download

The above mentioned genomes, annotated genes, markers, unigenes, ESTs and pathways can be downloaded in bulk at this database. In addition, SNPs, indels and novel genes identified from genome resequencing of 115 cucumber accessions (Qi et al. 2013) can also be downloaded.

Community

In this section, cucurbit related news and conferences are posted. The news includes updates of the contents and tools at this database, newly released data sets and publications for cucurbit crops.

Other Plant Databases that Provide Useful Information for Cucurbit Species

Plant Genome Duplication Database (PGDD)

PGDD (Lee et al. 2012) (http://chibba.agtec.uga.edu/duplication/) is a public database to identify and catalog plant genes in terms of intra-genome or inter-genome syntenic relationships. Current efforts focus on flowering plants with available whole genome sequences. Currently, two cucurbits (cucumber and watermelon) genomes have been added to this database. User can visualize the syntenic blocks between cucumber and watermelon, as well as between cucumber or watermelon and any other plant species available in the database.

Plaza

PLAZA (Proost et al. 2014) (http://bioinformatics.psb.ugent.be/plaza/) is an access point for plant comparative genomics to centralize genomic data produced by different genome sequencing initiatives. It integrates plant sequence data and comparative genomics methods and provides an online platform to perform evolutionary

analyses and data mining within the green plant lineage (Viridiplantae). Users can search for genes or explore gene families and genomic homologies using different analysis tools provided in the database. Currently, melon and watermelon genomes are available in this database. In addition, phylogenetic comparisons between melon and cucumber genes can be found at PhylomeDB v4 (http://phylomedb.org).

iTAK

iTAK (Zheng et al. 2016) (http://bioinfo.bti.cornell.edu/tool/itak) provides access to the identified transcription factors (TFs), transcriptional regulators (TRs) and protein kinases (PKs) in plants. More than 70 families of TFs and 20 families of TRs have been characterized in plants. Plant protein kinases are identified by detecting query sequences for kinase domains (PF00069 and PF07714) defined in the Pfam database. The identified protein kinases are classified into gene families by comparing their sequences to a set of Hidden Markov Models (HMMs). Predicated genes in cucumber, melon and watermelon genomes are used to identify TFs, TRs and PKs in this database.

Plant Transcription Factor Database (PlantTFDB)

PlantTFDB (Jin et al. 2014) (http://planttfdb.cbi.pku.edu.cn/) identified 129,288 transcription factors from 83 plant species and classified them into 58 families. For each family, a brief introduction and key references are presented. For each identified TF, functional domains, 3D structures, GO term annotations, plant ontology (PO), expression information, expert-curated functional description, regulation information, interaction, conserved elements, references, and annotations in various databases such as UniProt, RefSeq, TransFac, STRING, and VISTA are provided. The evolutionary relationships among identified TFs are also inferred. In addition, users can search interesting information based on various queries or perform sequence similarity search by BLAST. The TFs have been identified for cucumber, melon and watermelon in the database.

Plant MITE Database (P-MITE)

P-MITE (Chen et al. 2014b) (http://pmite.hzau.edu.cn/) is a database of miniature inverted transposable elements (MITEs) de novo identified from 41 plant genomes. So far, it comprises more than two million MITE-related sequences which can be classified into over 3000 sub-families. In this database, users can check the similarity of interesting sequences with MITEs by BLAST and download MITE sequences for a given species. The database contains MITEs for cucumber, melon and watermelon.

Concluding Remarks

In an era of big data, the exponential growth of genomic data requires revolutionary measures for data management and dissemination. Online databases have become the most important and broadly used approaches for archiving, integrating and making accessibility of the vast amount of sequencing data. The importance of bio-curation has been recognized by the whole scientific community (Howe et al. 2008). Since 1980s, public databases for nucleotide, protein and structure have been established, followed by the emergence of model organism databases. Along with the rise of genomics in plants, only these databases could not meet the needs of researcher and breeders. Integrative databases for specific genus or family that contains economically important crops, such as CuGenDB, are more and more widely used by the scientific community. The advantages of these integrative databases include (1) providing genomic resources for domesticated crops along with related wild varieties; (2) offering genome-wide comparison between closely related crops; (3) integrating genomic sequences and genetic markers into powerful browsing and searching tools. Databases can largely accelerate genetics, genomics and breeding in plants.

In the future, the cucurbit databases will be improved to better serve the plant research community. After the squash and pumpkin genomes are published, a large amount of new resources will be integrated into the species-specific databases and CuGenDB, enhancing the power of browsing, searching and annotation tools. Cross-species genome synteny and genetic variation studies will enhance the understanding of evolution in cucurbits. In addition, there will be more genetic markers from comparative genomic studies and QTLs from breeding programs released into the databases. Furthermore, data generated from meta-genomics studies between cucurbit plants and microbes will be a very valuable input into the cucurbit databases. The databases described above will certainly be expanded and become more complex as cucurbit genetic and genomic data accumulate. The keys to keep their popularity in the scientific community are fast data updates, easy access, powerful browsing and searching tools and accurate gene annotations.

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Genetic Mapping of Complex Traits in Cucurbits

María José Gonzalo and Antonio J. Monforte

Abstract The broad phenotypic diversity displayed among species belonging to the *Cucurbitaceae* family for interesting agronomical traits is mostly under polygenic control. Important crops included in this family, such as melon, cucumber, watermelon and squash, have been studied intensively in the last decades to understand the genetic control of this diversity. The development of genomic sequencing projects for different cucurbit species has facilitated the generation of saturated genetic maps, making possible the consistent identification and localization of QTLs involved in interesting traits related to yield, fruit quality, fruit morphology, vegetative growth or disease resistance, among others. In the current chapter, the mapping approaches for genetic dissection of complex traits in the four major cucurbit species mentioned above has been compiled, including a summary of the identified QTLs for the most relevant traits for each species.

Keywords Quantitative Trait Loci • Molecular marker • Phenotypic diversity • Melon • Cucumber • Watermelon • Squash

Introduction

Cucurbitaceae species probably show the broadest phenotypic diversity among cultivated species. Three genera include the major cultivated cucurbits: *Cucumis* (melon and cucumber), *Citrullus* (watermelon) and *Cucurbita* (pumpkin and squash). The most polymorphic species is *Cucurbita pepo* L. Fruits range from very elongated to absolutely flattened or discoid; different colors vary from white to orange, through green; and sizes range from small-fruited ornamental gourds to large pumpkins or zucchinis (Paris 2008; chapter "Genetic resources of pumpkins and squash, Cucurbita spp."). *Cucurbita maxima* Duchesne includes large size pumpkins, with giant pumpkins exceeding 460 kg (being the largest of all fruits), and a wide range of morphotypes with different flesh colors (Ferriol et al. 2004). *Cucurbita moschata* Duchesne contains medium to large- size pumpkin, butternut, crookneck and long types

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(Robinson and Decker-Walters 1997). Watermelon (*Citrullus lanatus* subsp. *vulgaris* L.) cultivars are also phenotypically diverse in fruit shape, weight, rind thickness, sugar content and flesh colors (Levi et al. 2004; Lewinsohn et al. 2005; chapter "Genetic resources of watermelon"). *Cucumis melo* L. (melon) cultivars are also diverse for fruit traits as size (from few grams to several kilograms), shape (round to very elongated), internal (white, green, orange) and external (white, yellow, red, green) color, ripening behavior (climacteric, non-climacteric), aroma, sweetness (Pitrat 2008; chapter "Melon genetic resources: phenotypic diversity and horticultural taxonomy"). *Cucumis sativus* L. (cucumber) also shows a high fruit trait variation such as size, shape, weight, and in quality traits as the flesh color, color uniformity, skin appearance, aroma and bitterness (Lu et al. 2015; Yuan et al. 2008a; Weng et al. 2015; chapter "Genetic resources of cucumber").

The genetic control of such impressive phenotypic diversity has been studied intensely during the last decades, gene lists are generated by several authors regularly in the Cucurbit Genetics Cooperative (http://cuke.hort.ncsu.edu/cgc/cgc-genes/genelists.html). Most of the agronomically interesting traits are under complex multigenic control. Genome sequencing initiatives have been carried out in the most important cucurbit species (chapters "The melon genome", "The cucumber genome", "The watermelon genome", "Genetics and genomics of Cucurbita species"), making it now possible to utilize current genetic and genomic approaches to answer relevant questions about the genetic basis of complex traits in the *Cucurbitaceae* family. In the current article we will review the state of the art of the mapping approaches for complex trait genetic dissection in the major cucurbit crops: melon, cucumber, watermelon and squash.

Melon

Populations and Genetic Maps

The first map including molecular markers (Restriction Fragment Length Polymorphisms, RFLP, and Random Amplified Polymorphic DNA, RAPD), as well as disease resistance and morphological markers, was developed by Baudracco-Arnas and Pitrat (1996), although this map did not cover the 12 melon chromosomes. Full coverage molecular maps were developed in parallel using mostly RFLP (Oliver et al. 2001) and RFLP combined with AFLP (Amplified fragment length Polymorphisms) by Perin et al. (2002a). During the 2000s decade, SSR (Single Sequence Repeats) markers were included in the mapping projects (Danin-Poleg et al. 2002; Fernandez-Silva et al. 2008; Fukino et al. 2008; Gonzalo et al. 2005). At the end of the decade, SNP (Single Nucleotide Polymorphisms) markers were also incorporated (Deleu et al. 2009; Harel-Beja et al. 2010). These maps were done mostly independently, with a limited number of common markers, making difficult map comparison. Diaz et al. (2011), under the International Cucurbit Genomics Initiative (ICuGI), integrated the six major international maps in a consensus linkage map including 1,592 markers, 686 of them being sequence characterized markers

(SSRs, SNPs and indels). The ICuGI map also included the Quantitative Trait Loci (QTL) mapped previously. Later on, SNP markers were used to develop high density maps to anchor the melon genome scaffolds (Argyris et al. 2015; Garcia-Mas et al. 2012). The SNP map was merged with the ICuGI map recently (Diaz et al. 2015), resulting in a final map with 836 SSRs and 1,014 SNPs anchored in the melon genome, which can be obtained from https://melonomics.net/.

Linkage disequilibrium (LD) mapping in germplasm collections has been evaluated more recently. LD decays rapidly; Tomason et al. (2013) using SSR markers and a relatively relaxed threshold suggested a drastic decay within 5 cM, whereas Esteras et al. (2013) and Leida et al. (2015) studying LD in local regions using SNPs and a more restricted criteria concluded that LD decayed within a few kilobases. More experiments are necessary including a broader array of germplasm and higher SNP density to provide a robust estimate. Nevertheless, both Leida et al. (2015) and Tomason et al. (2013) found association between markers and several traits, showing that genome-wide association analysis (GWAS) is feasible in this species.

Mapping Quantitative Traits

Perin et al. (2002b) published the first article on QTL mapping in melon (in this case studying ovary and fruit morphology), using a full coverage linkage map in RIL populations. Since then, different population types have been used: F_2, RIL, DHL, introgression lines (ILs) and association panels (Table 1). Diaz et al. (2011) integrated the previously mapped QTL in the consensus ICuGI map. We will use this work as the basis of the current review, updating it with the new results published since then (Table 1). We have selected 56 traits based on their relevance and classified them in six categories: Yield (including earliness), fruit morphology, fruit color, fruit quality, vegetative growth and disease tolerance.

Yield Related Traits

A total of 34 yield related QTLs (12 for earliness, 22 for yield) have been identified in only four experiments. QTLs have been located in all chromosomes, being more frequently mapped on chromosomes 2, 6 and 8, although, due to the low number of experiments, it is not possible to make any inference on the possible relevance of detected QTLs in the phenotypic variability within melon germplasm.

Fruit Morphology

Fruit morphology can be subdivided in four major subcategories: fruit weight (FW), fruit shape (FS), internal fruit morphology (cavity or flesh content) and external rind patterns (ribs and sutures). A total of 120 QTLs from 12 investigations (Table 1) have been described. QTLs for internal fruit morphology and external skin patterns

Table 1 Summary table of QTLs identified in melon for relevant fruit agronomic traits classified in six categories. The number of QTLs identified and the references of the corresponding publications are listed for each selected trait

Fruit agronomic traits		QTLs	References[a]
Yield traits	Earliness	12	A
	Yield	22	G, U
Fruit Morphology	Fruit Weight	31	A, C, G, K, S, U, X
	Fruit Shape	58	A, C, H, K, L, M, O, S, U, X
	Internal Morphology	25	E, H
	Rind pattern	6	K
Fruit Color	Rind color	8	A, C
	Flesh color	16	A, C, K, U, Y
Fruit Quality	Sugars	75	A, C, E, F, H, K, S, U, X, Y
	Ripening	8	B, Q, V
	Acidity	9	E, W, AC
	Carotenoids	11	I, K
	Physical	17	B, H, K, X, Y
Vegetative	Root	17	D
	Plant	10	G, S, AB, AD
Disease tolerance	Fungus	26	J, P, Z
	Insect	7	N
	Virus	5	R, T
TOTAL		363	

[a]A: Monforte et al. 2004; B: Moreno et al. 2008; C: Eduardo et al. 2007; D: Fita et al. 2008; E: Obando-Ulloa et al. 2008; F: Obando-Ulloa et al. 2009; G: Zalapa et al. 2007; H: Paris et al. 2008; I: Cuevas et al. 2008; J: Yuste-Lisbona et al. 2011; K: Harel-Beja et al. 2010, L: Perin et al. 2002a;M: Perin et al. 2002b; N: Boissot et al. 2010; O: Diaz et al. 2014; P: Fukino et al. 2008; Q: Vegas et al. 2013; R: Guiu-Aragones et al. 2014; S: Xu Unpublished; T: Palomares-Rius et al. 2011; U: Ramamurthy and Waters 2015; V: Perin et al. 2002c; W: Sherman et al. 2013; X: Tomason et al. 2013; Y: Leida et al. 2015; Z: Perchepied et al. 2005b; AB: Fukino et al. 2012; AC: Cohen et al. 2012; AD: Hwang et al. 2014

have been studied in a much reduced number of experiments, so we will focus on FW and FS. A total of 58 QTLs for FS and 31 for FW have been described so far. Interestingly, FS QTL have been reported relatively frequently on chromosomes 1, 2, 8, 11 and 12, defining 5 Meta-QTLs what represent the best candidates to underlie the FS diversity in melon germplasm. Regarding FW, a total of 4 Meta-QTL can be defined on chromosomes 2, 3, 8 and 11. FW and FS QTLs on chromosome 2 most likely are pleiotropic effects of the *andromonoecious* (*a*) gene that determines melon flower sex expression. Melon plants may be monoecious or andromonoecious depending on the presence/absence of stamens in the female flowers. Stamen development inhibits the ovary longitudinal elongation, making the mature fruit rounder and shorter than fruits developed from ovaries without presence of stamens. By investigation of the co-localization of those Meta-QTLs with orthologs of genes involved in tomato fruit morphology, including members of the *FASCIATED*, *CNR/FW2.2*, *SlKLUH/FW3.2*, *OVATE*, *LOCULE NUMBER* and *OVATE* gene

families, Monforte et al. (2014) concluded that members of *CNR/FW2.2* and *SlKLUH/FW3.2* gene families are strong gene candidates for melon FW QTLs, whereas *OVATE* members were candidates for melon FS QTLs.

Fruit Color

Recently, Feder et al. (2015) identified a Kelch domain-containing F-box protein regulating naringenin chalcone accumulation in melon rind producing the change from white to yellow rind. This gene co-localizes with external color (ECOL) QTLs on chromosome 10 mapped previously by Monforte et al. (2004) and Eduardo et al. (2007). These authors mapped four additional QTLs for ECOL, indicating that this trait is also under polygenic control.

Flesh color (FCOL) is controlled by two major genes: green flesh (*gf*, Hughes 1948) and white flesh (*wf*, Iman et al. 1972) on chromosomes 8 and 9, respectively. These genes have been mapped frequently in diverse mapping population as categorical traits or QTLs. The gene *wf* has been recently isolated, corresponding to a homolog of the cauliflower *BoOr* gene with the capacity of inducing β-carotene accumulation (Tzuri et al. 2015). In fact, QTLs for β-carotene accumulation have been mapped in the same region of the chromosome 9 previously (Cuevas et al. 2008). Nevertheless, 12 additional FCOL QTLs have been mapped in chromosomes 1, 2, 3, 4, 6, 7, 10 and 11 (Eduardo et al. 2007; Harel-Beja et al. 2010; Leida et al. 2015; Monforte et al. 2004; Ramamurthy and Waters 2015) that are involved in color intensity, modulating the effects of the major genes.

Fruit Quality

Several factors involved in fruit quality have been studied: organoleptic (sugar content, acidity, volatiles), nutritional (carotenoids), shelf life (ethylene and ripening) and physical (whole fruit and flesh firmness).

Ripening behavior among melon cultivars ranges from non-climacteric (*inodorus* as "Piel de Sapo" or honeydew) to fully climacteric (*cantaloupe* as charentais, *reticulatus* as Western Shippers), with accessions with intermediate behavior (Leida et al. 2015). Fruit quality traits as fruit softening, postharvest shelf life and aromas are influenced by the climacteric/non-climacteric behavior (Ayub et al. 1996; Obando-Ulloa et al. 2008) while others as sugar and carotenoid accumulation are independent (Ayub et al. 1996). Ethylene production is controlled by two mayor genes, *Al-3* and *Al-4*, mapping on chromosomes 8 and 9, but also by additional QTLs on chromosomes 1, 2, 3, 6, 11 and 12 (Leida et al. 2015; Moreno et al. 2008; Perin et al. 2002c; Vegas et al. 2013). QTLs on chromosomes 3 and 6 can independently induce climacteric ripening (Vegas et al. 2013).

Melon germplasm can be divided into sweet and non-sweet melons, depending on the accumulation of sucrose (Burger et al. 2002). Seventy-five QTLs have been mapped in all chromosomes, being more frequent in chromosomes 2, 3, 5 and 8,

possibly representing QTLs involved in variability for sugar accumulation. No clear co-localization of QTLs with genes involved in sugar metabolism has been found (Harel-Beja et al. 2010).

Another important organoleptic trait is acidity; melons belonging to varieties *flexuosus* and *acidulous* are acidic compared with sweet melons. A single gene on chromosome 8 controls this trait (Burger et al. 2002; Harel-Beja et al. 2010; Sherman et al. 2013). It has been recently isolated, and found to be a member of the pfam 03547 membrane transporter family (Cohen et al. 2014). Additional QTLs modulating the flesh acidity also have been found (Cohen et al. 2012).

QTLs for carotene content co-locate with the major flesh color genes *gf* and *wf* on chromosomes 8 and 9, respectively. Also, a QTL for carotenoid content is mapped on chromosome 6 in three studies, in the same region as FCOL QTL, supporting the importance of carotenoids in the flesh color variability (Cuevas et al. 2008; Harel-Beja et al. 2010; Monforte et al. 2004).

Genes involved in the synthesis of volatile precursors have been identified in melon (Portnoy et al. 2008; Gonda et al. 2013; Tang et al. 2015). Volatile synthesis depends strongly on ripening behavior; volatiles typical of climacteric melons are acetate and non-acetate esters and alcohols, whereas aldehydes, organic acids and terpenes are typical of non-climacteric varieties (Obando-Ulloa et al. 2008; Shalit et al. 2001). However, mapping of QTLs involved in volatile synthesis have been approached scarcely (Obando-Ulloa et al. 2010).

Physical attributes, as whole fruit firmness (WFF) and flesh firmness (FF) have been studied in a very limited number of investigations. FF QTLs on chromosomes 3 and 6 co-localize with climacteric QTLs, being most likely a consequence of pleiotropic effects of climacteric ripening on fruit firmness.

Vegetative Traits

Fita et al. (2008) found 17 genomic regions involved in diverse root architecture components. Regions on chromosomes 3 and 9 affected a larger number of root architecture components. The possible relationship between root architecture and biotic or abiotic stress tolerance still needs to be assessed. Other vegetative traits studied are: leaf area (Diaz et al. 2011, 2 QTLs on chromosomes 3 and 7), lateral branching (Zalapa et al. 2007, 5 QTLs on chromosomes 2, 6, 8, 9 and 11), short lateral branching (Fukino et al. 2012, 2 QTLs on chromosomes 3 and 11) and short internode length (Hwang et al. 2014, 1 QTL on chromosome 7).

Disease Resistance

Several disease resistance genes in melon have been isolated in the last years (Brotman et al. 2013; Dogimont et al. 2014; Joobeur et al. 2004; Nieto et al. 2006), however, a large number of disease resistances are under polygenic

control. Resistance to *Aphis gossypii* infestation is controlled by the recently identified gene, *Vat* (Dogimont et al. 2014), although Boissot et al. (2010) identified three additional QTLs in chromosomes 4 and 9. Two epistatic interactions also appear to be involved in the resistance, depending on the aphid strain (Boissot et al. 2010). A similar situation was found with *Fusarium oxysporum* f. sp. *melonis* (FOM) resistance: the gene *Fom-1* (Brotman et al. 2013) provides resistance to FOM races 0 and 2, the gene *Fom-2* (Joobeur et al. 2004) to races 0 and 1. On the other hand, FOM race 1.2 is very complex, controlled by up to 9 QTLs and 4 epistatic interactions (Perchepied et al. 2005a). Three major genes/QTLs involved in powdery mildew (PM) resistance have been mapped in chromosomes 2, 5 and 12, but with differences between works: Yuste-Lisbona et al. (2011) detected a single QTL in chromosome 5, Perchepied et al. (2005b) detected two in chromosomes 5 and 12, and Fukino et al. (2008) in chromosomes 2 and 5. In the case of resistance to *Cucumber mosaic virus* (CMV), one gene on chromosome 12 (*cmv1*, Essafi et al. 2009) confers total resistance only to specific CMV strains. Resistance to other strains is controlled by at least three genes, one of them is the *cmv1* itself that must be combined with other genes located in chromosomes 3 and 10 (Guiu-Aragones et al. 2014). QTLs on chromosomes 7 and 9 involved in resistance to *Bemisia tabaci* were mapped by Boissot et al. (2010). Downy mildew tolerance is also under complex genetic control, involving up to 12 QTLs (Perchepied et al. 2005b) and resistance to *Watermelon mosaic virus* is controlled by a single QTL on chromosome 11 (Palomares-Rius et al. 2011).

Cucumber

Populations and Genetic Maps

The first maps generated in cucumber resulted in unsaturated linkage maps (Bradeen et al. 2000; Fazio et al. 2003; Kennard et al. 1994; Serquen et al. 1997). The sequencing of the cucumber genome (Huang et al. 2009) enabled the development of new SSR and SNP markers and facilitated the generation of saturated linkage maps using co-dominant markers (Miao et al. 2011; Zhang et al. 2012). Moreover, two consensus maps derived from the cross between Gy14 and PI 183967 inbreed lines have been developed in cucumber using SSR markers for map integration (Yang et al. 2012; Zhang et al. 2012). The consensus map constructed by Zhang et al. (2012) included 1,369 loci in 7 chromosomes and spanned 700.5 cM covered in a 97.5 % with SSR markers. Recently, Rubinstein et al. (2015) utilized a SNP genotyping array with SNPs distributed with a median interval of 2 kb, to construct a high density genetic map with 11,156 SNPs. Also, Zhou et al. (2015), by re-sequencing 147 RILs, obtained a 116,000 SNP genetic map. In both cases, the concordance of physical and genetic positions was very high, indicating the high accuracy of the maps.

Mapping Quantitative Traits

The first QTL mapping reported in cucumber identified fruit quality traits (Kennard and Havey 1995) and relevant agronomic characters (Serquen et al. 1997) in F_3 and RIL populations respectively. Both types of populations show high power of QTL detection and have been used extensively in cucumber for the analysis of complex traits along with F_2 and BC (Backcross). In the last decade, the search of QTLs in cucumber had been the objective of several genetic studies. Unfortunately, the comparison of identified QTLs among experiments becomes a difficult task due to the use of populations derived from crosses between cucumber lines with different genetic backgrounds, the heterogeneity of the molecular markers used in map generation and the lack of integration between the maps from each experiment without agreement in the chromosome identification. For that reason, an extra effort to integrate the mapped QTLs has to be done similar than the work made in other cucurbits as melon by Diaz et al. (2011). Thus, in order to diminish the error in the QTLs localization and to confirm their consistency, most of the experiments have been carried out in different environments, years and/or populations. In this work, cucumber QTLs localized with robust and breeder friendly co-dominant markers have been compiled. In total, we have selected 39 traits (Table 2) based on their relevance and classified them in five categories: Yield (including earliness and flowering), fruit morphology, fruit quality, vegetative growth and disease tolerance.

Table 2 Summary table of cucumber QTLs involved in relevant agronomic traits classified in five categories. The number of QTLs identified and the references of the corresponding publications are listed for each trait

Fruit agronomic traits		QTLs	References[a]
Yield traits	Earliness	17	A, B, C, G
	Yield	14	A, B, E
	Flowering	9	A, B, S
Fruit Morphology	Fruit Weight	14	B, D, F, G
	Fruit shape	35	A, B, D, E, F, G, K
	Internal Morphology	24	B, E, F
Fruit Quality	Bitterness	2	I
	Fragrance	1	J
Vegetative	Plant	28	A, B, K
Disease tolerance	Fungus	46	L, M, N, O, P, Q, T
	Virus	4	R
TOTAL		194	

[a]A: Fazio et al. 2003; B: Yuan et al. 2008b; C: Lu et al. 2014; D: Wei et al. 2014; E: Weng et al. 2015; F: Yuan et al. 2008a; G: Bo et al. 2015; I: Zhang et al. 2013b; J: Yundaeng et al. 2015; K: Li et al. 2008; L: Pang et al. 2013; M: Zhang et al. 2013a; N: Yoshioka et al. 2014; O: Nie et al. 2015; P: He et al. 2013; Q: Zhang et al. 2014; R: Sugiyama et al. 2015; S: Bu et al. 2016; T: Fukino et al. 2013

Yield Related Traits

A total of 40 yield related QTLs (17 for earliness, 14 for yield and 9 for flowering) have been identified in six different experiments (Table 2). For earliness, 17 QTLs were identified for four traits related with harvest and first female flowers emergence time (Bo et al. 2015; Fazio et al. 2003; Lu et al. 2014; Yuan et al. 2008b). The experiments were carried out under different growing conditions detecting QTLs in different environments for all the characters analyzed. In three out of the four experiments, QTLs related with earliness (*ant6.1*, *fffn2.1*, *fft1.1*) were localized in the same genomic region near the *F* locus associated with gynoecy (Bo et al. 2015; Fazio et al. 2003; Yuan et al. 2008b). In the case of yield, the QTLs identified spanned all over the genome, being distributed in 6 of the 7 linkage groups. However, due to the lack of consensus in mapping, similarity of the QTLs found in the different populations was not possible (Fazio et al. 2003; Weng et al. 2015; Yuan et al. 2008b). Finally, flowering has been included in yield, since this trait directly influences fruit number. In the different studies carried out in cucumber, consistent QTLs involved in flowering were localized in the same genomic region for all the analysed populations (Bu et al. 2016, Fazio et al. 2003; Yuan et al. 2008b). The *sex6*.1 QTL identified by Fazio et al. (2003) was tightly linked to *sg6.2* affecting degree of femaleness (Bu et al. 2015), suggesting the detection of the same QTL involved in number of female nodes on the main stem.

Fruit Morphology

In cucumber, the most important studies related with fruit morphology have been carried out in three subcategories: fruit weight (FW), fruit shape (FS) and internal fruit morphology (flesh thickness and seed cavity). For FW, a total of 14 QTLs have been identified in four different experiments (Table 2) (Bo et al. 2015; Yuan et al. 2008a, b; Wei et al. 2014). In order to validate the QTLs found in each study, the fruit weight analysis was carried out under different environmental conditions (Yuan et al. 2008a, b) and in various mapping populations from the same crosses (Bo et al. 2015; Yuan et al. 2008a). Despite the difficulty of comparison between experiments, two QTLs affecting FW localized in LG3 by Wei et al. (2014), *fw3.1* and *fw3.2*, were detected in the same genomic region in previous experiments (Yuan et al. 2008b).

The following subcategory, FS, is a usual objective in cucumber breeding programs by their close association with the local markets requirements. Several studies of QTLs affecting fruit shape in cucumber have been carried out, identifying 35 QTLs in only 6 experiments (Table 2). QTLs involved in fruit shape were distributed through the whole genome spanned by all seven chromosomes (Bo et al. 2015; Fazio et al. 2003; Yuan et al. 2008a, b; Wei et al. 2014; Weng et al. 2015). Weng et al. (2015) localized QTLs involved in fruit shape and size in the consensus genetic map, considering QTL to be the same if they co-localized in the same chromosome

block. Thus, 12 consensus QTLs for cucumber fruit shape related traits (*FS1.1*, *FS1.2*, *FS2.1*, *FS2.2*, *FS3.1*, *FS3.2*, *FS3.3*, *FS4.1*, *FS5.1*, *FS6.1*, *FS6.2*, *FS7.1*) were established. These consensus QTLs act alone or in combination with other QTLs to regulate cucumber fruit growth and development. For example, *FS4.1* and *FS5.1* both played important roles in ovary length and fruit diameter growth, but only *FS4.1* influenced fruit length. The QTLs *FS2.1* and *FS2.2* affected fruit radial growth, *FS3.2* and *FS3.3* are involved in fruit elongation while the remaining QTLs play roles in both processes.

Another important fruit morphology attribute subject to the market requirements is the internal fruit shape, here divided in two traits: fruit flesh thickness and seed cavity diameter. For both traits, 24 QTLs were identified in three different experiments (Bo et al. 2015; Yuan et al. 2008b; Wei et al. 2014). In the case where comparison was possible, QTLs detected in LG1 and 2 for fruit flesh thickness (*fft1.1*, *fft 2.1*) and in LG1 and 6 for seed cavity diameter (*scd1.1*, and *scd6.1*) showed consistency at position and effect throughout the different mapping populations and seasons (Yuan et al. 2008a, b).

Fruit Quality

Although morphological markers for traits related with fruit appearance such as *ss* (small spines), *D* (dull skin), *U* (uniform immature fruit color) were localized in cucumber genome (Yuan et al. 2008b), research on QTLs related with fruit quality is scarce in cucumber except for the traits related with fruit morphology (see above). Regarding organoleptic characteristics, two works related with the identification of QTL associated with bitterness and fragrance have been published recently. Bitterness is an undesirable trait in cucumber. Zhang et al. (2013b) localized two QTLs on chromosome 5 (*bi-3*) and 6 (*bi-1*) associated with this trait in a population derived from the cross of two parents without fruit bitterness. Both QTLs explained close to 25 % of the phenotypic variance and showed opposite effects. On the other hand, fragrance is an unusual and a value-added trait for cucumber. In this species, a major QTL explaining 43 % of the fragrance variation was localized on chromosome 1 (*qFgr*) and detected in two different populations derived from the same cross (Yundaeng et al. 2015).

Vegetative Traits

Plant architecture has been an objective of breeding in cucumber since these characteristics are closely linked with the yield potential of the crop and are highly influenced by the environment. Thus, understanding the genetic control of traits related with the vegetative growth is crucial to improve the yield in this species. Besides the QTL analysis of Fazio et al. (2003) and Yuan et al. (2008b) for vegetative traits, Li et al. (2008) did a comprehensive study identifying 12 QTLs for traits such as lateral branch number, main stem diameter, internode length and petiole length, using a F_3 population. The comparison of the QTLs identified in the three experiments allowed localization of QTLs affecting the same traits in the same

genomic position. The lateral branch number (*qLBN-2)* localized near the *F* gene in all the experiments (Fazio et al. 2003; Li et al. 2008, Yuan et al. 2008b). Furthermore, the position was similar to other QTL mapped in the interval of genes *F* and *de* (determinate growth habit) in an experiment with different marker types and populations (Serquen et al. 1997), suggesting the identification of a homologous QTL in different genetic backgrounds.

Disease Resistance

Among the 15 disease resistance genes reported by Call and Wehner (2011) three were involved in virus resistance: *Cmv* (Resistance to *Cucumber mosaic virus*), *Wmv* (Resistance to *Watermelon mosaic virus*) and *zymv* (Resistance to *Zucchini yellow mosaic virus*). Moreover, a single recessive gene, z*prsv*^{02245}, controlling the resistance to *Papaya ringspot virus* (PRSV) in cucumber has been reported recently (Tian et al. 2015). Resistance to another virus affecting cucumber, MYSV (*Melon yellow spot virus*), is under polygenic control. Sugiyama et al. (2015) reported four QTLs associated to MYSV resistance in cucumber. Two QTL, located in chromosome 1 (*Swf-1*) and 3 (*Swf-3*), showed a major effect in the resistance explaining 20 and 22 % of the phenotypic variance respectively.

The rest of the resistance genes published were related to control of fungus or bacterial diseases such as scab, bacterial wilt or target leaf spot (Call and Wehner 2011). However, some of the most common fungal diseases in cucumber have polygenic control. In the last years, 46 QTLs involved in resistance to fungus were identified in seven different experiments. Among these diseases, the two most extensively studied were downy mildew (DM) and powdery mildew (PM). In the case of DM, 19 QTLs involved in the response against the fungus infection have been reported in three different experiments (Pang et al. 2013; Yoshioka et al. 2014; Zhang et al. 2013a). A QTL localized on chromosome 5, *dm5.1*, showed the largest effect in disease resistance, and was observed in all the experiments. Also a QTL located on chromosome 1, *dm1.1*, was detected in the same position in two of the studies (Yoshioka et al. 2014; Zhang et al. 2013a). For PM, 25 QTLs were identified in 3 experiments. The position of two of the QTLs, *pm5.1* and *pm5.2*, localized on chromosome 5 was highly consistent with other experiments (Fukino et al. 2013; He et al. 2013; Nie et al. 2015).

Watermelon

Populations and Genetic Maps

Navot et al. (1990) reported the first linkage study of isozyme markers, defining a total of seven linkage groups. Hashizume et al. (1996) published the first low density genetic map with RAPDs, RFLPs and isozymes. Higher density molecular maps were obtained by Hashizume et al. (2003) and Levi et al. (2001, 2004, 2011).

Ren et al. (2012) developed a large number of SSR, InDel and Structural Variation (SV) markers to construct the first high density map with a total of 698 loci and a marker density of 0.8 cM/markers that was used to order and orient the genomic scaffolds of the genome sequence (Guo et al. 2013). Sandlin et al. (2012) constructed the first consensus SNP map consisting of 378 SNPs markers with a density of 5.1 cM/marker. Ren et al. (2014) integrated Ren et al. (2012) and Sandlin et al. (2012) maps to obtain a final map with 953 sequence characterized makers and a density of 0.6 cM/marker. Finally, Ren et al. (2015) developed a high density SNP map (3,465 SNPs) by DArTseq.

Nimmakayala et al. (2014) and Reddy et al. (2015) assessed LD mapping in watermelon germplasm collections. The former used GBS to construct a matrix of 5,254 informative SNPs, allowing a deeper study of LD across the watermelon genome. The average LD decay was 100 kb, but it was not uniform across the genome, decreasing to as little as 5 kb in SNPs located within exons. This value is in a similar range to the LD estimates found by Esteras et al. (2013) and Leida et al. (2015) in melon. Interestingly, large LD blocks were found on watermelon chromosomes 3, 6 and 9, of both wild African and cultivated germplasm, indicating that those blocks are not due to selection sweeps by the domestication selection. In both cases, association of phenotypic traits to several markers were found, initiating the implementation of GWAS in watermelon.

Mapping Quantitative Traits

The first QTL mapping report was carried out by Hashizume et al. (2003) studying fruit quality traits such as hardness of rind, flesh juice, flesh color and rind color. In the last years, QTL mapping reports have quickly increased in this species (Table 3).

Table 3 Summary table of QTLs affecting agronomic traits in watermelon. The number of QTLs identified and the references of the corresponding publications are listed for each selected trait

Fruit agronomic traits		QTLs	References[a]
Flowering	Flowering time	7	L
	Sex expression	4	G
Fruit Morphology	Fruit Weight and Shape	38	H, I, K, R
Fruit color	Flesh and Rind color	8	A, F, Q
Fruit Quality	Sugar Flesh content	25	A, F, H, J, K, S
Seed traits		21	H, I, J, K, L, M
Disease tolerance		7	E
TOTAL		110	

[a]A: Hashizume et al. 2003; E: Lambel et al. 2014; F: Liu et al. 2015; G: Prothro et al. 2013; H, I, J, K: Ren et al. 2014; L: McGregor et al. 2014; M: Meru and McGregor 2014; Q: Kim et al. 2015; R: Reddy et al. 2015; S: Nimmakayala et al. 2014

Flowering Traits

Two flowering traits have been genetically dissected in watermelon: sex expression and flowering time. Cultivated watermelons express mainly monoecious flowers, whereas andromonoecy is common in wild watermelons. The genetic control of flowering types was already attributed to a single gene (*a*) in watermelon by Rosa (1928). Prothro et al. (2013) found a major QTL on chromosome 3, that may correspond to the *a* gene and co-localizes with an ACS gene homolog, as it has been found previously in melon and cucumber (Boualem et al. 2008, 2009).

The genetic control of flowering time was studied by McGregor et al. (2014). A major stable QTL was mapped on chromosome 3, plus additional minor QTLs on chromosomes 2 and 11. Proposed candidate genes for the major QTL were *flowering locus T* and *tempranillo 1* that were located in the QTL region.

Fruit Morphology

A total of 38 QTLs involved in different aspects of fruit morphology have been described in three different mapping populations (Sandlin et al. 2012; Ren et al. 2014) and one association panel (Reddy et al. 2015). QTLs for FW have been located in chromosomes 2, 3 and 5. Interestingly, FW QTLs have been reported in chromosome 2 in several mapping populations. Regarding FS, QTLs have been mapped in chromosomes 2, 3 and 10. The FS QTL on chromosome 3 overlaps with the major sex expression QTL (Prothro et al. 2013), so probably this QTL is due to pleiotropic effects of sex expression on FS as has been reported previously in melon (Abdelmohsin and Pitrat 2008; Perin et al. 2002b). Internal fruit morphology (rind thickness) has been also studied successfully, with QTLs being described on chromosomes 2, 5, 6. The QTL on chromosome 2 has been detected in several studies. These works suggest that QTLs on chromosome 2 and 3 may have an important role in watermelon fruit morphology diversity.

Fruit Color

Liu et al. (2015) identified a major QTL controlling both red color and lycopene accumulation on chromosome 4. A lycopene β-cyclase located in the QTL region, is a quite reasonable candidate gene for this QTL. Hashizume et al. (2003) identified a QTL for rind color, although as their map has not been integrated with the watermelon genome, it is not possible to compare this result with subsequent works. On the other hand, a major gene involved in rind pattern was located in chromosome 6 by Kim et al. (2015) and a putative good diagnostic marker was developed for the Jubilee-type stripe pattern.

Fruit Quality

QTLs for sugar flesh content have been mapped in chromosomes 1, 2, 3, 6, 7 and 8 in four different mapping populations (Sandlin et al. 2012; Ren et al. 2014; Liu et al. 2015). The most consistent QTL among populations and environments was located on chromosome 2, probably indicating that this QTL was important during domestication and diversification.

Seed Traits

The seeds of some watermelon cultivars are consumed in some West African countries as a source of proteins, carbohydrates, oil and vitamins. The seed types used for such purposes are large and flat and named with the term "egusi". Cultivars producing egusi seeds are classified as *C. lanatus* ssp. *mucosospermus* var. *egusi*. The egusi seed trait is controlled by a single recessive gene (*eg*) that was mapped by Prothro et al. (2012a, b) and Ren et al. (2014) on chromosome 6. Meru and McGregor (2013) more deeply studied genetic control of seed traits variation within normal and egusi background, in order to eliminate the effect of the *eg* locus. They found a QTL on chromosome 6 for kernel percentage and seed size, and other QTL for seed length, demonstrating that this region contains seed trait QTLs from diverse genetic backgrounds. A QTL on chromosome 6 (likely associated to the *eg* locus) controls the increase/decrease of oleic/linoleic acids, respectively, being the oleic increase induced by the egusi allele (Meru and McGregor 2014). QTLs for accumulation of other fatty acids as palmitic, stearic and oleic were also found in chromosomes 2 and 3, 8 and 2 and 8, respectively.

Disease Resistance

The genetic control of watermelon disease resistance with complex inheritance has been scarcely addressed in this species. Lambel et al. (2014) studied the genetics of resistance to *Fusarium oxysporum* race 1 finding a major QTL on chromosome 1 and some few minor QTLs, showing that a single gene could be used to develop resistant cultivars. Some candidate genes were defined for that QTL, specially a pathogenic-related gene sequence that encodes a glucan endo-1,3-β-glucosidase precursor and others encoding acidic class III chitinases.

Cucurbita

Despite of the economic and social importance, few genomics tools are available for *Cucurbita* species. The first genetic maps were developed using RAPD and AFLP markers (Brown and Myers 2002; Lee et al. 1995; Zraidi et al. 2007),

making it practically impossible to perform comparisons. Gong et al. (2008a, b) published the first SSR based map in a cross between Pumpkin x Crookneck cultivars. Blanca et al. (2011) reported a collection of nearly 20,000 SNPs, from where they developed an Illumina GoldenGate 384-SNP platform. This platform and some previously published SSRs were used to obtain a medium saturated linkage map in a *C. pepo* subsp. *pepo* var. Zucchini x *C. pepo* subsp. *ovifera* var. Scallop F_2. A total of 304 SNPs and 11 SSRs were distributed into 22 major linkage groups with an average distance among markers of 6.06 cM (Esteras et al. 2012). The last linkage map reported for this species was generated using the genotyping-by sequencing approach and contains 458 bin-markers across 20 linkage groups (Zhang et al. 2015).

QTL analysis was performed for traits related to vine, flowering and fruit traits in the former population. A total of 48 QTLs were detected, 15 were classified as major QTLs ($R^2 > 25\,\%$). The effects of 11 of the major QTLs and six minor QTLs were verified in subsequent backcross populations. Thus, a cluster of QTLs controlling several flowering traits was detected in LG 3, with Zucchini alleles associated with the early appearance of male and female flowers. Another cluster in LG 6 controlled fruit shape traits, in this case, the Zucchini allele contributed to produce elongated fruits, whereas the Scallop allele produced wider fruits with wider cavities. Also a QTL for fruit elongation was found in LG 18 and Scallop alleles in LG 5 and 11 increased the number of locules and ribbing intensity modifying fruit morphology. Finally, Zucchini alleles in LG 14 increased rind greenness and flesh color was controlled by QTLs on LG 20 and LG16 (Esteras et al. 2012). Regarding an important agronomic trait such as dwarf vine, three QTLs on linkage groups 1, 3 and 4 were identified by Zhang et al. (2015). The QTL located on chromosome 3, qCmB2, explained 21.4 % of the phenotypic variation. The genome of *Cucurbita pepo* is currently available at https://cucurbigene.upv.es/. This version of the genome contains 263 Mb assembled with an N50 of 1.73 Mb. The genome has been anchored to a genetic map of 9,350 markers developed by GBS in a RIL population (chapter "Genetics and genomics of Cucurbita species"). Certainly, the implementation of genomics tools in *Cucurbita* genetics and breeding will be speed up in the very next future after the release of this genome.

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Phloem Biology of the Cucurbitaceae

Robert Turgeon

Abstract Cucurbit phloem is especially interesting and unusual in several respects, which has made these plants the subject of intensive study by microscopists, physiologists and molecular biologists. Early light microscopists were attracted to cucurbits due to the large dimensions of the sieve tubes and sieve pores, which made them relatively easy to see (Esau, The phloem. Berlin/Stuttgart: Gebrüder Borntraeger, 1969). Hartig, the forest botanist who discovered the sieve element, turned to *Cucurbita* in his studies, describing many aspects of phloem structure including sieve plates, callose and "slime plugs." (See Esau, The phloem. Berlin/Stuttgart: Gebrüder Borntraeger, 1969 for the early history of phloem research). *Cucurbita* has also featured strongly in the analysis of phloem development. For example, Esau et al. (Bot Gaz 123:233–243, 1962) described the development of the sieve pores and the involvement of callose deposition in this process in *Cucurbita maxima*. A striking feature of cucurbit phloem is the presence of a unique array of extrafascicular (outside the vascular bundles) sieve tubes, which have been studied by several groups in recent years (Zhang et al. Proc Nat Acad Sci U S A 107:13532–13537, 2010; Gaupels et al. Plant Physiol 160:2285–2299, 2012; Zhang et al. Plant Physiol 158:1873–1882, 2012; Gaupels and Ghirardo, Front Plant Sci 4:187, 2013; Gaupels et al. Front Plant Sci 7:154, 2016).

Another interesting quirk is that cucurbit "phloem" exudes copiously when cut, which has led to its use in many metabolomic and proteomic analyses. It should be noted, however, that as early as 1944 Crafts and Lorenz thought the high nitrogen content of cucurbit exudate so unusual as to make it suspect as true, mobile phloem sap, a caution that has borne out in recent years (see below). In this chapter I will leave much of the early history of cucurbit phloem biology to Esau (The phloem. Berlin/Stuttgart: Gebrüder Borntraeger, 1969) and focus for the most part on several aspects of the subject addressed since the publication of her monumental work.

Keywords Phloem • Cucurbitaceae • P-protein • Sieve tube exudate • Stachyose • Extrafascicular

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Organization

The primary phloem in most members of the Cucurbitaceae is composed of bundles of sieve tubes and companion cells on both sides of the xylem (bicollateral, fascicular phloem) and an array of phloem elements outside the bundles (extrafascicular phloem) (Fig. 1). Bicollateral phloem is common to a number of other, unrelated plant families including, but not limited to, the Apocynaceae, Convolvulaceae, Onagraceae and Solanaceae. Extrafascicular phloem is only known in the Cucurbitaceae. In pumpkin, which has the complete array of extrafascicular elements, longitudinally oriented sieve tubes are found at the margins of the fascicular phloem, just inside the sclerenchyma ring (entocyclic sieve tubes), just outside the sclerenchyma ring (ectocyclic sieve tubes), and as a network of obliquely oriented commissural sieve tubes connecting the other types (Crafts 1932; Fig. 1).

Not all cucurbits have all of these extrafascicular elements, however. Fischer, as summarized by Esau (1969, pg 342–3), described six types of phloem in the

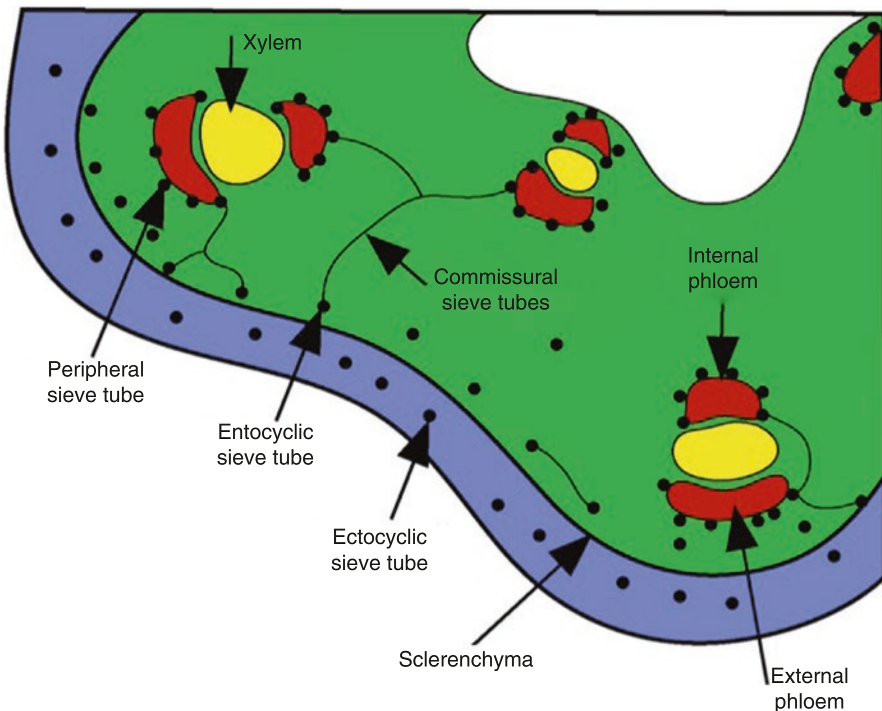

Fig. 1 Diagram of a transverse section of a pumpkin (*Cucurbita maxima*) stem illustrating the fascicular phloem, with internal and external bundles flanking the xylem, and several extrafascicular types of sieve tubes (peripheral, entocyclic, ectocyclic and commissural) (The figure is reproduced, with permission, from Zhang et al. (2012) (www.plantphysiol.org), Copyright American Society of Plant Biologists)

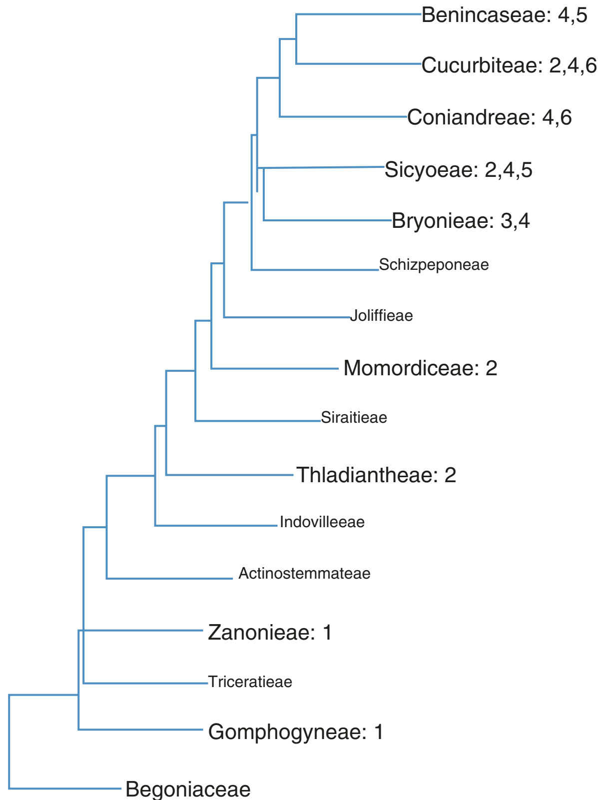

Fig. 2 Occurrence of extrafascicular phloem types, according to Esau (1969, pg 343), in the tribes of the Cucurbitaceae according to the phylogeny of (Schaefer and Renner 2011). *1 Alsomitra* type: Collateral vascular bundles (bicollateral bundles are present in all the remaining 5 types), no peripheral or commissural sieve tubes (STs); *2 Luffa* type: entocyclic STs possibly present; radial commissural STs only; *3 Bryonia* type: many entocyclic STS that become obliterated during tissue elongation, ectocyclic and commissural STs lacking; *4 Cyclanthera* type: numerous entocyclic STs, few commissural STs, no ectocyclic STs; *5 Lagenaria* type: abundant entocyclic and commissural STs; *6 Cucurbita* type: abundant extrafascicular phloem of all types

internodes of the Cucurbitaceae with differing degrees of complexity. *Alsomitra* has the simplest configuration with collateral rather than bicollateral bundles and no extrafascicular phloem. Jeffrey (1980) notes that the members of the Zanonioideae, which have typical cucurbitaceous features to a lesser degree than other tribes in the family, have late-forming internal bundles if they have any at all. The other types are all bicollateral and have increasingly complex extrafascicular phloem, with those of the Cucurbita type being the most complex. Overlaying these types on a phylogenetic map of the Cucurbitaceae (Schaefer and Renner 2011) (Fig. 2) indicates that there has been a progression in the complexity in more recently derived clades although the pattern is not simple. For example, in the Tribe Cucurbiteae the genus *Cucurbita* has the most complex phloem type with highly developed extrafascicular

phloem, but in *Cayaponia* and *Abobra*, also in the Cucurbiteae, ectocyclic sieve tubes are lacking and commissural sieve tubes are reduced. Whether these disparities are due to independent progressions or reversions in phloem evolution within the tribes is not clear and will require additional data to resolve. Some caution should be exercised since the analysis is based on Fischer's original data from 1884, which were obtained, obviously, without the benefit of electron microscopy. Many elements of the extrafascicular phloem, given the thinnest of the cells and their oblique orientation, can be difficult to see in light microscope sections.

Esau (1969, pg 165), working from the studies of Fischer, described the complex phloem of the *C. pepo* reproductive structures in detail. It is a general feature in the angiosperms that the orientation of xylem and phloem in vascular bundles of reproductive parts can be inverted due to the various ways in which the floral parts unite and this is also true in the cucurbits. Extrafascicular phloem is also abundant in reproductive structures of *C. pepo*, including the peduncle, receptacle (torus) and stigma of the flower, and it is extensive within the fruit.

The extrafascicular phloem is unique to the Cucurbitaceae and is generally considered to be a defense adaptation. Extrafascicular phloem exudates contain cucurbitacins, alkaloids and terpenoids to repel invaders (Konno 2011). Gaupels and colleagues (Gaupels et al. 2012, 2016; Gaupels and Ghirardo 2013) note that the extrafascicular system is similar to branched laticifer conduits and use the term latex to describe the exudate from these cells. The exudate is sticky, so it can trap insects or glue their mouthparts together, and the fluid from *Cucurbita maxima* contains at least 51 wound-related phloem proteins (Gaupels et al. 2012).

Are photoassimilates transported in the extrafascicular phloem? This is actually a difficult question to answer. Early autoradiographic studies indicated that when squash leaves are provided with $^{14}CO_2$, radiolabelled sugar is found downstream in both the fascicular and extrafascicular phloem (Webb and Gorham 1964). This suggests that the extrafascicular phloem participates in the long-distance transport of photoassimilate, but this may be too simplistic a view. It seems more likely that sugars and other nutrients are distributed from sources to sinks in the fascicular phloem and that some of this material is bled off into the extrafascicular system for the synthesis of defense compounds. Supporting this view, exudate from the extrafascicular phloem at the edge of the stem, furthest from the vascular bundles has little stachyose, the major transport sugar (see section "Minor Vein Structure and Phloem Loading"), but abundant hexose (Zhang et al. 2012). Also, when Zhang et al. (2010) applied carboxyfluorescein to leaves, the fluorescent tracer was carried long distances in the fascicular, but not the extrafascicular, phloem.

If it is correct that the extrafascicular phloem is not part of the long-distance transport network, what is the driving force for material exchange within the extrafascicular phloem? There is no satisfactory answer at present. Since the distances involved are relatively short, for example laterally from one vascular bundle to another, diffusion may play the predominant role, replacing sugars and other mobile phloem sap components as they are converted to other compounds. However, this subject is virtually untouched by experimentation at present.

The Sieve Tube

As noted above, microscopists have always found the exceptionally large sieve tubes of cucurbits attractive subjects of study (Fig. 3). Mullendore et al. (2010) compared the sieve elements of seven angiosperm species and found that those in *Cucurbita maxima* were much wider (25.7 μm at the sieve plate) than those in non-cucurbitaceous species examined (2.0 μm in *Arabidopsis thaliana* to 16.2 μm in *Phaseolus vulgaris*). The sieve elements of *C. maxima* are also long, and the sieve plate pores are few in number, but wide. As a result, sieve tube specific conductivity, a measure of the effectiveness of fluid transport, is at least four times that of the other species in the study. In addition, the sieve plate pores tend to be smaller at the plate margins and larger at the center where maximum flow velocities occur, features that facilitate laminar flow. Interestingly, flow velocity measured by MRI velocimetry was lowest for *C. maxima* indicating an inverse relationship between conductivity and velocity. Transport velocity differs between vascular bundles (Savage et al. 2013).

"Phloem" Exudate

Perhaps the greatest technical problem in phloem biology is in obtaining authentic, mobile content from sieve tubes. Gaining intravenous access in animals by venipuncture is generally a simple process because the fluid passes through a relatively large extracellular space. But in plants the sieve tubes are living entities that are

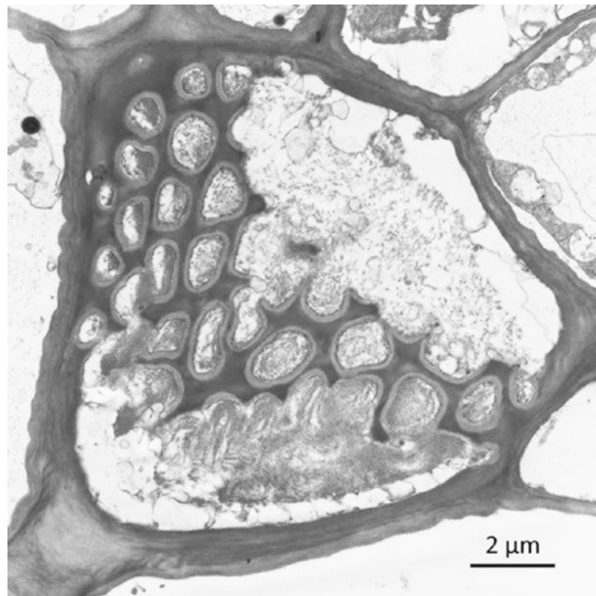

Fig. 3 Cucumber (*Cucumis sativus*) sieve plate from the external phloem of the petiole. The sieve pores are large and partially filled, in this image, with proteinaceous material

sequestered, under considerable pressure, and delicate. There is as yet no technique that samples the phloem adequately. Even stylectomy, the laborious and technically challenging, method of cutting embedded aphid stylets to obtain minute quantities of phloem sap is problematic given that aphids undoubtedly alter phloem physiology and sap content to encourage continued flow. Given the problems inherent in other methods, it is seductively easy to cut a cucurbit stem and obtain large quantities of fluid exuding from what first appears to be the phloem of fascicular bundles.

Exudate from severed cucurbit phloem has been used in a number of studies with the intention of assaying metabolites, RNA and protein. However, the very low concentrations of transport sugars in cucurbit exudate (Richardson and Baker 1982, Tolsikov et al. 2007) suggest that it is not pure long-distance transport sap. Indeed, it has been shown recently that this exudate actually comes from several sources including the fascicular phloem, the extrafascicular phloem, the xylem, and cut cells at the surface of the wound (Zhang et al. 2010, 2012; Gaupels et al. 2012). To illustrate how difficult it is to equate the exudate with authentic sap, consider that when the petiole of a pumpkin leaf labeled with $^{14}CO_2$ is cut, fluid can discharge from the wound surface for many minutes, but radiolabel exudation stops much more quickly (Zhang et al. 2012). Over half of the radiolabel that will exude does so within 10 s and the rate of radiolabel efflux continues to fall exponentially. Rapid blockage of the fascicular phloem after cutting was also noted by Zhang et al. (2010) using video microscopy. To reduce contamination from cut surfaces it is common practice to blot away the first half-minute or so of exudate, which means that almost all mobile material from the sieve tubes is discarded before sampling begins. Also, calculations of phloem volume and experiments that allow phloem and xylem transport to be distinguished from one another indicate that almost all the exudation that occurs after the first few minutes is from the water transport column under positive pressure, not the phloem (Zhang et al. 2012). It seems, therefore, that phloem exudate from cut cucurbit tissue can, under some circumstances yield sap that flows from fascicular sieve tubes, in practice this fluid may be heavily contaminated with material from the extrafascicular phloem, cut cells and the xylem.

P-Protein

One of the striking features of cucurbit phloem is the accumulation of protoplasm in the form of plugs at the sieve plates in tissue preserved for microscopy. Light microscopists in the early days of phloem research called this material “slime,” (see Esau 1969 for a comprehensive review of the early literature). The slime plugs are primarily proteinaceous in composition, as seen by staining with mercuric bromophenol blue, and come out in the exudate that erupts from severed phloem (Kollmann et al. 1970; Williamson 1972). As early as the middle of the nineteenth century it was thought that the slime plugs are not present under normal conditions; rather, the various components line the sieve tube periphery and accumulate at the sieve plates as a result of injury (see (Cronshaw and Sabnis 1990). This is a reasonable

supposition since damage to the long-distance transport system could potentially result in excessive loss of nutrients and provide an entrance route for invading pathogens. To determine if the accumulation of slime against the sieve plates is caused by injury Eschrich (1963) injected a variety of fixatives, including potassium permanganate, into the hollow core of the *Cucurbita ficifolia* petiole and observed relatively open pores with minimal accumulation of slime, supporting the concept that the plates are normally open. This conclusion has been largely validated by additional studies (e.g, Evert et al. 1972) although the degree of protein blockage of sieve plate pores under normal physiological conditions is still a matter of debate.

Phloem-specific proteins (P-proteins) are found in the sieve elements of all dicotyledonous, and many, but not all, monocotyledonous plants. The term, "P-protein" historically refers to distinct proteins that accumulate as aggregates (P-protein bodies) in sieve elements during differentiation and which may, or may not, disperse when the sieve element matures (see Anstead et al. 2012). The most common P-proteins found in sieve tubes of most species belong to the "sieve element occlusion-related" (SEOR) family (Froelich et al. 2011; Ernst et al. 2012; Knoblauch et al. 2014). The SEOR proteins were first identified in legumes as distinct bodies known as forisomes (Knoblauch et al. 2001) but have since been recognized as a broad class of phloem proteins in non-Fabaceae plants, including the cucurbits (Ernst et al. 2011). However, the most abundant P-proteins in cucurbits, known as PP1 and PP2 (see below) are not members of the SEOR family.

Cucurbit phloem exudate contains many additional proteins of various types (Walz et al. 2004; Lin et al. 2009) in addition to the SEOR and PP1/PP2 protein classes. Kehr (2006) compiled a list of phloem sap proteins from various species that may be involved in wound and defense reactions. In cucurbit sap, from one species or another, one finds enzymes that are reactive oxygen/redox related such as thioredoxin h, kinases, phytohormone-related proteins such as ACC oxidase, protease inhibitors and others. Squash phloem exudate contains an aspartic acid proteinase inhibitor (SQAPI), a member of a small family of approximately 10 highly homologous proteins (Christeller et al. 1998, 2006). SQAPI genes are also found in other members of the Cucurbitaceae and in wider members of the Cucurbitales. Gene promoters from variants of this gene family in *C. maxima* drive gene expression in tobacco leaf phloem (Anandan et al. 2009). Trypsin inhibitor, which prevents proteolosis of trypsin or chemotrypsin, is also detected in cucurbit exudate (Murray and Christeller 1995). Balachandran et al. (1997) have shown that phloem proteins have the capacity to increase the size exclusion limit of plasmodesmata between mesophyll cells.

The functions of P-proteins are still not fully understood but it is widely assumed that they participate in wound healing and defense. For example, burning the tips of *C. maxima* leaves sends electropotential waves (EPWs) along phloem of the main vein resulting in rapid coagulation of sieve element proteins followed by gradual occlusion by of the pores by callose approximately 10 min later (van Bel 2006; Furch et al. 2010). However, Knoblauch and van Bel (1998) imaged P-protein aggregates on the sieve plates of intact and apparently uninjured fava bean (*Vicia fava*) plants, which were minimally wounded in preparation for confocal micros-

copy. Knoblach et al. (2014) present a detailed argument against the common, intuitive view that P-protein seals the phloem to prevent photoassimilate loss, challenging the conclusions of Ernst et al. (2012) on tobacco and Jekat et al. (2013) on Arabidopsis who demonstrated an increase in sugar loss in plants of both species in which SEOs were depleted by mutation. Knoblach et al. (2014) point out that SEOs do not seal the phloem entirely and furthermore the loss of sugar from peripheral organs does not substantially impair fitness. While these are reasonable cautions, it seems to this author that sealing against sugar loss is probably a multi-faceted phenomenon in which sieve tube plugging by SEOs may be an important, though perhaps only partial, component. Zhang et al. (2012) make the case that a major wound-healing function of P-protein in cucurbits is to seal damaged xylem by spreading out over the entire cut surface to form a dry, protective film. The water column is often under positive pressure in cucurbits and can lose very large volumes when cut. This could be an especially critical issue in this family, many members of which are xerophytes.

PP1 and PP2

Phloem proteins 1 and 2 (PP1 and PP2) were initially characterized in cucurbit phloem sap (Walker 1972; Beyenbach et al. 1974; Kleinig et al. 1975; Sabnis and Hart 1976, 1978, 1979; Read and Northcote 1983a, b). PP1 may be unique to cucurbits (Clark et al. 1997) but PP2 is widely found in angiosperm and gymnosperm genera (Dinant et al. 2003). The synthesis of these proteins is developmentally regulated at defined stages of phloem differentiation (Dannenhoffer et al. 1997). They form discrete bodies that, at least in the phloem of the vascular bundles, dissociate at sieve element maturity and take up a parietal position against the cell membrane (Shah and Jacob 1969; Eschrich 1970; Evert et al. 1972). In the extrafascicular phloem the protein bodies often fail to disperse (Cronshaw and Esau 1968). Both PP1 and PP2 coagulate in the presence of oxygen (Alosi et al. 1988; Golecki et al. 1998). Interestingly, the exuded sap from stems gels rapidly upon exposure to air, but the sap from squash or pumpkin fruit does not (Alosi et al. 1988).

PPI, the phloem filament protein, is an especially abundant P-protein (Lin et al. 2009). The PP1 from pumpkin is a monomeric 96-kD protein that forms soluble polymers (Read and Northcote 1983a). The PP1 from *C. maxima* has a 2430 bp coding sequence with no introns and encodes a 809 amino acid polypeptide. This polypeptide is highly repetitive with four 200-aa domains that have commonality with cysteine proteinase inhibitors (Clark et al. 1997). PP1 can be broken down by proteases in aphid saliva suggesting that the aphid uses this strategy to suppress a plant defense response (Furch et al. 2014). The calculated molecular mass of the monomer is 95.4 kDa although the measured mass can differ substantially (Leineweber et al. 2000). It produces insoluble aggregates when the tissue is cut and plugs injured sieve tubes (Read and Northcote 1983a; Knoblauch and van Bel 1998; Golecki et al. 1999; Will and van Bel 2006).

PP1 is transported in the phloem (Golecki et al. 1999; Leineweber et al. 2000). Golecki et al. (1999) detected PP1, probably in monomeric form, in the scions of Cucurbita/Cucumis grafts, although there was no evidence for mRNA movement. Interestingly, the protein was detected in the extrafascicular, as well as the fascicular, phloem, indicating, as with dye movement studies, that the two systems are connected. In the extrafascicular phloem most of the transported protein was detected in the SEs but there was some weak staining in companion cells as well, indicting SE-to-CC mobility.

PP2 has been studied more extensively than PP1. PP2 is a homodimeric 48-kD lectin that specifically binds poly(β-1,4-*N*-acetylglucosamine) (Bostwick et al. 1992; Bobbili et al. 2014). PP2 proteins are widespread in the plant kingdom, including cereals. The two subunits from melon take the form of a 154-aa 17-kDa protein and a 26-kDa component. Within the *Cucumis* genus the 17-kDa lectin is highly conserved in contrast to the 26-kDa form that is highly variable (Dinant et al. 2003). Comparing PP2 from Cucurbitales and Apiales revealed four conserved motifs (Dinant et al. 2003). PP2 mRNA is detected only in companion cells (Bostwick et al. 1992) but the protein is found in both the companion cell and sieve element. Interestingly, antibodies to PP2 stain the extrafascicular phloem more consistently and more densely than the companion cells of the phloem bundles (Smith et al. 1987). PP2 accumulates in the SE, apparently due to movement of the protein from cell to cell through plasmodesmata (Balachandran et al. 1997) allowing it to traffic between companion cell and sieve element and migrate to sinks (Bostwick et al. 1992; Dannenhoffer et al. 1997; Golecki et al. 1999). P-proteins can also cross intergeneric graft boundaries (Golecki et al. 1998, 1999). Recent mutational analysis of PP2 from *C. maxima* has identified amino acid residues that interact with chiooligosaccharides (Bobbili et al. 2014).

PP2 interacts with a variety of RNAs and has been implicated in long distance movement of viruses and viroids (Gómez and Pallás 2001; Owens et al. 2001). The interaction between PP1 and PP2 appears to be non-covalent and transient (Dinant et al. 2003). As with PP1, Golecki et al. (1999) detected movement of PP2 protein, presumably as the dimer, across the union of *Cucurbita*/*Cucumis* grafts. No PP2 mRNA movement was seen. In the extrafascicular phloem the protein was readily detected in both the SEs and CCs, indicating that the protein moves more readily through the plasmodesmata that connect these cells than does PP1.

Minor Vein Structure and Phloem Loading

Cucurbits employ a phloem loading mechanism distinct from the one common to the majority of crop plants. In most herbs, sucrose produced in the mesophyll migrates from cell to cell until it reaches the minor veins, then exits the cells and enters the cell wall space, the apoplast. From the apoplast it is loaded into the minor vein phloem by transporters loaded on the plasma membrane of the phloem cells (De Schepper et al. 2013; Slewinski et al. 2013; Savage et al. 2015; Yadav et al.

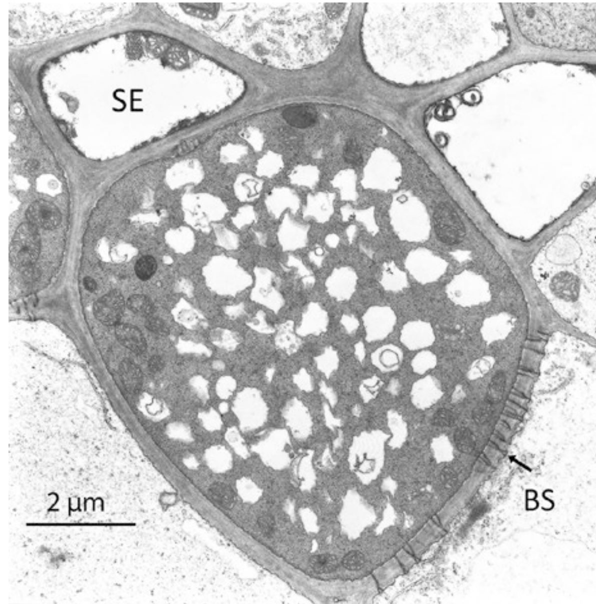

Fig. 4 Cucumber (*Cucumis sativus*) intermediary cell in the phloem of a minor vein. The Intermediary cell is adjacent to a sieve element (*SE*). The cytoplasm of the intermediary cell is dense, with many small vacuoles, and many branched plasmodesmata (*arrow*) link the cell to a bundle sheath (*BS*) cell

2015). In cucurbits by contrast, sucrose does not enter the apoplast, rather it migrates from the mesophyll all the way into the phloem through plasmodesmata, an entirely symplastic route (Turgeon and Hepler 1989; Liesche and Schulz 2013).

At first a symplastic phloem loading mechanism seemed unlikely given that the concentration of sugar in the phloem is much higher than in the mesophyll and small molecules pass through plasmodesmata passively. The solution to this apparent paradox is that cucurbits translocate raffinose and stachyose, especially the latter, with a smaller amount of sucrose. When sucrose enters the companion cells of the minor veins it is converted to these larger sugars, which cannot pass back through the same plasmodesmata into the mesophyll due to their size (Turgeon and Gowan 1990; Dölger et al. 2014). This size-dependent loading mechanism is known as the polymer trap.

Polymer trapping has been identified as the phloem loading mechanism in at least 15 families of dicots and in every case the sugars transported are raffinose and stachyose (Turgeon et al. 2001). In all cases, certain cell structures and physiological characteristics are shared with the cucurbits. The minor vein companion cells (Fig. 4) are large and dense with ribosomes and mitochondria (Turgeon et al. 1993). The vacuoles are numerous and small and the plastids rudimentary. The plasmodesmata linking the companion cells to the bundle sheath are extremely numerous, probably more so than at any other interface in plants, and clustered in distinct fields. They are highly branched, more so on the companion cell side. Given the unique characteristics of these minor vein companion cells, they are commonly called “intermediary cells.” The minor veins of cucurbits are simple in structure compared to most other plants, consisting primarily of two intermediary cells with adjacent sieve tubes, and no phloem parenchyma cells (Turgeon et al. 1975). This

simplified structure is common to most other polymer trap plants, such as *Coleus blumei* (Fisher 1986). The bicollateral condition extends into the minor veins. The abaxial and adaxial phloem of *Ecballium* minor veins are connected by anastomoses (see Esau, 1969 pg 156), which suggests exchange of photoassimilate between the two parts of the bicollateral system.

Why has the polymer trap evolved in certain groups? There is no definitive answer presently but it probably does not involve particularly favorable properties of raffinose and stachyose beyond their size, allowing the size discrimination mechanism to work. A more likely possibility is that some useful compound(s), perhaps defense related, is transported from the mesophyll into the phloem through the plasmodesmata (Davidson et al. 2011). The presence of open pores at this boundary would then preclude transporter-mediated loading since the loaded sucrose would leak back into the mesophyll. To date no molecule driving the evolution of polymer trapping has been identified in the cucurbits but certain of the Lamiaceae, which also load by polymer trapping, export iridoid glycosides, defensive compounds approximately the same size as sucrose (Gowan et al. 1995; Voitsekhovskaja et al. 2006).

Although polymer trapping is the primary phloem loading mechanism in cucurbits, there is ample evidence that at least some sucrose is loaded via the apoplast, so called “mixed” or “heterogeneous” loading (van Bel 1993). First, in all RFO-transporting lineages, “ordinary” companion cells, with few plasmodesmata linking them to surrounding cells, are present in minor veins in addition to the intermediary cells (Turgeon et al. 1975; Turgeon et al. 1993). In the Scrophulariaceae, species-specific variation in the proportion of intermediary cells to ordinary companion cells is correlated with the proportion of RFOs transported with respect to sucrose (Turgeon et al. 1993). The proportion of intermediary cells decreases with increasing vein size such that the largest minor veins are composed of many ordinary sieve element/companion cells complexes with a single pair of intermediary cells on their flanks. In the major veins, which probably load very little, if any, sucrose from the mesophyll, the intermediary cells are absent. Do the ordinary companion cells in the minor veins of RFO plants load sucrose from the mesophyll? Several lines of evidence indicate that they do (reviewed in van Bel 1993; Oparka and Turgeon 1999; Knop et al. 2004; Slewinski et al. 2013; Öener-Sieben et al. 2015). This reinforces the concept that the polymer trap mechanism has been overlaid, in an evolutionary sense, on the apoplastic mechanism for an additional function, perhaps to allow specific, and to date unknown, molecular species to gain access to the phloem without having to pass through the apoplast. The cucurbits have many more secrets to tell.

Conclusions

Cucurbits continue to be model plants for phloem biologists. The highly interesting P-proteins and other sieve tube proteins will continue to reveal secrets about plant defense, and these revelations may in turn suggest new strategies for crop protection. In terms of the mechanics of phloem transport, the extreme size of the sieve

tubes and sieve pores of cucurbits requires that these plants be considered in theoretical analyses.

A continuing problem in cucurbit phloem biology is the difficulty of genetically transforming members of this family. Progress has been made (e.g. Sui et al. 2012) but the process is still inefficient. When transformation technologies in the family become routine, cucurbits will become even more important in the basic science of phloem biology.

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Sex Determination in Cucumis

Natalia Yaneth Rodriguez-Granados, Afef Lemhemdi, Fadi Abou Choucha, David Latrasse, Moussa Benhamed, Adnane Boualem, and Abdelhafid Bendahmane

Abstract The *Cucurbitaceae* family is widely recognized for their highly diverse sexual systems. Due to this variability and the agricultural importance of some of its members, cucurbits have been used as a plant model for understanding sex determination in the kingdom. Several studies in important members of this family such as melon and cucumber, have supplemented plant biologists with meaningful findings regarding the main factors influencing flower sexuality. Sex determination and the evolution of sexual systems comprise different factors, ranging from genetic components involved in ethylene biosynthesis to transcription regulators and epigenetic processes.

In this chapter, we present an integrative explanation of the mechanisms governing sex determination in different members of the *Cucurbitaceae* family. For this purpose, several studies on the field will be integrated in order to provide the main fundaments of flower development and sexual systems in cucurbits. Starting with the basics of floral ontogenesis, this chapter will discuss the genetic models regulating sex determination and the evidenced factors influencing flower sexuality. At last, we mention the nebulous aspects of the sexual systems present in cucurbits and the importance of considering them for future research.

Keywords Sex determination • *cucurbits* • monoecy • dioecy • ethylene • epigenetics • TILLING

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Introduction

Considering sexual reproduction as the main motor of evolution is not an overestimation. Its presence in the 99.9 % percent of the organisms, from prokaryotes to eukaryotes, clearly explains the impact it has on species variability and adaptability (Otto and Lenormand 2002; de Visser and Elena 2007). This later conclusion has arisen from the cornerstone of biology laid by the most memorable scientists.

The contribution of sexual reproduction in species fitness relies on the emergence and evolution of sex determination mechanisms. Sexual systems vary widely among all the domains of life. The extensive research on the field, especially in animals, has elucidated several sex determination mechanisms such as haplodiploidy, paternal genome loss, male heterogamety, female heterogamety, polygenic and environmental sex determination, among others (Werren and Beukeboom 1998).

Despite its shorter trajectory, the biology behind sex determination in the plant kingdom has been an intriguing process initially assessed by different scientists such as Linnaeus, Darwin and Camerarius, which started describing the plant sexuality and its diversity within the kingdom (Zarsky and Tupy 1995).

In contrast to animals, hermaphroditism (defined as the development of bisexual flowers with both stamen and carpel sexual organs) occurs in the vast majority of flowering plants or angiosperms. However, a small percentage, around 10 % of the species, diverged from this ancestral trait and developed unisexuality by several independent evolutionary events (Barrett 2002; Ming et al. 2011).

Unisexuality, presented generally as dioecy or monoecy, relies on the spatiotemporal activation or repression of genetic pathways that leads to a sex-specific phenotype. Due to its long evolutionary trajectory, which comprises several independent events, plant sexual systems present a wide-range of regulatory mechanisms that give rise to different distributions of gamete-producing organs at both individual and population levels (Ming et al. 2011).

The genetic network behind sex determination in plants is complex and highly regulated at both temporal and spatial axes during development. An increasing research on the field has supplemented previous findings with the discovery of several sex determination mechanisms used by different plant species; however, numerous aspects of this important process remain poorly characterized.

A clear example of the relevant advances of the scientific community regarding this biological question is the understanding of sex determination in *Cucurbitaceae* family. Important members of this family are cucumber (*Cucumis sativus*), the honey melon (*Cucumis melo*), the watermelon (*Citrullus lanatus*) and the squash (*Cucurbita pepo*). Both cucumis species, cucumber and melon, have been recognized for presenting a highly polymorphic sexual system that makes them an ideal model for understanding the genetic and epigenetic mechanisms of sex determination (Ming et al. 2011; Zhang et al. 2014).

Flower Ontogenesis

The *Cucurbitaceae* family present intraspecific sexual systems that are coordinated by conserved molecular pathways that lead to different phenotypic outputs depending on the species (Fig. 1a, b).

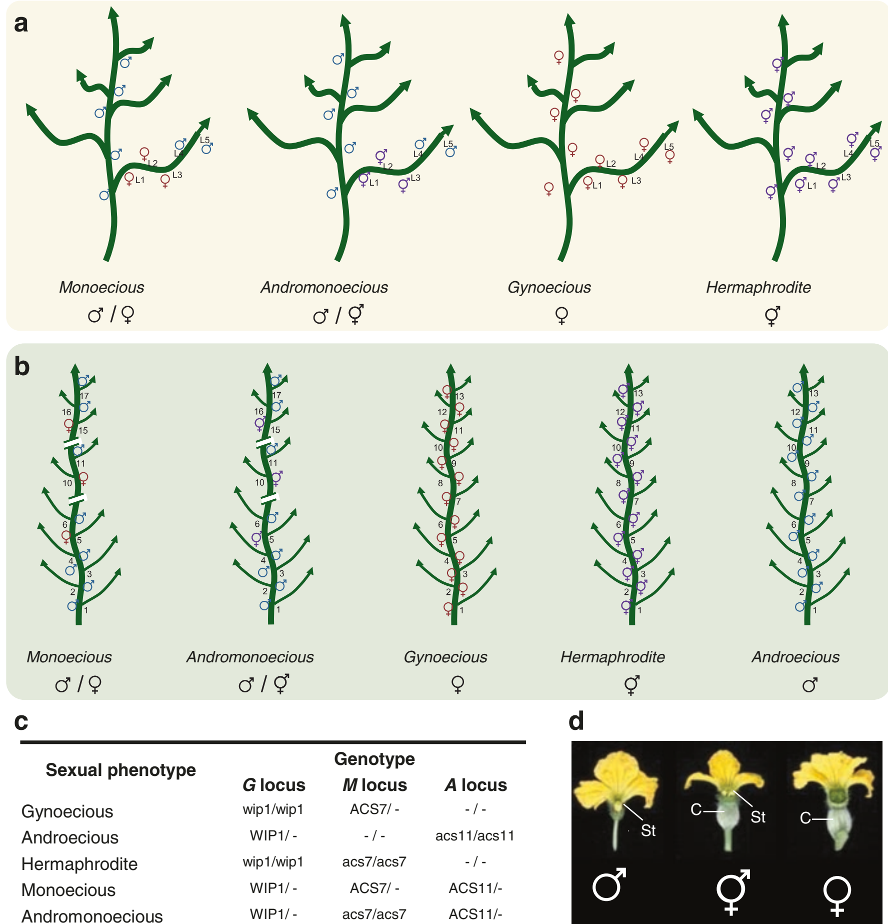
In important members of this family, such as melon and cucumber, sex determination has been evidenced to rely on the developmental arrest of sex-specific organs at early stages of the flower development.

Previous studies in cucumber, have followed flower development and divided this process into 12 different stages. Firstly, the floral meristem is initiated in the axils of leaf primordia (Stage 1). Floral meristem then broadens and leads to the sequential initiation of the sepal, petal, stamen and, finally, carpel primordia (stages 2–5, respectively). From stages 6–12, flower ontogenesis can take two different paths depending on the genotype and developmental fate of the flower (Fig. 1c, d). Bisexual flowers proceed with the primordia differentiation of both stamina and pistils, giving rise to a hermaphrodite or perfect flowers. In contrast, unisexual flowers controls primordia differentiation through the activation or inactivation of genes required for stamen and pistil development, thereby promoting the exclusive formation of female or male imperfect flowers (Bai et al. 2004).

Mechanism of Sex Determination in Cucurbits

It is generally believed that sex determination is mainly regulated by a complex genetic cascade. Frankel and Galun (1977) suggested that transcription of "key" sexual genes can elicit the expression of others placed downstream the cascade, thus promoting, as a final product, the development of pistillate, staminate or hermaphrodite flowers. Under this scenario, it can interpreted that independent from the flower sexuality fate, all the genetic components involved in sex determination are present simultaneously in the genome of all types of flowers. Thus, the selective development of bisexual or unisexual (male or female) flowers relies on different regulation mechanisms that selectively direct the expression of sex determining genes (Frankel and Galun 1977).

The identification of such genes is not an easy task since they are part of complex pathways. Placing the genetic components in the proper position of the pathway is even more difficult; however, once its relation with sex determination is observed, assessing its biological function is usually the first approach. Those biological functions are tightly related with the establishment of cell identity, a process that relies on the integration of internal and external signals. This multifactorial dependence makes sex determination a complex and highly interconnected network. The extensive research on the field have revealed the role of diverse variables such as environmental conditions, gene expression regulation, epigenetics and plant hormone production (Piferrer 2013; Ming et al. 2011).



Sexual phenotype	Genotype		
	G locus	*M* locus	*A* locus
Gynoecious	wip1/wip1	ACS7/ -	- / -
Androecious	WIP1/ -	- / -	acs11/acs11
Hermaphrodite	wip1/wip1	acs7/acs7	- / -
Monoecious	WIP1/ -	ACS7/ -	ACS11/-
Andromonoecious	WIP1/ -	acs7/acs7	ACS11/-

Fig. 1 Schematic representation of sexual morphs and genotypes present in melon and cucumber. (**a**) Sexual morphs in melon. Monoecious melon lines develop male flowers in the main stem. In the lateral branches, female flowers are located in the first three nodes followed by male flowers in the subsequent nodes. Andromonoecious lines present the same distribution but instead of female, hermaphrodite flowers develop. Gynoecious and hermaphrodite lines develop only female and hermaphrodite flowers, respectively. (**b**) Sexual morphs in cucumber. Monoecious lines in cucumber present a sequential distribution starting with the development of male flowers in the first 4 nodes followed by a female flower at the fifth. Andromonoecious lines present the same organization except that hermaphrodite flowers develop instead of female. Gynoecious, androecious and hermaphrodite lines develop only female, male or hermaphrodite flowers, respectively. (**c**) Genotype and sex loci combination of cucumber and melon sexual systems. Minus lines denotes the existence of any allelic variant at their corresponding locus (Adapted from Boualem et al. 2015). (**d**) Male (*left*), hermaphrodite (*middle*) and female (*right*) flowers found in melon. *St* stamina, *C* carpel

In cucurbits, some of these variables have been elucidated and placed in an evolutionary context, thereby providing a well supported genetic system governing sex determination. It has been previously found that flower sexuality in cucurbits is determined by the interplay of three different loci: *androecious (A)*, *gynoecious (G)*

and *monoecious (M)* (Poole and Grimball 1939). The genetic components at the gene level were posteriorly discovered by the integration of co-segregation analyses, cloning techniques and, forward and reverse genetics.

The transition from bisexual floral bud to the development of male or female reproductive structures, is thought to occur as a result of the developmental selective arrest governed by a genetic pathway that includes genes involved in ethylene biosynthesis, transcription factors and epigenetics (Boualem et al. 2008, 2009, 2015; Martin et al. 2009; Ming et al. 2011). The combination and interaction of their corresponding alleles can lead to a variety of sexual phenotypes (Fig. 1c).

Hormones

Recent studies have discovered the contribution of hormones such as ethylene in sex determination (reviewed in Yamasaki et al. 2005; Dellaporta and Calderon-Urrea 1993). Treatments of monoecious melon with ethylene or its precursors lead to production of unisexual female plants. Treatments of gynoecious melon with the ethylene perception inhibitors, like silver nitrate, lead to production of bisexual flowers (Byers et al. 1972; Yin and Quinn 1995).

Consistent with ethylene being a feminizing agent, we previously demonstrated that two different genes, Cm*ACS11* and Cm*ACS7*, play a key role in the development of sex-specific floral organs. Both genes are members of the 1-aminoacyclopr opane-1-carboxylic acid synthase (ACS) gene family, which catalyze important steps of the ethylene biosynthesis pathway (Wang et al. 2002; Boualem et al. 2008, 2015).

The Monoecious Gene

The first discovered regulators were the *ACS7* gene in melon (*CmACS7*) and its homologues in cucumber, Cs*ACS2,* in watermelon, *ClACS7,* and in squash, *CpACS27A* (Boualem et al. 2008, 2009; 2016; Martinez et al. 2014; Ji et al. 2015; Monzano et al. 2016). These genes, commonly known as the *Monoecious (M)* gene, have been observed to be necessary for female flower development in monoecious and gynoecious lines. This concluding remark arose from reverse and forward genetic approaches as well as gene expression profiles, which consistently indicate that the *M* gene acts a stamina inhibitor.

Forward genetics demonstrated that melon andromonoecy and hermaphroditism, in natural populations, is related to a missense mutation that affects the functionality of the protein. This lines, which presents the recessive *m* allele, showed a A57V transition that negatively alters enzymatic activity, since it prevents the formation of hydrogen bonds between the enzyme and the substrate (Boualem et al. 2008, 2009). Solid evidences were obtained through the screening of monoecious genotype *(MMGG)* population of ethyl methane-sulphonate (EMS) mutants. Interestingly, a

missense mutation causing a G19E amino acid change at a highly conserved region of the *M* gene was shown to lead to andromonoecy (Boualem et al. 2008, 2009) (Fig. 2a, b).

Similar mechanisms where posteriorly found in cucumber and watermelon. After the determination of the cucumber and watermelon homologues to the Cm*ACS7* gene in melon, diverse genetic studies showed that the loss of CsACS2/ClACS7 enzymatic activity is the main difference between the *M* and *m* alleles. As in melon, stamen development in hermaphrodite flowers of hermaphrodite and andromonoecious accessions relies on the reduction of the enzymatic activity of CsACS2/ClACS7, thus highlighting its importance in the repression of stamen primordia differentiation during female flower development (Boualem et al. 2009, 2016). As expected, in melon and cucumber, the *ACS7/ACS2* gene is specifically expressed in the carpel primordia of female flowers in gynoecious and monoecious lines at stage 4 when flower sexuality determination is thought to begin (Boualem et al. 2008, 2009, 2016).

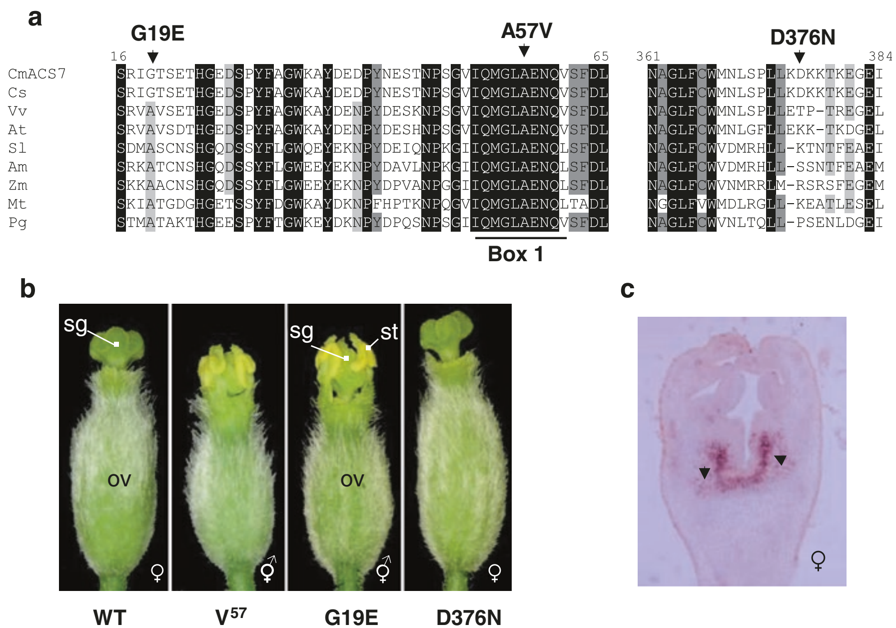


Fig. 2 Functional characterization of CmACS7. (**a**) Multiple sequence alignment of CmACS7 and V57 isoform to homologous proteins from Cs (*Cucumis sativus*), Vv (*Vitis vinifira), At* (*Arabidopsis thaliana), Sl* (*Solanum lycopersicon),* Am (*Antirrhinum majus*), Zm (*Zea mays), Mt. (Medicago truncatula)* and Pg (*Picea glauca).* Conserved domains identified through the alignment are indicated and named as box 1. Position of A57V natural isoform and EMS-induced mutations at *ACS7* are shown above the alignment. (**c**) Melon flower types observed in Monoecious (WT), Andromonoecious (carrying the natural A57V transition), and EMS mutants G19E and D376N. *Sg* stigma, *St* stamina, *Ov* ovary (Modified from Boualem et al. 2008)

The Androecious Gene

CsACS11, *CmACS11* or *androecious* (*a*) gene has been recently proposed to be the key regulator of the genetic pathway. This enzyme has been found to control female flower development by repressing the carpel-inhibitor *CmWIP1* transcription factor. The cloning and sequence analyses of the *CsACS11* gene in cucumber androecious lines have evidenced a non-synonymous nucleotide deletion, Δ843, within exon 3 of *ACS11* (Fig. 3a). This deletion leads to a premature stop codon that abolish the enzymatic activity of the protein (Boualem et al. 2015). Plants from a TILLING (Targeting Induced Local Lesions in Genomes) collection presenting mutations at conserved regions of *ACS11*, suggested that *ACS11*-mediated ethylene biosynthesis

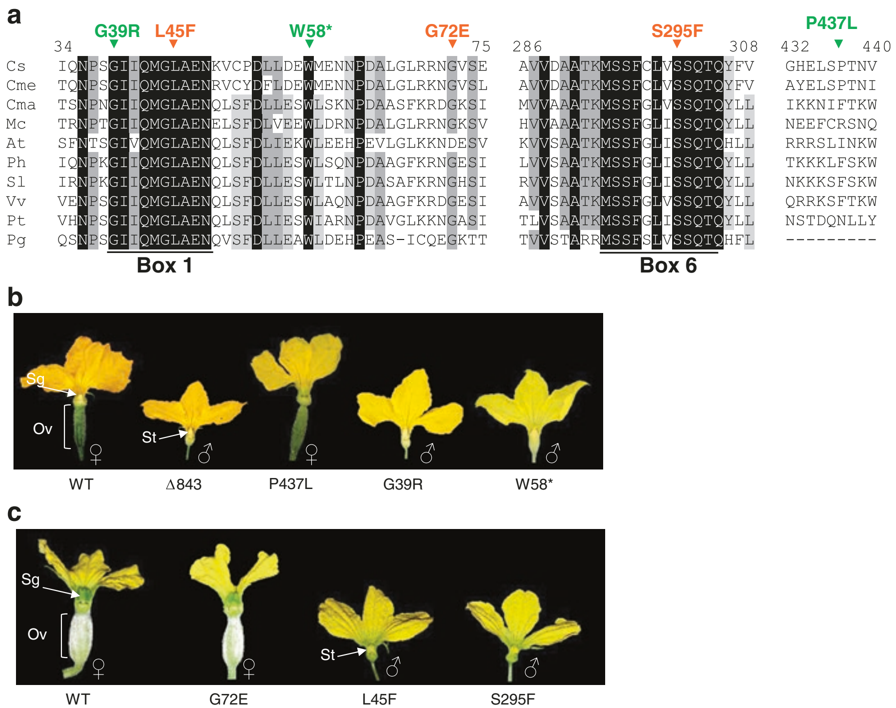


Fig. 3 Functional characterization of ACS11. (**a**) Multiple sequence alignment of CsACS11 (*Cucumis sativus*) to homologous proteins from Cme (*Cucumis melo)*, Cma (*Cucurbita maxima*), Mc (*Momordica charantia),* At (*Arabidopsis thaliana),* Ph (*Ptunia hybrida),* Sl (*Solanum lycopersicum)*, Vv (*Vitis vinifira)*, Pt (*Populus trichocarpa),* and Pg (*Picea glauca).* Conserved domains identified through the alignment are indicated and named as boxes 1 and 6. Genetic modifications of TILLING mutants at *ACS11* are shown in green and orange for cucumber and melon, respectively. (**b**) Cucumber flower types observed in Monoecious (WT), Androecious (carrying the natural deletion D843), and EMS mutants G39R, W58*, and P437L. (**c**) Melon flower types observed in Monoecious (WT) and EMS mutants G72E, L45F, and S295F (Obtained from Boualem et al. 2015)

is required for female flower development in monoecious and gynoecious lines (Fig. 3b, c) (Boualem et al., 2014). Transcriptional analyses in *Cmacs11* mutants showed that *ACS11* and *WIP1* gene expression profiles are antagonistic: *Cmacs11* loss-of-function mutants presented a reactivation of *CmWIP1* expression. The generation and phenotyping of *wip1acs11* double mutants further analyzed this inhibitory interaction. As *Cmwip1* single mutants, the *wip1acs11*double mutant leads to gynoecy, thus suggesting that *CmWIP1* is epistatic to the *ACS11* gene (Boualem et al. 2015).

Transcriptional Regulation

The importance of different members of the transcriptional machinery in sex determination and flower development has been assessed in several species. Studies in different plant models such as *Arabidopsis thaliana* and *Oryza sativa*, have elucidated a bunch of genes directing flower development and organ identity (Guo et al. 2015).

As in animals, homeotic genes are the key components controlling plant ontogenesis; flower development is not an exception and in fact, it comprises numerous regulatory pathways. In flower development, all these genes are part of the ABC model of flowering (Bowman et al. 1989; Coen and Meyerowitz 1991), lately extended into a system composed by five major classes (A, B, C, D and E) of homeotic genes (Angenent and Colombo 1996; Theissen and Saedler 2001). Development of sex-specific organs relies on the combinatorial and differential expression pattern of such homeotic genes along time and space: stamen development relies on the cooperative action of class B, C and E genes (forming AGAMOUS-SEPALLATA-APETALLA3-PISTILLATA complex), whereas carpel and ovule identity depends on the expression of C, D and E genes (forming AGAMOUS-SEPALLATA and SEPALLATA-SEEDSTICK complexes for carpel an ovule development, respectively) (Bowman et al. 2012; Guo et al. 2015).

Considering previous studies on *AGAMOUS* homeotic gene and its relation to flower development (Yanofsky et al. 1990), the characterization of stamen- and pistil-specific transcriptional regulators is a crucial step towards the understanding of sex determination. Despite of their importance, a great part of the current knowledge comes from *Arabidopsis* and, in cucurbits most of the molecular pathways they trigger are still unknown.

The Gynoecious Gene

Despite of the unresolved aspects mentioned above, the regulation of flower sexuality at the transcriptional level in cucurbits has been attributed to *CmWIP1*, a zinc-finger-type transcription factor that, when expressed, is able to promote carpel

abortion and thereby, the development of unisexual male flowers. *CmWIP1* is mainly expressed in the carpel primordia of male flowers at stage 6, when the developmental arrest of inappropriate sexual organs occurs. Expression level of the *CmWIP1* gene is significantly low in flower buds committed to be female, thus suggesting the existence of upstream mechanisms responsible for regulating *CmWIP1* expression and carpel inhibition (Martin et al. 2009). Loss-of-function mutants of *CmWIP1* obtained through the TILLING approach showed a gynoecious phenotype with a complete feminization of male flowers from monoecious lines (Martin et al. 2009) (Fig. 4a).

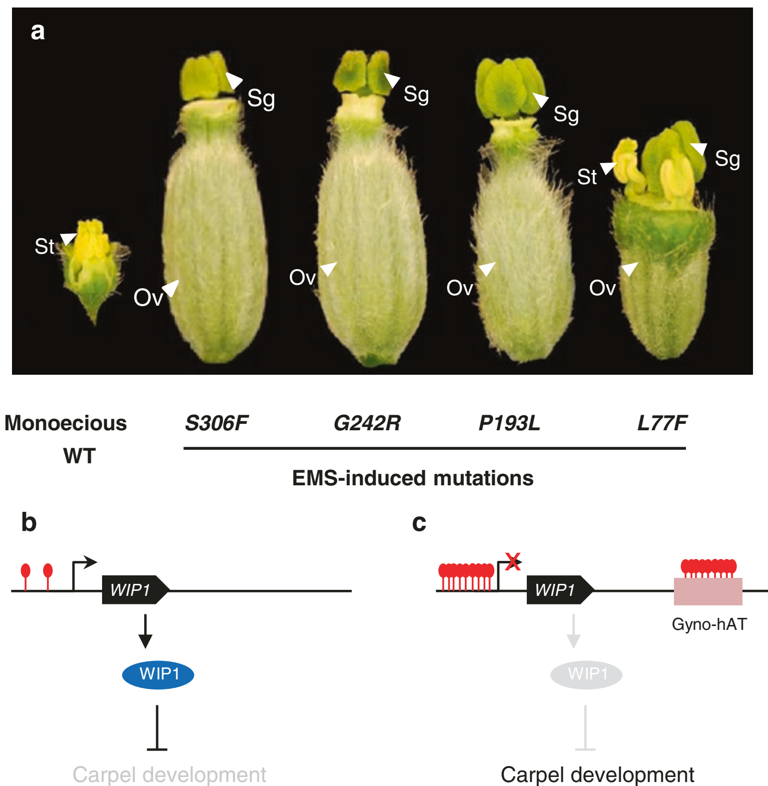


Fig. 4 Functional characterization of Cm***WIP1***. (**a**) Melon flower phenotypes observed in Monoecious (WT) and EMS mutants S309F, G242R, P193L, L77F (Obtained from Martin et al. 2009). (**b**, **c**) Representation of the epigenetic events behind the regulation of the *CmWIP1* gene. (**b**) Cm*WIP1* transcription factor gene is expressed in the early stages of flower development to block the carpel development, leading to the formation of a male flower. (**c**) A *hAT* transposon, called *GynohAT*, is inserted close to the Cm*WIP1* locus. The epigenetic control exerted on this transposable element leads to hypermethylation of the Cm*WIP1* promoter, inhibiting its expression, allowing carpel to develop and the formation a female flower. Red lines and balls in *GynohAT* or Cm*WIP1* promoter indicate cytosine methylation in a CGN context

Epigenetic Regulation

Several studies on animals have reported the importance of different epigenetic processes involved in gene dosage compensation, sex determination and development of reproductive structures (Werren and Beukeboom 1998; Fraser and Heitman 2005; Brockdorff 2011; Piferrer 2013).

In melon, the epigenetic mechanism behind carpel formation corresponds to a *Gyno-hAT* transposon insertion necessary for the initiation and maintenance of DNA methylation at the promoter region of *CmWIP1.* Sequence analyses of a collection composed by 497 different accessions demonstrated that the insertion of the *Gyno-hAT* transposon occurs exclusively in gynoecious and hermaphrodite lines. This insertion has been proved to correlate with an increased DNA methylation spreading in gynoecious lines when compared with DNA methylation levels in monoecious plants (Martin et al. 2009) (Fig. 4b, c).

DNA methylation and transposon insertion have been, so far, the main epigenetic processes involved in sex determination of melon. Nevertheless, the roles of additional epigenetic changes at both gene and genome-wide levels are still unknown.

A, M, G Molecular Interaction and Evolution of Cucurbit Sexual Systems

A well-structured and fundamented model was proposed after integrating the genetic and epigenetic aspects of sex determination in cucurbits: the expression of the carpel inhibiting *WIP1* depends on the non-expression of *ACS11*, and the expression of the *ACS7* relies on the ACS11-mediated repression of *WIP* (Boualem et al. 2015) (Fig. 5). According to this model, flower sexuality developed from modifications of a sexual system composed by the same sex-determinant genes. These modifications reside on the differential expression of functional and nonfunctional (allelic variants) proteins codified by the *A, M* and *G* genes. Hermaphrodite lines arose from the simultaneous inactivation of stamen, *ACS7*, and carpel, *WIP1*, inhibitors (Fig. 5b). Meanwhile, male flower development in monoecious and andromonoecious lines results from the non-expression of *ACS11* thus, *WIP1* gene is expressed and it can repress carpel development and *ACS7* expression (Fig. 5a). In contrast, female flower development in monoecious plants is promoted by the expression of *ACS11*, which represses *WIP1* expression and allows the expression of *ACS7* (Fig. 5c). The andromonoecious phenotype, emerges from the expression of a non-functional *ACS7* isoform giving rise to hermaphrodite flowers instead of female (Boualem et al. 2015) (Fig. 5b).

Conventionally, dioecy in angiosperms is believed to arise from monoecy. Male and female sterile mutations are thought to mediate the transition from monoecy to dioecy. The genetic components regulating sex determination in cucurbits seems to follow this evolutionary pathway. The latter conclusion derived from two consecutive crosses of female (*wip1/wip1, acs11/acs11*) and male (*WIP1/wip1, acs11/acs11*)

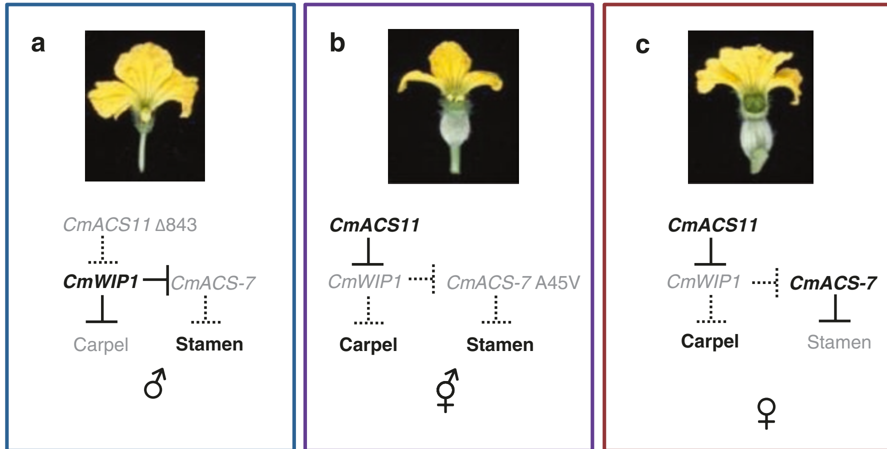


Fig. 5 Sex determination pathway in Cucumis species. Genetic pathway leading to the development of male (**a**), hermaphrodite (**b**), and female flowers (**c**) (Adapted from Boualem et al. 2015)

plants. This segregation analyses showed that a combination of genes regulating monoecy can lead to the emergence of dioecy (Boualem et al. 2015).

Conclusions and Future Perspectives

The cloning and exhaustive characterization of sex determining genes in the *Cucurbitaceae* family has provided the scientific community with solid evidences of how monoecy and dioecy developed in an evolutionary context. Both genetic and epigenetic factors are influencing the gene expression pattern and inhibiting activities of the members belonging to the genetic pathway of different sexual systems.

Even though an important part of the pathway is elucidated; the events occurring downstream are still unknown. Robust evidences have shown the importance of homeotic genes in flower ontogenesis and their regulation by several mechanisms. Assessing the targets of both *G* and *M* genes would link sex determination with flower ontogenesis, both processes being of great importance for crop improvement.

Molecular characterization of *WIP* transcription factors family have shown its high potential of forming complexes with other proteins (Appelhagen et al. 2010), however, the nature and biological function of such proteins remain poorly characterized in cucurbits; and, its relation with sex determination is even more nebulous.

Since unisexuality is present at the flower (monoecious) and individual (dioecious) level, it would be of great interest to study the spatial regulation of sex determining genes. This implies the study of epigenetic events involved in cell identity and determination of sex-specific floral organs. It is well known that histone modi-

fiers, remodeling complexes and other epigenetic players such as ncRNAs can target homeotic genes and fine-tune their expression (Guo et al. 2015).

Taking into consideration the technical advances available in the present and near future, forthcoming studies can be optimized by the use of specific cell populations; the determination of their gene expression regulation, before and after the establishment of their fate, will solve pieces in the puzzle that cannot be explain by classical genetics. Assessing the missing pieces of the puzzle can contribute to an integrative perspective of crop improvement, where different genetic or exogenous manipulations on plants genome and epigenome can increase the maintenance and inheritance of key phenotypes required for the high quality and quantity of production in an auto sustainable manner, which nowadays seems to be a suitable strategy to fight against climate and demographic abrupt changes occurring in our present and future.

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Genomic Analysis of Cucurbit Fruit Growth

Rebecca Grumet and Marivi Colle

Abstract Fruit development in cucurbit species follows the canonical progression of ovary development, fruit set, expansive fruit growth, and maturation and ripening. This commonality, however, belies tremendous morphological diversity. Variation in timing, amount, and orientation of cell division and cell expansion pre- and post-anthesis, as well as factors influencing carpel number, floral sex, photosynthetic capacity and trichome development all drive extreme variability in fruit size and shape. New genomic approaches utilizing recently assembled draft genomes for the four major cucurbit crop species (*Cucumis sativus*, *Cucumis melo*, *Citrullus lanatus*, *Cucurbita spp*), next generation high throughput sequencing, molecular mapping methods, transcriptomic analyses, gene cloning, and transgenic approaches are all contributing to an increased understanding of the key processes underlying cucurbit fruit development. Extensive quantitative trait locus (QTL) analyses have identified numerous QTL for features such as ovary length, width, and shape, and fruit length, width, shape, flesh thickness and cavity diameter. Most recently, multipronged approaches combining mapping, sequence, and transcriptional analyses have allowed for identification specific candidate genes influencing cucurbit fruit morphology.

Keywords Cell division • Cell expansion • Cucurbitaceae • Fruit development • Fruit shape • Fruit size • Ovary • QTL • Transcriptome

Introduction

Cucurbit fruits – fantastical in shape and legendary in size – have well earned their place in mythology and lore. Pumpkin contests produce individual fruits exceeding one ton in weight while wings, warts, ridges and spines adorn

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squashes, melons and cucumbers. Not surprisingly, fruit size and shape is a defining feature classifying different classes of cucurbits. For example, melons (*Cucumis melo*) are classified into eleven cultivated types, such as *conomon*, *monmoridica, flexousus*, *cantalupensis*, and *dudaim*, each of which exhibits typical size and shape characteristics (Monforte et al. 2014; Pitrat 2016). Similarly, *Cucurbita pepo* subspecies *pepo* is classified into eight morphologically distinct cultivar groups including members with round (pumpkin), elongated (crookneck, straightneck, cocozelle, vegetable marrow and zucchini) and complex (scallop, acorn) fruit shapes (Chap. 6, Paris 2016). Archeological and anthropological evidence suggests that the main driver of the extreme phenotypic variation observed in *Cucurbita* has been human intervention, as wild *Cucurbita* fruits are typically small and round (Chap. 6, Paris 2016).

In this chapter we will explore the genetic basis driving morphological variability in cucurbit fruit as is now beginning to be elucidated using genomic approaches. Fruit development is classically characterized as proceeding through four stages: ovary development; fruit set; expansive fruit growth; and maturation and ripening (Gillaspy et al. 1993; Okello et al. 2015). Cucurbit fruits also follow this canonical progression (Monforte et al. 2014). Fruit size and shape are established during the first three stages of development (ovary development, fruit set and expansive fruit growth), resulting from a combination of factors occurring pre-anthesis in the developing ovary of the female flowers and post-anthesis in the growing fruit. The primary drivers of growth and morphology are timing, amount, and orientation of cell division and expansion, and the factors that regulate those processes.

Ovary Development

Members of the Cucurbitaceae family are characterized by an enlarged, inferior ovary that exhibits distinctive shape in the developing flower bud prior to anthesis (Fig. 1) (Goffinet 1990; Robinson and Decker-Walters 1997). Distinctive ovary shape, which was documented nearly a century ago in a series of classical works by Sinnott (e.g., 1936), indicates that fruits are shaped by critical factors acting early in floral development. Recent analyses of segregating populations of melon and cucumber have shown strong correlations between shape of the ovary and subsequent shape of the fruit, and that shape features, which are highly heritable, are largely independent of environmental influence (e.g., Perin et al. 2002; Ramamurthy and Waters 2015; Weng et al. 2015). Consistent with the defining action of ovary development, quantitative trait locus (QTL) analyses have found co-localization of major QTL for ovary length, width and shape with fruit length, width and shape at several loci in melon (Perin et al. 2002), and for ovary length and diameter with mature fruit length and diameter in cucumber (e.g., Weng et al. 2015).

Fig. 1 Cucurbit ovaries exhibit distinct shapes consistent with final fruit shape. Clockwise from top left: Chinese long and picking cucumber (*Cucumis sativus*); scallop squash (*Cucurbita. pepo*), acorn squash (*C. pepo*), pumpkin (*C. pepo*); melon (*Cucumis melo*)

Role of Cell Division in Establishing Fruit Shape and Size in the Developing Ovary

Cell division pre-anthesis influences fruit size, as the number of cells in the ovary pericarp at anthesis serve as the basis for succeeding cell divisions upon pollination (Bohner and Bangerth 1988). Some of the first published studies of ovary development in cucurbits found that early ovary growth is due to an increase in cell number, and that ovaries of larger fruited pumpkin (*Cucurbita pepo*) had a larger number of cells that were approximately the same size as cells in ovaries of smaller fruited pumpkins (Sinnott 1939). Similarly, cell count analysis in cucumber showed that

increase in ovary size pre-anthesis is primarily driven by cell division (Colle 2015). During the period from 7 days pre-anthesis to anthesis, cucumber ovaries showed an seven to eightfold increase in cell number in the longitudinal axis, while cell size remained largely constant. Cell number differences influencing fruit shape also were evident at this stage. Cultivars differing in fruit length had two to threefold differences in ovary cell number along the longitunidal axis as early as 7 days pre-anthesis, with increasing differences in cell number as flowers approached anthesis (Colle 2015).

Cell proliferation is regulated by a number of factors including cyclins, cyclin-dependent kinases (CDKs), and CDK inhibitors, that control phases of the cell cycle (G1, S, G2, and M) (Inze and De Veylder 2006). Transcriptomic analysis comparing near-isogenic cucumber lines differing in fruit length at 4 days pre-anthesis showed increased expression of four cyclin genes in the long-fruited line relative to a shorter fruited line (Jiang et al. 2015). Consistent with differences in cell division, the GO term most enriched for differences between the two lines was 'microtubule-based movement' (Jiang et al. 2015). Microtubules play a critical role in mitosis by facilitating alignment of chromosomes at the spindle equator (Ganguly and Dixit 2013). Mitotic kinesins, in turn, play critical roles in chromosome organization during mitosis via regulation of cytoskeleton microtubule dynamics (Ganguly and Dixit 2013). Variation in expression levels of kinesins, which are subject to regulation via CDK-mediated phosphorylation, also was also observed in developing cucumber ovaries in correlation with differences in cell division and fruit size (Yang et al. 2013).

In tomato, the best studied model for fleshy fruit development, several genes regulating fruit shape and size have been identified including, *Fw2.2*, *Fw3.2* (*SlKLUH*), *OVATE*, *SUN*, *Slelf1*, *FAS* and *LC* (van der Knapp et al. 2014). As in cucurbits, shape and size differences are apparent at anthesis, indicating action early in ovary development. *Slelf1*, *OVATE*, *SUN*, *LC*, and *FAS*, have been implicated to act during floral meristem formation or gynoecium initiation (van der Knapp et al. 2014; Wu et al. 2015; Xu et al. 2015a, b). Not surprisingly, several of the tomato fruit size and shape genes affect amount or orientation of cell division. *Fw2.2*, a member of the Cell Number Regulator family, and *Fw3.2/SlKLUH*, a CYP78A cytochrome P450 enzyme, affect overall fruit size by regulating cell number (Chakrabarti et al. 2013; Guo and Simmons 2011). *Slelf1* causes elongated fruit (elf) by an increase in cell layer in the proximal region of the ovary (Chusreeaeom et al. 2014), while mutation within the *OVATE* gene, a transcriptional repressor that has been implicated in both cell division and cell elongation, causes a transition from round to pear-shaped fruit (Huang et al. 2013; Liu et al. 2002; Wang et al. 2011; Wu et al. 2015). Alleles of the *SUN* gene, which cause elongated fruit, encode a protein containing the IQ67 calmodulin binding domain that influences the direction of cell division; *SUN* appears to act both pre- and post-anthesis (Huang et al. 2013; Tanksley 2004; Wu et al. 2011, 2015; Xiao et al. 2008). Fruit shape and size are also influenced by genes modulating locule (carpel) number, a feature established during floral meristem development. Locule number (*LC*) is an ortholog of *WUSCHEL*, likely influencing meristem size and floral organ number (Munos et al.

2011; Rodriguez et al. 2011), while *FASCIATED* (*FAS*) is a member of the CLAVATA receptor complex family (*SlCLV3*) associated with enlarged meristems (Cong et al. 2008; Huang et al. 2013; Huang and van der Knaap 2011; Rodriguez et al. 2011; Xu et al. 2015a, b).

Recently completed cucurbit genome sequences allow identification of homologs of the various tomato fruit size and shape genes. The tomato fruit size and shape genes are members of large gene families, and multiple family members also have been identified in the melon and cucumber genomes (Monforte et al. 2014; Weng et al. 2015). In melon, 24 members of the *SUN* family (*CmSUN*), 21 of the ovate family proteins, *OFP* (*CmOFP*), four of the *CmCLV3*, nine of the cell number regulator *CNR* (*CmCNR*), five of the *KLUH*/*CYP78A* (*CmCYP78A*), and ten of the *WOX* (*CmWOX*) families have been identified (Monforte et al. 2014). All of the 74 melon homologs also mapped to the cucumber genome (Bo et al. 2015; Weng et al. 2015). The homologs of fruit size and shape genes are distributed over all the twelve of the melon chromosomes and all seven of the cucumber chromosomes. Similar to members of fruit size and shape gene families in tomato (Huang et al. 2013), several homologs in melon and cucumber also appear to cluster or are located close to each within specific chromosomal regions (Monforte et al. 2014; Weng et al. 2015). For example, cucumber chromosome six contains a cluster including homologs of *OVATE*, *SUN*, and *FAS* (Weng et al. 2015). Other clusters occur on chromosomes 1, 3, and 5. In addition, three members of the *OVATE* family are located within a small region of cucumber chromosome 1. Whether these homologs of tomato fruit size and shape genes play similar roles in cucurbit fruit development remains to be determined. Intriguingly, several of these gene family members have been located in regions associated with fruit shape and size QTL in cucumber and melon (Colle 2015; Monforte et al. 2014; Weng et al. 2015).

Carpel Number, Floral Sex Expression, and Ovule Number

Among the floral and ovary development factors that can influence cucurbit fruit morphology are carpel number, floral sex expression, and ovule number. Carpel or locule number is an important factor influencing fruit size in several species, including tomato (Monforte et al. 2014; van der Knapp et al. 2014). Cucurbit ovaries are typically composed of three or five fused carpels, a feature which is established during early floral organ differentiation (Goffinet 1990, Robinson and Decker Walters 1997). Alleles of the *Pentamerous* (*p*) gene in melon specify production of three vs. five carpels (Perin et al. 2002). Not surprisingly, in a segregating melon population of a cross between a three-carpel, round-fruited Vedrantais melon, and a five-carpel oblong-fruited PI, QTL for ovary length, width, and shape, and fruit length, width, and shape, were found close to the *p* locus on chromosome 12 (Perin et al. 2002). This region, however, does not appear to contain homologs of the known tomato carpel-regulating genes, *FAS* or *LC*, suggesting a novel mechanism influencing establishment of carpel number in melon (Monforte et al. 2014).

Although carpel number can influence cucurbit fruit shape, it should be noted that is possible to have extreme variation in fruit shape in the absence of variation in carpel number, as can be observed between round *cantalupensis* melons and highly elongate 'snake melons' of the *flexuousus* group, both of which possess three carpels (Ramamurthy and Waters 2015). Indeed, despite extensive variation for fruit shape among melon groups and varieties (Paris 2016, Chap. 4), very few have five carpels. Extreme differences in size also can occur in the absence of differences in carpel number. Comparison of locule number of three pumpkin types (*Cucurbita maxima*) derived from 'Hubbard' showed that 'Hubbard' averaged three locules, while the larger fruited 'Mammoth' and 'Atlantic Giant' typically had four locules (Savage et al. 2015). Although this observation suggests that locule number contributes to increased size, enormous differences in size also occur between the two four-locule types. 'Mammoth' has a record weight of 183 kg, while 'Atlantic Giant' is the variety that produces world-record size 1000 kg pumpkins.

Sex type of the carpel-bearing flowers, i.e., female vs. bisexual, can also influence fruit shape. The major floral sex expression genes, *A* and *G* in melon, and *F* and *M* in cucumber, also can influence fruit shape. *F* in cucumber and *A* in melon suppress stamens in carpel-bearing flowers, resulting in female rather than bisexual flowers (Grumet and Taft 2011; Perl-Treves 1999). Elongated ovaries and fruits are frequently associated with female flowers while rounder ovaries are associated with bisexual flowers in *Cucumis* and *Cucurbita* (e.g., Iezzoni et al. 1982; Peterson et al. 1983; Pitrat 2013; Wall 1967; Manzano et al. 2011). Application of ethylene increases femaleness as evidenced by increased production of carpel-bearing flowers and/or conversion of bisexual to male flowers (Grumet and Taft 2011; Perl-Treves 1999). The cucumber *F* and *M* genes and melon *A* all encode ethylene production enzymes, and expression of melon *G*, encoding the transcription factor, wound inducible protein 1 (WIP1), is regulated by ethylene production (Boualem et al. 2008, 2009, 2015; Martin et al. 2009; Switzenberg et al. 2014; Trebitsh et al. 1997). Transgenic studies modulating ethylene perception and production in developing floral primordia of melon also have shown that ethylene can influence ovary and fruit shape (Switzenberg et al. 2015). Transgenic melons exhibiting increased ethylene production and conversion of bisexual to female flower buds, exhibited elongated ovaries and fruit relative to their non-transgenic counterparts. However, the relationship between ethylene, floral sex, and shape in cucurbits remains to be resolved. A new allele of the *M* locus of cucumber, *m-1*, has been recently identified that results in hermaphrodite flowers with elongated fruit, and in progeny of *m* and *m-1* plants, variable fruit shape was observed on a single plant (Tan et al. 2015). Similarly, elongated ovaries and fruit were observed in the transgenic ethylene overproducing melon plants, whether they formed female or bisexual flowers (Switzenberg et al. 2015). These results suggest transient balance of hormones at different points in development can influence fruit shape independent of floral sex, and that fruit shape does not result from pleiotropic effects of accommodating the stamen whorl during floral development. Elongated fruit shape also occurs in the non-ripening, ethylene perception inhibition mutant of tomato, *Never-ripe 2* (Barry et al. 2005), further suggesting that ethylene can play a role in regulation of fruit

growth. Earlier and higher expression of the ethylene response factor, *CsERF3*, was observed during the exponential growth stage of 'Chinese Long 9930' cucumber relative to the short pickling type cucumber, 'GY14' (Colle 2015), both of which produce female flowers. *CsERF3* is located within a fruit length QTL *mfl3.1* identified by Bo et al. (2015) and adjacent to *FS3.1* of Weng et al. (2015).

Ovule number, which ultimately delineates maximum seed number, is an additional ovary- determined factor that can influence fruit size and shape. In the *Cucumis* species, melon and cucumber, seeds are arranged within the fruit along the longitunidal axis. A QTL located on linkage group eight in melon drove both increased fruit length and increased seed number seed number (Fernandez-Silva et al. 2009). In cucumber, ovule or seed number also was positively correlated with ovary and fruit length, and QTL for ovule/seed number in cucumber co-localized with QTL for fruit length in cucumber on chromosomes 1 and 6 (Weng et al. 2015). At this time we can cannot distinguish whether increased ovule number drives increased ovary length, or if increased ovule number results from the additional space provided by increased ovary length. In various *Cucurbita pepo* fruit types (pumpkin, vegetable marrow, zucchini, and cocozelle) increasing seed number was generally correlated with increased fruit size as measured by weight, although genetic drivers for seed number difference were not identified, and may reflect pollination success rather than ovule number (Nerson 2005). It will be of interest to determine whether orthologous genes influence ovule number along the longitudinal axis in both melon and cucumber, especially as the location of the ovule number QTL on cucumber chromosome 6 (Weng et al. 2015) and melon chromosome 8 (Eduardo et al. 2007; Fernandez-Silva et al. 2009) fall in an region of broad synteny between the two species (Huang et al. 2009).

Fruit Growth Post-anthesis

Fruit growth post-anthesis results from a combination of cell division and cell expansion (Gillaspy et al. 1993). Cell division typically predominates during the first few days post-pollination with cell expansion subsequently playing the predominant role during exponential fruit growth. This pattern of rapid cell division during early fruit growth followed by a period of cell expansion has been observed in cucumber, melon, watermelon, and pumpkin (Boonkorkaew et al. 2008; Fu et al. 2008; Hu et al. 2011; Monforte et al. 2014; Wechter et al. 2008). For example, in cucumber, peak cell division typically occurs during the first 4–5 days post pollination and subsequent rapid fruit expansion is largely completed by 2 weeks post-pollination (Ando and Grumet 2010; Boonkorkaew et al. 2008; Marcelis and Hofman-Eijer 1993). In melon the peak division lasted for 5–10 days and maximal expansion completed in 2–3 weeks (Higashi et al. 1999; Monforte et al. 2014).

While both cell number and cell size can influence fruit size, their relative roles can vary. In melon, the length of the period of cell division following pollination was shown to be a key factor influencing final fruit size, with the major difference

in size resulting from final cell number in the pericarp tissue (Higashi et al. 1999). Cucumbers that differed in fruit shape also showed differences in rate and duration of cell division, leading to differences in cell number in the longitudinal versus transverse direction (Colle 2015). Length of the period of fruit expansion also can vary. Peak expansion for the melon cultivar ‘Piel de Sapo’ occurred between approximately 4 and 14 days post pollination, although in large fruited cultivars, growth may continue until harvest (Monforte et al. 2014). Variation in fruit size in different cucumber varieties also resulted from differences in both cell number and cell size (Jiang et al. 2015; Yang et al. 2013). Perhaps most dramatically, growth achieved by giant pumpkins (*Cucurbita maxima*), is manifested as an increase in both the period of cell division and cell expansion (Hu et al. 2011; Nakata et al. 2012). Cell division in pumpkin fruits ranging in size from 320 to 640 kg, continued for as long as 10–20 days post pollination (a time when fruit expansion is largely complete in cucumber and melon), while exponential increase in pumpkin circumference continued until approximately 50 days, and increase in weight until approximately 60 dpp (Hu et al. 2011). Cell volume in giant pumpkins was estimated to be approximately eightfold larger than in standard pumpkin (Nakata et al. 2012).

Regulation of Cell Division

As would be expected for a period of rapid cell division, transcriptomic analysis of developing cucumber fruit showed significantly increased representation of genes associated with cell organization and biogenesis, and DNA or RNA metabolism at 0 and 4 days post pollination (Ando et al. 2012). The period of rapid cell division was accompanied by increased peak expression of the cell cycle genes, cyclins and cyclin-dependent kinases (Cui et al. 2014; Fu et al. 2008), and a large number of cyclin- and cyclin dependent kinase-related gene family members were nearly exclusively expressed (>90 % of transcript reads) in samples at 0 and 4 dpp relative to 8, 12 and 16 dpp time points (Ando et al. 2012). Cyclin and CDK genes are distributed among the cucumber chromosomes (Cui et al. 2014), including some located in QTL regions associated with fruit size on chromosomes 1, 3, 4, 5 and 6 (Colle 2015). The conserved kinesin motor domain was identified in 47 genes in the cucumber genome, ten of which showed peak expression during the phase of rapid cell division 1–3 dpp (Yang et al. 2013). Comparison of cucumber lines with different fruit size showed differences in kinesin gene expression corresponding with differences in cell number during the period of rapid cell division in the first few days post-anthesis. While it is anticipated that such cell cycle-related genes would exhibit increased expression during rapid cell division, factors regulating the cell cycle genes in cucurbit fruits remain to be determined.

In many fruit tissues, endoreduplication, chromosome multiplication in the absence of cell division, is also associated with early fruit growth (Okello et al. 2015). This has been documented in tomato, where increased cell DNA content is correlated with increased cell size (e.g., Cheniclet et al. 2005). Similarly, ovary cells

of developing cucumber fruit exhibit increased polyploidy from predominatly diploid at anthesis, to majority tetraploid at 2–4 days post-anthesis (Fu et al. 2010). By 6–8 days post anthesis, concomitant with increased cell size, nuclear content of approximately 80 % of the ovary cells was either 4C, 8C or 16C. In apple, increased ploidy through endoreduplication was considered a contributing factor to variation in fruit size (Malladi and Hirst 2010); however, whether endoreduplication plays a role in variation in cucurbit fruit size is not clear at this time. Examination of a wide range of *Cucurbita pepo* and *Cucurbita maxima* species did not find a correlation between fruit size and nuclear content (Tatum et al. 2006). Despite the very large cell size in giant pumpkins, there was not a significant difference in nuclear content relative to cells from standard size pumpkins (Nakata et al. 2012). In watermelon, autotetraploidy was not associated with larger fruit size relative to diploids (Davis et al. 2013), and transgenic tetraploid melons produced smaller fruits than their diploid counterparts (Papadopoulou et al. 2005). Thus, while limited information is available about ploidy level in cucurbit fruit tissue, and there may be examples where endoreduplication plays a role in enhanced fruit cell size and growth, these observations suggest it may not be a widespread component causing increased cucurbit fruit size.

Factors Affecting Cell Expansion

Amount and orientation of cell division lays the framework for fruit shape and size, but it is cell expansion that is ultimately responsible for dramatic increases in fruit size. Okello et al. (2015) argue that cell expansion is often underestimated as a factor driving fruit growth. Sinnott (1939) found that, in watermelon, fruit size is primarily driven by enormous increase in cell size, with final cell volume approximately 350,000-fold greater than meristematic cell volume. Less dramatically, a small-fruited cucumber cultivar, ‘1971 B-2’, showed only threefold increase in cell size between 5 and 16 dpp vs. 30-fold for several other varieties (Yang et al. 2013). Studies with cucumber also showed that in conditions where assimilates were not limiting, duration of the period of cell expansion could be increased (Marcelis and Hofman-Eijer 1993), and that low cell number in early fruit development could be compensated for by an increase in cell size later in development (Marcelis 1993).

At a mechanical level, the primary driver of cell expansion is turgor pressure leading to loosening of the cell wall accompanied by deposition of new cell wall material (Guerriero et al. 2014; Okello et al. 2015). Extensive expression of cell wall-related genes has been observed during expansive growth in watermelon and cucumber (Ando and Grumet 2010; Guo et al. 2011; Wechter et al. 2008). Among the transcripts highly expressed at this stage are genes that are annotated to promote cell loosening and expansion, production of and cross-linking of cell wall structural components and cellular adhesion, such as extensins, expansins, cellulose synthases, pectin modifying genes, arabinogalactan proteins, and xyloglucan endotransglosylases.

The uptake of water necessary to create turgor pressure stimulating cell expansion is driven by reduced solute potential (i.e., accumulation of photosynthates, primarily sucrose) within the cell. Thus optimal growth depends on sufficient availability of both water and photoassimilate. The parent plant must be able to both produce and transport the necessary photosynthate in response to the demands of the sink activity of the developing fruit. Numerous reports have shown the importance of adequate photoassimilate as evidenced by effects of loss of photosynthetic tissue due to disease, insect damage, or experimental defoliation. Analyses of competition between fruit on the same vine have shown that for cucumber, approximately ten leaves are needed to provide full fruit growth (Zhang et al. 2015). Studies of giant pumpkins have varied in their conclusions regarding the role of variation in leaf tissue in driving fruit size. Larger and thicker leaves were observed for 'Atlantic Giant' vs. a pumpkin variety with standard size fruit (Nakata et al. 2012), while comparison of progenitor 'Hubbard' squashes or smaller fruited pumpkins with 'Atlantic Giant' did not observe significant differences in primary photosynthetic capacity, as measured by total plant leaf area or photosynthetic rate (Savage et al. 2015). Although adequate production of photosynthate by leaf source tissue is clearly critical for fruit growth, and leaf size, number and arrangement, are under genetic control, capacity for photosynthetic productivity will not be examined in this chapter.

The comparison of 'Hubbard' squashes, smaller fruited pumpkins, and 'Atlantic Giant' pumpkins suggested that increased fruit growth resulted from differential partitioning of photosynthate. One of the largest differences between the giant pumpkins and Hubbard squashes was phloem size and structure, suggesting importance of increased capacity to transport photoassimilate into the growing fruit tissue (Savage et al. 2015). In the giant pumpkins a greater proportion of the cross section of petioles and pedicels was devoted to phloem tissue. Cucurbit species are characterized by a unique and functionally divergent network of extrafascicular phloem external to the vascular bundles and dispersed throughout the cortex of the stem and peduncle, as described more fully in Chap. 16 (Turgeon 2016). They employ a symplastic sugar loading strategy facilitated by specialized phloem companion intermediary cells with highly branched plasmodesmata connecting to adjacent bundle sheath cells (Liesche and Schulz 2013; Rennie and Turgeon 2009).

Studies of cucumber growth showed elevated expression of cucurbit specific phloem proteins during exponential growth (Ando et al. 2012). Highly expressed proteinaceous phloem filaments, comprised of the cucurbit specific PP1 proteins, and the more widely distributed PP2 phloem lectin proteins (Dinant et al. 2003), are primarily associated with the extrafasicular phloem (Zhang et al. 2010). Strong expression of phloem protein genes during rapid growth has been observed in other studies, including PP1 expression in green stage watermelon fruit (Dannenhoffer et al. 1997; Dinant et al. 2003; Wechter et al. 2008). Furthermore, expression of genes associated with phloem unloading, including raffinose family oligosaccharide transporters, and acidic alpha-galactosidase enzymes associated with hydroysis of stachyose to sucrose, increased in peduncles of developing cucumber fruit as they as they achieved exponential growth (Cheng et al. 2015; Zhang et al. 2015).

Availability of the full genome sequence of cucumber has contributed to understanding of the phloem unloading process in fruit by facilitating the identification and verification of activity of raffinose family oligosaccharide transporters (Cheng et al. 2015).

Along with sugar transport, uptake of water is essential for fruit growth. Increase in cell size is typically accompanied by extensive vacuolization as has been observed in mesocarp cells of watermelon and cucumber (Ando and Grumet 2010; Wechter et al. 2008). Aquaporins are a class of proteins that facilitate transport of water across cellular membranes (Maurel et al. 2015). The two largest sub-families of aquaporins in plants are plasma membrane intrinsic proteins (PIPs) for uptake into the cell, and tonoplast intrinsic proteins (TIPs) for uptake into the vacuole. Cucumber PIP family members exhibited both tissue- and developmental-specific gene expression (Shi et al. 2015). During a time course of cucumber fruit development, different PIPs showed a variety of patterns of expression, including oscillating transcript levels (Shi et al. 2015). Several genes had peaks of expression occurring at anthesis while others peaked during exponential growth. The majority had very low levels of expression during the first sample time post-anthesis, presumably when most cells were actively dividing rather than expanding. In addition to the PIPs, two highly expressed TIPs also showed peak expression during exponential growth (Ando et al. 2012).

Other genes with peak abundance during the exponential growth phase of cucumbers and watermelons included cytoskeleton related proteins such as tubulins and actin-related proteins, (Ando and Grumet 2010; Wechter et al. 2008), but more surprising, was the observation that genes with unknown function or without Arabidopsis homologs, dominated those genes most highly expressed during the exponential growth phase of cucumber fruit (Ando et al. 2012). The predominance of transcripts without Arabidopsis homologs or with unknown predicted functions during the peak exponential growth stage may reflect fewer studies to date about this phase of growth, or unique features of cucurbits.

As is the case for the period of cell division in cucurbit fruit development, the genes regulating cell expansion in cucurbits also remain to be discovered. Genomic and transcriptomic analyses may facilitate this process. A recent study combined specific length amplified fragment sequencing (SLAF-seq) with classical SSR-based QTL analysis to define a narrow genomic region associated with cucumber flesh thickness (Xu et al. 2015a, b). Availability of whole genome sequence then allowed identification of 20 genes located in within the region defined by the major QTL. Within that group was a single gene, *Csa2M05670.1*, that exhibited differential expression between the thick and thin mesocarp lines during fruit development. Peak expression of this gene, which potentially encodes a SET domain histone modifying factor, occurred during the period of peak fruit expansion, from 6 to 9 dpp. Consistent with differential expression, alleles from the thick and thin mesocarp lines differed due to a small deletion within the promoter region. Similarly, in cucumbers differing for fruit length, differential expression during the exponential growth phase was observed for a homolog of the Arabidopsis transcription factor gene, *AtHB2*, located within the region of cucumber fruit size QTL, FS3.1 (Colle 2015;

Weng et al. 2015). *AtHB2* encodes an HD-Zip homeobox protein first identified for its role in orientation of cell elongation associated with shade avoidance response (Carabelli et al. 2013). The *CsHB-2* allele in the long-fruited variety, had a deletion within the GAGA element in the 5' NTR, suggesting a possible change in regulation (Colle 2015).

Finally, while there are factors that drive rapid fruit growth, there must also be factors that signal the end of fruit expansion. Transcriptomic analysis of developing cucumber fruit has implicated potential involvement of several transcription factors accompanying the transition away from exponential growth (Ando et al. 2012). Cucumber fruit genes that exhibited sharply increased transcript levels occurring at the end of the period of rapid expansion were significantly enriched for several homeodomain, NAC domain, translationally controlled tumor protein and TAZ domain factors that have been annotated to be associated with development in Arabidopsis, suggesting a developmental switch point leading to the final maturation phase of fruit growth. This last phase of fruit development provides a period of time during which seed development is completed prior to the final investment in ripening. Ripening and associated quality components, such as colors, sugars, and aroma are described in Chaps. 19 (Yano and Ezura 2016; Gur et al. 2016).

Identification of QTL Associated with Cucurbit Fruit Size and Shape

Several studies have identified QTL for fruit traits that influence size and shape in cucurbits. In cucumber, the first moleuclar marker-based linkage map, which was developed using a population derived from interspecific and intraspecific hybridization of feral and cultivated cucumber, facilitated the identification of loci associated with fruit traits such as fruit length, diameter, length/diameter ratio (fruit shape), and seed cavity size/diameter ratio (Kennard et al. 1994; Kennard and Havey 1995). In addition, a QTL for fruit weight was identified using a denser linkage map (Serquen et al. 1997; Dijkhuizen and Staub 2002). Improvements in molecular marker technology led to the development of relatively saturated genetic maps and construction of linkage groups that correspond to the seven chromosomes of cucumber enabling better detection and description of fruit related QTL (Fazio et al. 2003; Yuan et al. 2008a, b). Moreover, with the recent availability of genome sequence (Huang et al. 2009) and increased computing speed, highly dense linkage maps have been developed providing better resolution and more accurate mapping of fruit trait related QTL as well as identification of new QTL for ovary length, ovule number, ovary diameter, fruit length, fruit diameter, seed cavity diameter, flesh thickness, and fruit weight (Bo et al. 2015; Miao et al. 2011; Qi et al. 2013; Wei et al. 2014; Weng et al. 2015, Xu et al. 2015a, b, Zhou et al. 2015). The most recent maps are characterized by a large number of well-spaced markers along all seven linkage groups allowing for better assignment of potential QTL locations. Despite availability of highly saturated maps, the number of QTL for specific fruit trait in

cucumber vary among studies, which may be due to differences in the mapping population, developmental stages of fruit examined and environmental effects.

Fruit size and shape appear to be highly quantitative traits controlled by QTL distributed throughout the genome. For example, multiple studies have found QTL for fruit length on chromosomes 1, 3, 4, 6 and 7 (Bo et al. 2015; Qi et al. 2013; Wei et al. 2014; Weng et al. 2015; Yuan et al. 2008a, b). On the other hand, QTL for fruit diameter were located in chromosomes 1, 2, 4, 5 and 6. The QTL for length/diameter ratio mapped to chromosomes 1, 4, 5, and 7 in three different genetic studies (Fazio et al. 2003; Serquen et al. 1997; Yuan et al. 2008a, b). Fruit weight QTL also mapped to chromosomes 1, 2, 3, 4 and 6 (Bo et al. 2015; Serquen et al. 1997; Wei et al. 2014; Yuan et al. 2008a, b). For seed cavity and fruit flesh thickness, QTL were detected in chromosomes 3 and 6, and chromosomes 1, 2, 3, 5 and 6, respectively (Weng et al. 2015, Xu et al. 2015a, b, Yuan et al. 2008a, b). Despite the variation in the QTL detected for fruit size and shape related traits, as well as difficulty in comparing the exact regions where QTL map due to non-colinearity of markers among different studies, the location of the QTL, in several cases, appears to be consistent and reproducible (Bo et al. 2015).

Most of the mapping studies in cucumber were conducted by phenotyping either fruit at harvest and/or mature stage. However, previous reports in other plant systems, such as tomato, showed that genetic factors regulating size and shape may act early during ovary development (Huang et al. 2013; van der Knapp et al. 2014; Xiao et al. 2009). In cucumber, QTL for ovary length, ovary diameter, and ovule number were also identified (Weng et al. 2015). Comparison of the location of these ovary QTL with the QTL detected using immature and fruit showed that several co-localized. For example, QTL for ovary length and immature and mature fruit length were located in chromosomes 1, 3 and 4 and were on the same chromosome block (Weng et al. 2015). Correlation analysis of fruit traits also showed that ovary length and immature and mature fruit length are highly correlated suggesting that these QTL are involved in regulating fruit length in cucumber. On the other hand, for both ovary and fruit diameter, QTL were found in chromosomes 2, 5, 6 and 7 however no significant correlation was observed between these two fruit traits suggesting that ovary and immature and mature fruit diameter might be under different genetic control (Weng et al. 2015).

A number of mapping studies to identify QTL for fruit traits have also been performed in melon and watermelon (Chap. 15, Gonzalo and Monforte 2016). For example in melon, Diaz et al. (2011) identified a total of 101 QTL for fruit length, fruit diameter, fruit shape and fruit weight using different mapping populations. Additional fruit size and shape QTL were also detected in different genetic studies of melon (Eduardo et al. 2007; Fernandez-Silva et al. 2010; Monforte et al. 2004; Perin et al. 2002; Ramamurthy and Waters et al. 2015). QTL for fruit related traits also have been identified in watermelon (Ren et al. 2014; Reddy et al. 2015; Sandlin et al. 2012; Tanaka et al. 1995). The recent genome sequencing of cucumber, melon and watermelon has provided information on the evolutionary history of these species including period of divergence, chromosomal fusion, duplication, inter and intrachromosomal rearrangements and genome synteny (Garcia-Mas et al. 2012;

Guo et al. 2013; Huang et al. 2009). Despite the structural changes and variations, it appears that many chromosomal regions are syntenic among cucumber, melon and watermelon. It will be of interest to determine whether chromososmal regions associated with fruit size and shape QTL are syntenic among cucurbit species and whether the different cucurbit species share common genetic mechanisms regulating fruit size and shape.

Seed Development and Parthenocarpy

Fruit growth post-anthesis for most fruits depends on successful pollination, and fruit size and weight are often a function of the number of successful fertilizations that have occurred in the ovary (Bohner and Bangerth 1988; Ledenunff et al. 1993). Observations in cucumber and tomato have shown that the number of fertilized ovules determined the initial growth rate of the ovary and amount of cell division (Gillaspy et al. 1993; Varga and Bruinsma 1990). Early studies in cucumber indicated that fertilization occurs in ovules near the apical end of the ovary within 72 h after pollination (Young 1943), and start of fruit growth is apparent as early as 24 h after pollination (Fuller and Leopold 1975). Developing seeds generate hormonal signals that promote cell division and enhance sink strength (review: McAtee et al. 2013). Auxins, cytokinins, gibberellins (GAs), and brassinosteroids all have been implicated to facilitate fruit set in cucurbits, although there is debate as to which hormones are most critical, and their respective roles in promoting cell division vs. inhibiting abscission of a non-pollinated flower (Boonkorkaew et al. 2008; Fu et al. 2008). Transcriptomic analysis of fruit set suggested sequential up-regulation of auxin-associated genes, followed by cytokinin-, then GA-associated genes (Li et al. 2014). Conversely, ethylene production post-anthesis has been associated with fruit abortion in zucchini (Martinez et al. 2013, 2014). While seed development is typically important for fruit set and development in cucurbit species, there are notable, economically important exceptions, parthenocarpic cucumbers and seedless watermelon.

Parthenocarpy allows for continued growth of the ovary, even in the absence of fertilization. Parthenocarpic fruit set is especially important for greenhouse production in the absence of insect pollinators. While most widely used for cucumber, parthenocarpy is also of value for off-season greenhouse production of zucchini in Europe (Martinez et al. 2014). It is also increasingly considered potentially valuable in field conditions where there are insufficient pollinators. Despite the ability for successful parthenocarpic fruit set, cell number, size, and fruit fresh weight were observed to increase faster and to a greater extent in flowers of parthenocarpic cucumber cultivars that were pollinated rather than left unpollinated (Boonkorkaew et al. 2008; Varga and Bruinsma 1990). Studies examining the effect of hormonal treatments on inducing parthenocarpic fruit set have shown that treatment with CPPU [1-(2-chloro-4-pyridyl)-3-phenyurea] was associated with earlier and/or enhanced auxin and sugar import and accumulation in the developing fruit (10 days post anthesis), but reduced sugar accumulation and smaller fruit size later in development (15–35 daa), suggesting that sink

strength was influenced by the presence of seeds, and that the effect of the CPPU treatment was transitory (Li et al. 2002, 2011). Sequential application of CPPU promoted resumed growth in the parthenocarpically set fruit.

Consistent with the role of hormones in promoting fruit set, and the ability of exogenous auxins, cytokinins and brassinosteroids to stimulate parthenocarpic fruit set, auxins and cytokinins increased more rapidly and to a greater extent in cultivars that set fruit parthenocarpically, with three to fivefold greater levels of IAA, zeatin and isopentyl adenine at 4 dpp (Boonkorkaew et al. 2008). Biosynthesis of elevated levels of brassinosteroids were also found to be essential for successful parthenocarpic fruit set (Fu et al. 2008), and parthenocarpy in zucchini was associated with reduced ethylene production (Martinez et al. 2014). Examination of gene expression showed that parthenocarpic cucumber fruit exhibited a similar profile of increased hormone-related gene expression during the first 8 days post-anthesis as did fertilized fruit, when compared to non-pollinated, non-parthenocarpic fruit (Li et al. 2014). While the genetic underpinnings of parthenocarpy remain to be defined, the above studies suggest they are likely to involve hormonal factors or their regulation. Continuous variation for level of parthenocarpy among diverse cucumber germplasm, and analysis of segregating progeny from crosses between parthenocarpic and non-parthenocarpic lines, indicate involvement of several genes, including a small number of major genes (Yan et al. 2008, 2009). Initial QTL analysis similarly suggested potential involvement of multiple components (Sun et al. 2006). More recently, six parthenocarpy QTL have been located on cucumber chromosomes 2, 5, 6 and 7 (Weng et al. 2016).

Another crop for which seedless fruit are highly prized is watermelon. The popularity of seedless watermelon, especially in the United States, has steadily increased over the past decade (Freeman et al. 2007; McGregor and Waters 2014). Seedless watermelon fruit are the result of triploid maternal plants that do not produce viable seeds due to unpaired chromosomes. Unlike parthenocarpy described above, fruit set on triploid watermelon plants requires active pollination and fertilization of the ovules. However, because triploid cultivars, which are the product of tetraploid by diploid crosses, produce very little viable pollen, field production systems include the planting of pollinizer plants to provide an adequate source of pollen. Most frequently the pollinizer plants are diploid watermelon cultivars, although some studies also have investigated the use of alternative species, such as bottle gourd (*Lagenaria siceraria*) (Dittmar et al. 2010; Sugiyama et al. 2015). Thus, seedless watermelon fruits do not result from a specific genetic propensity toward parthenocarpic fruit development, but are instead the result of the capacity for watermelon plants to develop fruit despite incompatible fertilizations.

Spines, Warts, and Stripes

Among the features for which cucurbit fruits can vary, are trichomes, spines, and warts. Preference for or against spines and warts can vary with market class and culinary preferences in different parts of the world. For some fruits, such as horned

melon (*Cucumis metuliferus*), spines and highly pronounced tubercuoles are a defining feature. Cucumber fruits produce two types of trichomes. Type I trichomes are small, multicellular, secretory trichomes with a three-to-five-cell base and four-to-eight-cell head (Chen et al. 2014). Type I trichomes are associated with cuticle formation, including secretion of silica dioxide, which covers the fruit surface with a fine white powder refererred to as 'bloom', causing the fruit surface to appear dull, rather than shiny. Type II trichomes (spines) are much larger, comprised of a base of hundreds of cells, which in turn, typically sit on a locally enlarged region of the fruit. The enlarged region, or tuberculole, causes a warty fruit surface. The top of a type II trichome is composed of a three to seven cell stalk with a sharp pointed apical cell.

Both types of trichomes, and the associated traits of wartiness or dullness of the fruit surface, can be simply inherited. Alleles such as dull fruit (*D*), glabrous (*gl*), heavy netting of fruit (*H*), prominant tubercuoles (*P*), intensifier of P (*I*), tuberculate (*Tu*), black spine (*B*, *B-2*, *B-3*, *B-4*), numerous spines (*ns*), spine frequency (s, s-2, s-3), and small spines (*ss*) in cucumber (Call and Wehner 2010) and warty fruit (*Wt*) in *Cucurbita pepo* (Paris and Brown 2004), and glabrous (*gl* and *gl-2*) in summer squash (Xiao and Loy 2007) have been long described. The cucumber *H*, *tu*, *D*, *ns*, and *ss* genes appear to be clustered in linkage group 5, along with some additional fruit surface traits such as ribbing, thick skin, and uniform fruit color, that are often inherited in set combinations characteristic of a given market class e.g., (Li et al. 2013; Miao et al. 2011).

Recent studies have identified several simply inherited genes associated with trichome formation and development in cucumber fruit. The glabrous – *CsGL1* and tiny branched hair – *tbh* genes influence trichome formation throughout the plant (i.e., leaves, stems, tendrils) as well as fruit (Chen et al. 2014; Li et al. 2015). Mutations in these genes result in fruit that is smooth, shiny, and wartless, indicating loss of both types of trichomes. Analysis of density and developmental stage of trichomes in the wild type and *tbh* and *csgl1* mutants indicates that TBH is associated with trichome formation, while CsGL1 is associated with subsequent trichome development (Chen et al. 2014; Li et al. 2015). Both mutations resulted in extensively modified gene expression. For the *csgl1* mutant, a multi-pronged approach including fine mapping, sequence analysis and transcriptional analysis, allowed for identification of the responsible gene (Li et al. 2015). Consistent with a potential regulatory role, *CsGL1* was found to encode a homeodomain leucine zipper (HD-ZIP) protein, although not one that had been previously associated with glabrous mutants in Arabidopsis or other species (Li et al. 2015). *CsGL1* was most highly expressed in the spines and warts of fruit, rather than vegetative tissues. The *csgl1* mutant allele is marked by a 2.6 kb deletion upstream of the start codon. The *Tu* gene responsible for tubercules on cucumber fruit also has been recently cloned and found to be a C2H4 zinc finger protein (Yang et al. 2014). Expression of *Tu*, which is epistatically regulated by *CsGL1*, is specific to fruit spine cells, and appears to promote cytokinin-driven division of underlying cells, resulting in multicellular tubercules on the fruit surface. The recessive, non-tuberculate allele results from a 4.9 kb deletion of the gene and surrounding promoter sequence.

In addition to presence or absence of spines, there also can be difference in whether the spines are heavily pigmented. The dominant locus, *B*, which confers black vs. white spines, is also associated with orange mature fruit color. *B* was located by successive fine mapping analysis to a small region on cucumber chromosome 4 (Li et al. 2013). Among the six candidate genes in that region was an R2R3-MYB transcription factor gene. As R2R3 type MYB transcription factors have been associated with regulation of flavonoid and anthocyanin biosynthesis in other systems (Hichri et al. 2011), and elevated expression of this gene was observed in fruits with black spines, the R2R3 MYB transcription factor was proposed to be a strong candidate for the *B* gene. The findings that many of these simply inherited traits appear to result from mutations altering expression of regulatory genes is consistent with a growing body of evidence suggesting that alterations in transcriptional regulation of development-associated genes is a key driver of phenotypic evolution in plants (Rodriguez-Mega et al. 2015).

Fruit striping patterns also result from the action of major genes. While some recent studies have begun to determine genomic regions and markers associated with these traits, at this time, less progress has been made in identifying specific underlying genes. *Cucurbita pepo* fruits such as pumpkins, squashes, and gourds often exhibit dramatic striping patterns. Most frequently the patterns result from ten alternating dark and light colored regions wherein the light colored regions are positioned above the main capillary vein tracts and dark regions in central areas between the vein tracts (Paris 2003). Appearance of the stripes is highly dependent on the stage of fruit development, and also can vary in expression among adjacent fruits on the same plant, indicating marked phenotypic plasticity. Variation in continuity within the stripe and distribution over the fruit surface has been observed to result from a series of multiple alleles at the *l-1* (*light coloration-1*) locus. The most recessive allele, *l-1*, confers light color over the full fruit surface, while the most dominant *L-1*, confers dark coloration over the full fruit surface. The dominant version of a second gene, *l-2*, causes stripes to be apparent throughout fruit development. Interaction between *l-1* and *l-2* can result in fruit exhibiting reciprocal striping with the dark colored regions positioned over the capillary vein tracts (Paris 2009). *L-1* and *L-2* segregate independently, but map positions for these genes have not yet been identified.

Watermelon fruit are also frequently characterized by a striped rind of alternating intensity of green color and striping patterns which can be a defining feature of market types (Guner and Wehner 2004). Depending on variety, the stripes can vary in width, pattern (unicolored, bicolored, marbled), intensity, and sharpness of the boundary between bands (Guner and Wehner 2004; Kim et al. 2015). Striping is dominantly inherited relative to non-striping and segregating progeny from parents with differing rind pattern types indicate that different band features such as depth of color, stripe boundary, and yellow vs. green banding can segregate independently (Kim et al. 2015; Yang et al. 2015). Bulked segregant analysis identified a region of chromosome 6 associated with deep green stripes and sharp boundaries (Kim et al. 2015). Consistent with this study was an analysis identifying microsatellite markers on chromosome 6 segregating with diffuse versus clearly defined stripes (Gama et al. 2015).

Conclusions

Fruit development is a highly complex process resulting from the intricate interplay of a large number of contributing components. Cucurbit species provide excellent systems from which to explore factors driving morphological diversity. As modern genomic approaches and tools allow us to unravel underlying mechanisms, it is anticipated that many new factors influencing fruit development will be discovered, providing new biological insights and opportunities to expedite breeding efforts for desired fruit traits.

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Fruit Ripening in Melon

Ryoichi Yano and Hiroshi Ezura

Abstract Ripening is a highly programmed developmental process that confers economically important properties to fruit. Although the key roles of the phytohormone ethylene and related transcription factors have been well studied in the regulation of fruit ripening in the model fruit, tomato, melon (*Cucumis melo* L.) is also recognized as an attractive alternative model because of the co-existence of climacteric and non-climacteric types, as well as the availability of the whole genome sequence and other rich genetic resources. In climacteric melon, genetic evidence demonstrates that ripening-associated biochemical changes are brought about by both ethylene-dependent and -independent pathways. Recently, genome, transcriptome, metabolite, and systems biology studies that have employed high-throughput analytical technologies have further investigated the molecular basis of fruit ripening in melon. This chapter is intended to combine the previous and current knowledge about melon fruit ripening with a main focus on molecular mechanisms.

Keywords Melon • Climacteric and non-climacteric ripening • Phytohormone • Ethylene • Carotenoid • Functional genomics

Abbreviations

1-MCP	1-methylcyclopropene
a	Andromonoecious
ACC	1-aminocyclopropane-1-1carboxylic acid
ACO	ACC oxidase
ACS	ACC synthase
Al	Abscission layer
AP2a	APETALA2 transcription factor APETALA2a
ATH	N-Acetyltransferase hookless
AuxRD	Auxin-responsive element
BSR-seq	Bulked segregant RNA-seq

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CDPK	Calcium-dependent protein kinase
Cnr	Colorless non-ripening
CRT	C-repeat
CRTISO	Carotenoid isomerase
CTR	Constitutive ethylene response
DEG	Differentially expressed gene
DMR	DEMETER-like DNA demethylase
DRE	Dehydration responsive element
DREB	DRE binding factor
EIN	Ethylene insensitive 3
EMS	Ethylmethane-sulfonate
EREBP	Ethylene-responsive element binding protein
ERF	Ethylene Responsive Factor
ETR	Ethylene-resistant
FUL	Fruitfulll
g	Gynoecious
gf	Green flesh
Gr	Green-ripe
NIL	Near-isogenic line
nor	Non-ripening
Nr	Never-ripe
pg2a	Polygalacturonase2a
PSY	Phytoene synthase
LG	Linkage group
Or	Orange
PDS	Phytoene desaturase
PSY	Phytoene synthase
QTL	Quantitative trait locus
RIL	Recombinant inbred line
rin	Ripening-inhibitor
RTE	Reversion to ethylene sensitivity
S-AdoMet	S-adenosyl-L-methionine
SAMase	S-AdoMet hydrolase
SBP	Squamosa-promoter binding protein
SEP	Sepallata
SGR	Stay-green
SNP	Single nucleotide polymorphism
TAGL	Tomato agamous-like
TCA	Tricarboxylic acid
TD-GC-MS	Thermal desorption gas chromatography mass spectrometry
TILLING	Targeting induced local lesion in genomes
UFGC–SAW	Ultra-fast gaschromatograph coupled with a surface acoustic wave sensor
wf	White flesh
ZDS	ζ-carotene desaturase
Z-ISO	ζ-carotene isomerase

Introduction

Fruit ripening is a highly complex and coordinated developmental process that induces changes in organoleptic properties, such as firmness, color, acidity, sweetness, aroma, and flavor (Brady 1987; Karlova et al. 2014). In nature, fruit ripening is thought to be essential for seed dispersal, and in many plants, changes associated with fruit ripening make fruit succulent and attractive to animals, thereby promoting long-distance dispersal of seeds. Domesticated fruit plants have been further selected by traditional farmers and, more recently, by modern breeding techniques to produce fruit that better satisfies human preferences. In most economically important fruit crops (including tomato and melon), sweetness, sourness, flavor, texture, and accumulation of health-promoting secondary metabolites (like flavonoids, carotenoids, and terpenoids) are important traits that increase the commercial value of produced fruits. Many of these economically desirable properties are induced by ripening; however, ripening often negatively affects shelf-life. In several fruits, such as cantaloupe melon and strawberry, the fruit quality rapidly deteriorates after harvest, which limits the market available to commercial producers. Because ripening is a key factor in determining fruit quality, there is both scientific and commercial interest in elucidating its molecular mechanisms.

Fleshy fruits are physiologically classified into two major categories: climacteric and non-climacteric. Climacteric fruits include tomato, apple, avocado, banana, passion fruit, certain groups of melon such as Cantaloupe and Charentais, and peach, and they differ from non-climacteric fruits in their ability to exhibit respiration bursts and autocatalytic ethylene production at the onset of fruit ripening (Abeles and Takeda 1990; Biale 1964; Paul et al. 2012; Pech et al. 2008; Lelièvre et al. 1997). In climacteric crops, any chemical perturbation of ethylene perception or transgenic suppression of ethylene biosynthesis or signal transduction prevents fruit from ripening and improves shelf-life, thus demonstrating that the phytohormone ethylene plays an essential role in climacteric fruit ripening. By contrast, non-climacteric fruits, such as strawberry, citrus, pineapple, and grape, do not exhibit respiration bursts upon ripening. Ethylene biosynthesis is not activated in non-climacteric fruits; in addition, exogenous application of ethylene fails to initiate ripening as it does in unripe climacteric fruits. Shelf-life is generally longer in non-climacteric fruits than in climacteric ones, although there are exceptions, such as strawberries and apple fruits, which have a relatively short or long shelf-life, respectively. On the other hand, the processes of some biochemical changes are common to both types. Fruit sweetness, for example, is generally the result of elevation in the level of mono- and disaccharides, which is brought about by the conversion of starch to sugars or by extracellular transport. Color changes are caused by alterations in chlorophyll, carotenoid, flavonoid, and other pigment contents of the plastids and vacuoles (Ayub et al. 1996; Lu et al. 2006; Powell et al. 2012; Tzuri et al. 2015; Yuan et al. 2015). In melon fruits, carotenoids are the predominant factor that confers color changes, and their accumulation is irrespective of ripening behavior.

Ethylene is a simple and diffusible compound, consisting of only two carbons and four hydrogens. It was the first gaseous phytohormone to be identified and is best known for its essential role in climacteric fruit ripening (Alexander and Grierson 2002; Karlova et al. 2014; Klee and Giovannoni 2011). In addition to fruit ripening, ethylene regulates a wide range of physiological and developmental processes, including organ senescence, abscission, seed dormancy, germination, root initiation, leaf expansion, flower development, and sex determination (Aloni 2013; Boualem 2008; Corbineau et al. 2014; Ju and Chang 2015; Koyama 2014; Switzenberg et al. 2015). Its biosynthetic pathway, which has been defined in both the model plant *Arabidopsis* and the model fruit plant tomato, involves two key steps (Kende 1993). The first committed step is the conversion of *S*-adenosyl-L-methionine (*S*-AdoMet) to 1-aminocyclopropane-1-1carboxylic acid (ACC) by ACC synthase (ACS, *S*-adenosyl-L-methionine methylthioadenosine lyase, EC4.4.14), and ACC is subsequently oxidized to form ethylene by ACC oxidase (ACO) (Fig. 1). The rate-limiting step of ethylene synthesis is the conversion of *S*-AdoMet to ACC by ACS. This is partly because ACS is labile after purification and is only present at low levels in plant tissues, characteristics which also indicate that ethylene biosynthesis is tightly controlled (Kende 1993). The regulation of the ACS and ACO expression has been investigated in many plants (Wang et al. 2002), and during ripening of climacteric fruits, the onset of ethylene production is generally associated with increased activities of ACS and ACO. This activation is brought about by both transcriptional up-regulation of corresponding genes and post-transcriptional regulation of the enzymes (Choudhury et al. 2013; Klee and Giovannoni 2011) (see below). A breakthrough in uncovering the ethylene signal transduction pathway was provided by the molecular genetic dissection of ethylene-response mutants in *Arabidopsis* (Bleecker and Kende 2000; Ju and Chang 2015). The *Arabidopsis thaliana* ETHYLENE-RESISTANT 1 (AtETR1) receptor is a homologue of the prokaryotic signal transducers known as two-component regulators and was the first ethylene receptor identified in the plant kingdom (Chang et al. 1993). In concert with the CONSTITUTIVE ETHYLENE RESPONSE 1 (AtCTR1) protein kinase, the AtETR1 receptor suppresses down-stream ethylene response in the absence of ethylene (Hua and Meyerowitz 1998; Kieber et al. 1993) (Fig. 2). Although the detailed mechanism has yet to be fully understood, it is known that the ethylene signal is transmitted from the endoplasmic reticulum (the site of ethylene perception) to the nucleus (the site of transcriptional response) (Alonso et al. 1999; Ma et al. 2006). Binding of ethylene to receptors leads to the stabilization of the ETHYLENE INSENSITIVE 3 (EIN3) transcription factor, which in turn activates down-stream ETHYLENE RESPONSIVE FACTOR (ERF) transcription factors (Chao et al. 1997; Solano et al. 1998). Interestingly, the basic mechanism of the ethylene signal transduction pathway is functionally conserved not only in plants but also even in algae (Ju et al. 2015); however, the outcomes mediated by the hormonal pathway are different between species. In particular, there are significant differences in fruit structure, as well as in ripening-associated biochemical changes, between the fruits of *Arabidopsis* and most fleshy-fruit plants (Karlova et al. 2014). Specifically, *Arabidopsis* has a dehiscent fruit type called a silique, which is

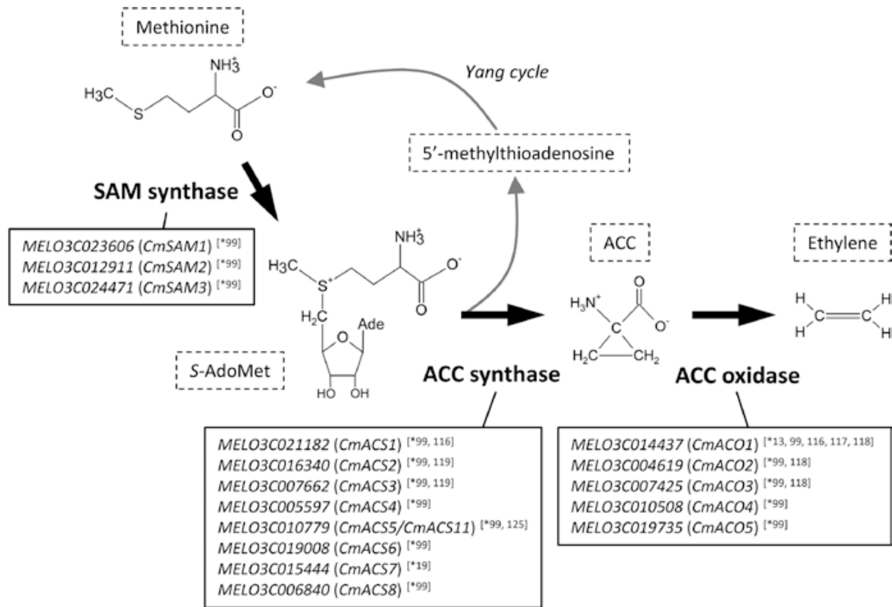


Fig. 1 *C. melo* genes relating to the ethylene biosynthesis pathway. *S*-adenosyl-L-methionine (*S*-AdoMet or SAM) is the precursor for ethylene biosynthesis and is synthesized from the amino acid methionine by the action of SAM synthase. Ethylene is synthesized from *S*-AdoMet by two key reactions, which are catalyzed by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase. The melon genome carries at least three, eight, and five copies of SAM synthase, ACC synthase, and ACC oxidase, respectively (Garcia-Mas et al. 2012). The asterisked numbers [13], [19], [99], [116], [117], [118], [119], and [125] indicate that the corresponding information was obtained from the references

representative of Brassicaceae and legume plants. Similar to climacteric fleshy fruits, *Arabidopsis* siliques are also known to exhibit respiration bursts and autocatalytic ethylene production (Kou et al. 2012). However, the ripening of dehiscent *Arabidopsis* fruit is facilitated by separation of the valves at an abscission layer (termed the dehiscence zone) that is formed at the valve-replum boundary. On the other hand, most fleshy fruits usually undergo a ripening process in which the biochemistry, physiology, and structure of the organ are altered to influence appearance, texture, flavor, and aroma in order to attract seed-dispersing organisms. Molecular mechanisms underlying such inter-species differences have yet to be defined.

The tomato fruit exhibits typical climacteric ripening and has been the model fleshy fruit plant (Klee and Giovannoni 2011). During the last two decades, molecular and genetic dissection of fruit-ripening mutants in tomato has greatly contributed to the elucidation of fruit ripening mechanisms (Fig. 3). These non-allelic, single mutants include *ripening-inhibitor* (*rin*), *non-ripening* (*nor*), *Colorless non-ripening* (*Cnr*), *Never-ripe* (*Nr*), and *Green-ripe* (*Gr*) (Manning et al. 2006; Martel et al. 2011; Osorio et al. 2011; Tigchelaar et al. 1973; Vrebalov et al. 2002; Wilkinson

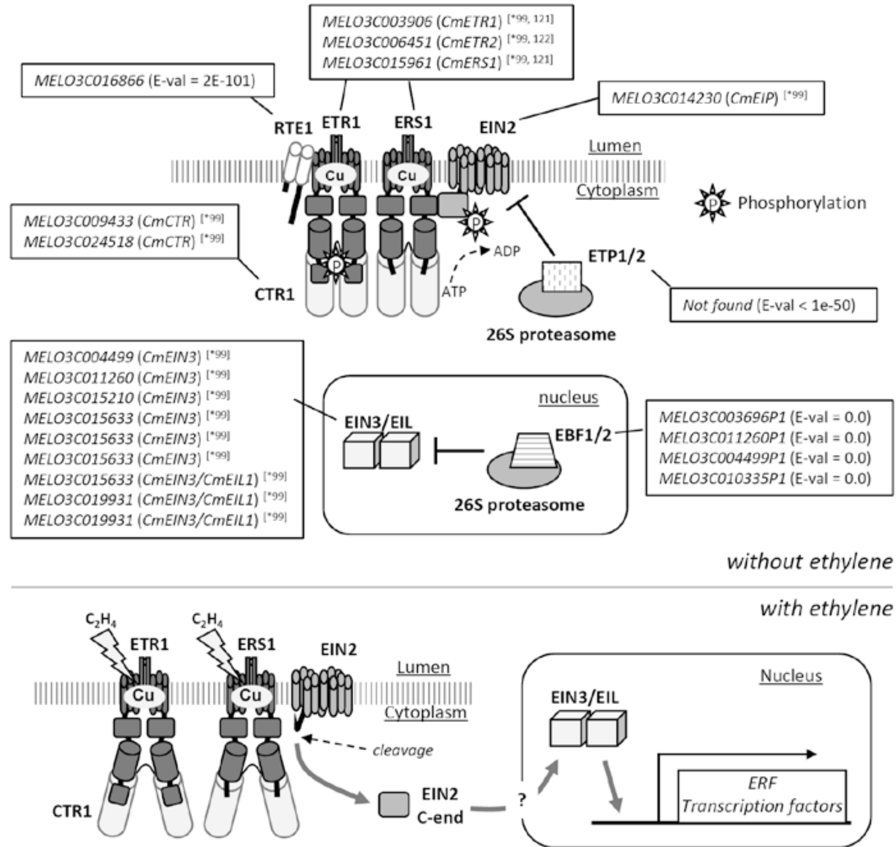


Fig. 2 *C. melo* genes relating to the early phase of ethylene signal transduction. According to the ethylene signal transduction model proposed in the model plant *Arabidopsis* (Ju et al. 2015), the formation of functional ethylene receptors (ETR1 and ERS1 homodimers) depends on REVERSION-TO-ETHYLENE SENSITIVITY1 (RTE1) in the absence of ethylene perception (top). Without ethylene, the ETR1 and ERS1 homodimers activate the CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1) protein kinase, which in turn phosphorylates the C-terminal domain of down-stream ETHYLENE INSENSITIVE 2 (EIN2). This phosphorylation results in degradation of EIN2 by the 26S proteasome via interaction with the F-box proteins EIN2 TARGETING PROTEIN 1 and 2 (ETP1/2). In the nucleus, the additional F-box proteins EIN3 BINDING F-BOX 1 and 2 (EBF1/2) target the EIN3 and EIN3-Like1 (EIL1) transcription factors for 26S proteasomal degradation, thereby preventing down-stream transcription. Upon binding of ethylene (represented by lightning bolts), the ethylene receptor is inactivated and no longer activates CTR1. The C-terminal domain of non-phosphorylated EIN2 is cleaved, thus transmitting a signal into the nucleus to induce 26S proteasomal degradation of the EBF1/2. This in turn stabilizes EIN3/EIL1 master transcription factors, thereby activating a transcriptional cascade that includes the downstream ERF transcription factor genes. In melon, three ethylene receptor genes have been previously cloned and reported in [121] and [122] (indicated by asterisked numbers in the figure). The melon homologues of additional components of ethylene signal transduction pathway have been identified (Saladie et al. 2015). The best possible matches from the melon genome (lowest E-value in BLAST search) for *Arabidopsis EBF1/2* and *RTE1* were identified using BLAST (software version 2.2.29+) with the *Arabidopsis* gene used as the query and the *C. melo* whole genome protein sequence dataset (version 3.5 obtained from the "Melonomics" website, https://melonomics.net/, (Garcia-Mas et al. 2012)) used as the database (*C. melo* genes with E-values). For *Arabidopsis* ETP1/2 genes, no melon homologues were identified with E-values < 1e-50

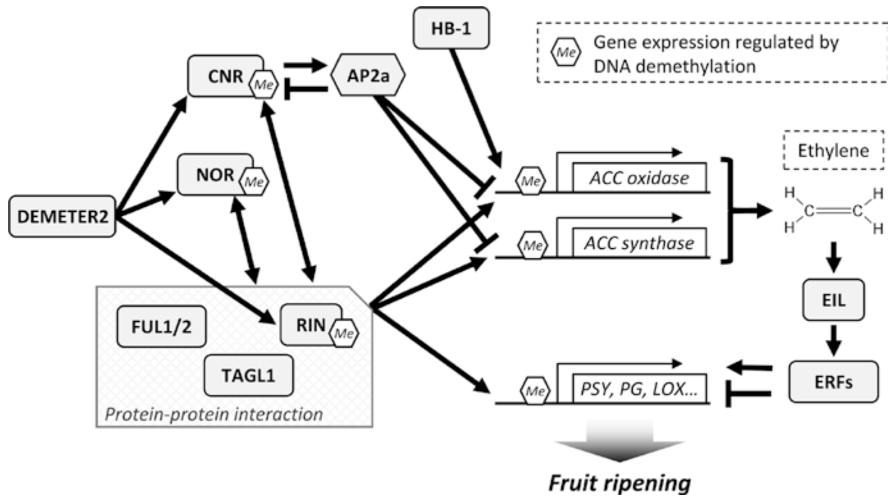


Fig. 3 Schematic overview of transcriptional network involved in the regulation of fruit ripening in tomato. Arrowheads represent positive regulatory interactions, and bar heads represent negative regulation. *RIPENING-INHIBITOR* (*RIN*), *COLORLESS-NONRIPENING* (*CNR*), and *NON-RIPENING* (*NOR*) are the central components involved in both ethylene-dependent and -independent regulation of fruit ripening in tomato. Any loss-of-function mutation in these genes results in reduced production of ethylene and carotenoids, as well as suppression of softening, thereby causing a delayed ripening phenotype (Manning et al. 2006; Martel et al. 2011; Tigchelaar et al. 1973; Vrebalov et al. 2002). *APETALA2a* (*AP2a*) is a negative regulator of ethylene production and ripening (Chung et al. 2010; Karlova et al. 2011), while *HB-1* directly up-regulates the expression of the 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase gene, thereby promoting ripening (Lin et al. 2008). FRUITFULL 1/2 (FUL1/2) and TOMATO AGAMOUS-LIKE 1 (TAGL1) directly interact with RIN and are also involved in the regulation of fruit ripening (Bemer et al. 2012; Itkin et al. 2009; Fujisawa et al. 2014; Shima et al. 2014; Vrebalov et al. 2002). DNA demethylase *DEMETER 2* has been shown to be required for epigenetic regulation of *RIN*, *NOR*, and *CNR* gene expression, as well as expression of carotenoid synthesis genes, such as *Phytoene synthase 1* (*PSY1*) (Liu et al 2015). Ripening-inducible reduction in the degree of DNA demethylation has also been observed in a wide range of ripening-inducible genes, including those for ethylene biosynthesis (ACC synthase and ACC oxidase), *Polygalacturonase* (*PG*), and *Lipoxygenase C* (*LOXC*) (Zhong et al. 2013)

et al. 1995; Yen et al. 1995), and they are classified into two categories. The first group includes *rin*, *nor*, and *Cnr*, which share common physiological characteristics; (a) mutant fruits develop to the mature green stage but do not advance to ripening; (b) mutant fruits fail to exhibit climacteric respiration bursts and ethylene production; (c) exogenous ethylene treatment cannot rescue the mutant phenotypes described above; and (d) despite failure to ripen, exogenous ethylene still manages to induce some ethylene-responsive genes (Eriksson et al. 2004; Giovannoni 2007; Klee and Giovannoni 2011; Martel et al. 2011). Thus, the concerted action of *RIN*, *NOR*, and *CNR* is essential for both ethylene-dependent and -independent regulation of fruit ripening. *RIN*, *NOR*, and *CNR* have been reported to encode a SEPALLATA (SEP)-type MADS-box domain transcription factor, a NAC domain

family transcription factor, and a SQUAMOSA-PROMOTER BINDING PROTEIN (SBP) domain transcription factor, respectively (Manning et al. 2006; Vrebalov et al. 2009). The RIN transcription factor directly binds to the promoter of ripening-inducible genes, such as the tomato (*Solanum lycopersicum*) ethylene biosynthesis genes *SlACS2* and *SlACS4*, the cell wall metabolism gene *Polygalacturonase2a* (*pg2a*), and the carotenoid biosynthesis gene *PHYTOENE SYNTHASE1* (*PSY1*), and also regulates their expression in a *CNR*-dependent manner (Fujisawa et al. 2012; Fujisawa et al. 2013; Martel et al. 2011). *Cnr* was identified as an epigenetic mutant, and DNA hypermethylation in the promoter of the mutant's *CNR* gene is associated with failure of ripening-inducible expression (Liu et al. 2015; Manning et al. 2006; Zhong et al. 2013). The second group of mutants includes *Nr* and *Gr*. Unlike the previous group, the *Nr* and *Gr* mutants can produce ethylene during fruit ripening; however, ethylene-responsive gene expression is impaired. This ethylene-insensitive phenotype cannot be restored by exogenous ethylene treatment, indicating that these mutants are impaired in either ethylene perception or signaling (Barry et al. 2005). Accordingly, *Nr* has been shown to encode an ethylene receptor, *SlETR3*, which is the tomato homologue of *AtETR1* (Wilkinson et al. 1995; Yen et al. 1995), and *Gr* has also been identified as a homologue of *Arabidopsis REVERSION TO ETHYLENE SENSITIVITY 1* (*AtRTE1*) (Barry and Giovannoni 2006). In *Arabidopsis*, the *rte1* mutation restores the ethylene insensitivity of *etr1* mutants, and functional AtRTE1 is involved in ethylene signaling through direct protein-protein interaction with ethylene receptors (Dong et al. 2010; Qiu et al. 2012; Resnick et al. 2006; Zhou et al. 2007). In tomato, other ethylene receptor genes, *SlETR1*, *SlETR4*, and *SlETR6*, have been shown to regulate fruit ripening, as well (Kevany et al. 2007; Mubarok et al. 2015; Okabe et al. 2011).

Additional important components such as the MADS-domain transcription factors *TOMATO AGAMOUS-LIKE 1* (*TAGL1*) (Itkin et al. 2009; Vrebalov et al. 2009), *FRUITFUL 1* (*FUL1*) and 2 (*FUL2*) (Bemer et al. 2012), *SlMADS1* (Dong et al. 2013), HD-Zip homeobox transcription factor *LeHB-1* (Lin et al. 2008), and APETALA2 transcription factor *APETALA2a* (*SlAP2a*) (Chung et al. 2010), and the NAC domain transcription factor *SlNAC4* (Karlova et al. 2011; Zhu et al. 2014) have also been identified in tomato and shown to play a role in fruit ripening. TAGL1 and FUL1/2 proteins, for example, directly interact with the RIN protein (Fujisawa et al. 2014; Leseberg et al. 2008; Martel et al. 2011), and they are also involved in the regulation of ethylene biosynthesis and carotenoid accumulation (Itkin et al. 2009), although there is a discrepancy regarding the involvement of *FUL1/2* in ethylene-dependent processes (Bemer et al. 2012; Shima et al. 2014). *LeHB-1* directly up-regulates *SlACO1* expression, and its silencing results in reduced ethylene biosynthesis, as well as delayed fruit ripening (Lin et al. 2008). *SlNAC4* is also a positive regulator of fruit ripening and involved in the regulation of ripening-associated gene expression including ethylene biosynthesis genes (Zhu et al. 2014). By contrast, *SlMADS1* and *SlAP2a* act as negative regulators of fruit ripening (Chung et al. 2010; Karlova et al. 2011). RNAi-mediated repression of *SlMADS1* or *SlAP2* results in early fruit ripening as well as increased ethylene production, in comparison to wild-type fruits. More recently, DNA demethylation was shown to

play a role in fruit ripening. Exogenous treatment of 5-azacytidine, a methyltransferase inhibitor that causes DNA hypomethylation, resulted in premature fruit ripening, indicating the positive effect of DNA demethylation on fruit ripening (Zhong et al. 2013). A bisulfate DNA sequencing approach further revealed that cytosine nucleotides around RIN targets are highly methylated in unripe tomato fruit, while, at the onset of ripening, demethylation progresses in association with the transcriptional activation of corresponding genes (Zhong et al. 2013). Moreover, RNAi-suppression of the DEMETER-like DNA demethylase gene *SlDMR2* results in delayed fruit ripening, consistent with hypermethylation and suppressed expression of *RIN, NOR, CNR*, and *PSY1* (Liu et al. 2015). The process of DNA demethylation itself is also regulated by *RIN* and *CNR*, as the genomic regions of many ripening-inducible genes are hypermethylated in *rin* and *Cnr* mutants (Martel et al. 2011). Taken together, this series of tomato studies has demonstrated that the concerted action of both master transcription factor regulators and epigenetic modification is essential for fruit ripening in tomato.

Although molecular and genetic studies in tomato have greatly contributed to the elucidation of fruit ripening mechanisms, evolutionary processes are expected to produce a wide variety of developmental manifestations in structures, designs, and functions of fleshy fruits. As such, it is potentially inadequate to extrapolate findings in tomato as universal to other fruit species, and the use of alternative model plant systems that differ from tomato in quantitative and/or qualitative aspects of ripening is necessary to provide novel insight into the general ripening process and to explain the disparity in fruit ripening processes among plant species (Ezura and Owino 2008; Paul et al. 2012). Recently, with the advance of genomics technology, such as next-generation sequencing, whole genome sequences have been uncovered and published for various fruit species. These include grape (Jaillon 2007), papaya (Ming 2008), cucumber (Huang et al. 2009), apple (Velasco et al. 2010), cacao (Argout et al. 2011), strawberry (Hirakawa et al. 2014; Shulaev et al. 2011), melon (Garcia-Mas et al. 2012), banana (D'Hont et al. 2012), kiwifruit (Huang et al. 2013), pear (Wu et al. 2013), sweet orange (Xu et al. 2013), watermelon (Guo et al. 2013), and pepper (Kim et al. 2014). Among them, melon has been recognized as an attractive alternative model to tomato for fruit ripening studies (Argyris et al. 2015; Ezura and Owino 2008; Pech et al. 2008).

In melon, the flesh, embryo, placenta, and seeds are well ordered, and its development can be clearly divided into ethylene-insensitive and -sensitive stages. In addition, melon is the most diverse species of the genus *Cucumis*, and there is a wide range of phenotypic variation in fruit size (several grams to kilograms), shape (round to elongated), skin color, flesh color (carotenoid content), and organoleptic properties (Burger et al. 2010; Leida et al. 2015; Stepansky et al. 1999a, b) among varieties and wild relatives. Most importantly, melon has advantages over the tomato in that it includes both climacteric and non-climacteric genotypes. Certain groups of melon such as *C. melo* var. *cantalupensis,* which possesses sweet and aromatic flesh characteristics, exhibits typical climacteric behavior (respiration burst and autocatalytic ethylene production) with short shelf-life (Hadfield et al. 1995). In contrast, other groups of melons, such as *C. melo* var. *inodorus*, do not

produce ethylene during ripening and generally have a slow ripening rate, which is associated with a long shelf-life and fewer postharvest losses during storage and transport (Stepansky et al. 1999a). Genetic studies have identified several quantitative trait loci (QTLs) involved in ethylene production and/or fruit ripening behavior (Moreno et al. 2008; Perin et al. 2002; Vegas et al. 2013). In addition, TILLING (targeting induced local lesion in genomes) mutant populations generated by ethylmethane-sulfonate (EMS) treatment (Dahmani-Mardas et al. 2010; Gonzalez et al. 2011; Tadmor et al. 2007) and Eco-TILLING (Nieto et al. 2007) are techniques that have been developed in order to promote functional genomics studies, not only in one representative variety but also in several varieties. The melon whole genome sequence has now enabled integrative, multi-omics study of fruit ripening, as well (Chayut et al. 2015; Freilich et al. 2015; Saladie et al. 2015). Accordingly, this chapter introduces and summarizes our accumulating knowledge of the molecular mechanisms of fruit ripening in melon.

Physiology of Melon Ripening

Climacteric ripening is traditionally defined by the occurrence of a respiration burst and/or autocatalytic ethylene production at the onset of the ripening stage (Biale 1964). In climacteric fruits, the respiration burst and ethylene production can be triggered and further enhanced by exogenous ethylene treatment (McMurchie et al 1972; Paul et al. 2012; Tucker 1993; Yamane et al. 2007). By contrast, non-climacteric fruits do not exhibit respiration bursts, and exogenous ethylene treatment stimulates neither respiration nor ethylene production (Atta-Aly et al. 2000). As described above, melon plants exhibit a wide range of phenotypic variation in ripening behavior: climacteric and non-climacteric. The *cantaloupensis* and *reticulatus* varieties tend to exhibit a respiration burst shortly after fruit maturity, whereas the respiration bursts of *inodorus* and *saccharinus* varieties tend to be prolonged or absent (Aggelis et al. 1997; Burger et al. 2010; Kendall and Ng 1988; Leida et al. 2015; Liu et al. 2004). Previously, respiration bursts were considered an artifact that was induced by fruit harvest (detachment from vine). This is partly because a rise in carbon dioxide concentration was observed in harvested fruits of some netted muskmelon and *inodorus* varieties only after a certain period of storage (Miccolis and Saltveit 1991; Pratt et al. 1977; Shellie and Saltveit 1993). However, the fruits of 'Charentais' melon, a *reticulatus* variety, were found to exhibit respiration bursts without detachment from the vine and at an extent that was comparable to that of harvested fruits. Thus, respiration bursts are recognized as a naturally occurring phenomenon that is associated with fruit ripening in climacteric melon varieties. In general, the rate of ethylene production is closely associated with several economically important fruit traits. While aroma production, abscission, and softening are positively associated with ethylene production, shelf-life is negatively associated. In the fruits of *cantaloupensis* and *reticulatus* varieties, climacteric ethylene production coincides with fruit maturity before abscission (Hadfield et al. 1995; Shellie

and Saltveit 1993). Despite the desirable aroma and flesh softness, fruit quality of these melons rapidly deteriorates after harvest. By contrast, *inodorus* melons, such as the 'Honeydew' cultivar, exhibit longer shelf-life and lower ethylene production than other varieties. In addition, *inodorus* melons fail to form an abscission zone at the timing of commercial harvest maturity making it difficult to judge the best timing of fruit harvest. Although *inodorus* melons are not sensitive to exogenous ethylene when compared with climacteric melons, treatment of the fruits with ethylene is sometimes recommended in order to obtain a more uniform and rapid ripening, as well as better development of color, wax, and aroma (Suslow et al. 2002). The ability to produce ethylene has also been suggested to correlate with rind type. The surface meshwork found in the fruit epidermis of melons consists of an elaborate system of lenticels that are derived from the subepidermal periderm (Webster and Craig 1976). It is thought that the netted rind might enhance gas exchange between the atmosphere and the melon mesocarp, thereby contributing to emission of ethylene molecules.

Genetic Regulation of Ripening Behavior in Melon

It is widely known that the ripening behavior of melon fruits (climacteric or non-climacteric) is genetically determined (Pech et al. 2008). Climacteric ripening is dominant over non-climacteric, and whenever climacteric and non-climacteric genotypes are crossed, fruits of the resultant F_1 progeny exhibit the climacteric phenotype. For example, crossing of the non-climacteric 'Honeydew' with the climacteric 'Charentais' produces hybrids of the climacteric type (Ezura et al. 2002).

Perin et al. (2002) identified and reported several QTLs associated with fruit abscission and ethylene production, using a recombinant inbred line (RIL) population derived from a cross between the non-climacteric 'Songwhan Charmi (PI 161375)' (*C. melo* subsp. *agrestis*, *conomon*group) and the climacteric 'Védrantais' (*C. melo* subsp. *melo*, *cantalupensis* group). The 'Védrantais' fruits exhibit a dramatic rise in ethylene production, as well as respiration, at the onset of ripening, while the 'PI 161375' fruits exhibit almost no increase in either phenotype. In 'PI 161375', exogenously applied ethylene also fails to stimulate abscission, loss of firmness, and ethylene production. By analyzing the segregation of the formation of the abscission layer (Al) of the peduncle and ethylene production, the climacteric character was found to be controlled by two independent loci, *Al-3* and *Al-4* on linkage group (LG) VIII and LG IX, respectively. The intensity of ethylene production was also controlled by at least four QTLs, which were localized in other genomic regions.

Moreno et al. (2008) also conducted a QTL analysis of fruit ripening behavior, using a near-isogenic line (NIL) derived from a cross between two non-climacteric melons, 'Piel de sapo' (*C. melo* subsp. *melo*, *inodorus* group) and 'PI 161375'. Although non-climacteric parents were used for NIL development, one QTL (*eth3.5*) in the line 'NIL SC3-5-1' was found to exhibit climacteric ripening behavior with increased respiration and ethylene levels, indicating that the non-climacteric

phenotype is brought about by different mechanisms in 'Piel de sapo' and 'PI 161375'. Recently, two additional QTLs (*eth3.5* and *ethqv6.3*) on LG III and LG VI, respectively, were reported to produce climacteric ripening independently (Vegas et al. 2013), and were found to additively affect both fruit abscission and ethylene emission. Taken together, there are at least several independent QTLs that confer climacteric fruit ripening in melon.

The highly complex nature of genetic structure in melon accessions has been recently uncovered using 251 single nucleotide polymorphism (SNP) datasets from 175 melon accessions. Leida et al. (2015) classified 175 melon accessions into seven sub-groups (populations) based on the SNPs' variation. This classification matched well with traditional classifications in some melon accessions. For example, 'Védrantais', a French *cantalupensis* group melon cultivar, was classified into a sub-group that consists exclusively of 'Charentais' varieties. Similarly, some *reticulatus* and *inodorus* cultivars, such as 'Dulce' and 'Piel de sapo', were classified into sub-groups that are representative of each group. On the other hand, more than half of the accessions expressed admixed genotypes, suggesting that mating events between populations may have occurred in the past. For example, the 'Earl's favorite' accession, which is a major breeding line in Japan and is traditionally classified in the *reticulatus* group, exhibited admixture of at least five populations. Although there is a strong genetic structure among melon accessions, which increases the chance of finding false-positive genotype-phenotype associations, accessions of the *ameri* group were suggested to be suitable for association mapping because of the lack of genetic structure and the presence of high phenotypic and molecular diversity. Some SNPs have been assessed and identified as potentially associated with variation in sugar accumulation and ripening behavior (Leida et al. 2015).

Regulation of Ethylene Biosynthesis Gene Expression in Melon

Two types of ethylene synthesis mechanisms are known in plants: system 1 and system 2 (McMurchie et al. 1972). System 1 ethylene synthesis is responsible for the basal level of ethylene production in vegetative tissues or unripe fruit and is regulated by a negative-feedback mechanism, wherein ethylene inhibits its biosynthesis. By contrast, system 2 ethylene synthesis operates during senescence of delicate flowers, such as carnation, or ripening of climacteric fruits, where a dramatic rise of ethylene production is observed. System 2 ethylene synthesis is regulated by a positive-feedback mechanism in which ethylene activates its biosynthesis (Nakatsuka et al. 1998; Lelièvre et al. 1997; Olson et al. 1995). In tomato, RIN and CNR-dependent upregulation of specific ACS and ACO isoforms (*SlACS2*, *SlACS4*, and *SlACO1*) is responsible for ethylene production during fruit ripening (Fujisawa et al. 2012; Fujisawa et al. 2013; Martel et al. 2011). In addition, epigenetic regulation mediated by DNA demethylation is involved in the transcriptional activation of ethylene biosynthesis genes, as well as the activation of master regulators, at the onset of fruit

ripening (Liu et al. 2015; Zhong et al. 2013). It is thought that this epigenetic regulation is a prerequisite for phase transition from the unripe stage (system 1) to the ripening-competent stage (system 2). Protein stability of some ACS isoforms is further regulated by phosphorylation of its C-terminal domain through mitogen-activated protein kinase and/or calcium-dependent protein kinase (CDPK) (Choudhury et al. 2013). In tomato, SlACS2 protein stability has been shown to be regulated by CDPK-dependent phosphorylation (Kamiyoshihara et al. 2010).

Although it is currently unknown whether molecular mechanisms similar to those observed in tomato are also involved in melon ripening, the melon genome carries at least eight ACS and five ACO genes (Balague et al. 1993; Boualem 2008; Garcia-Mas et al. 2012; Ishiki et al. 2000; Lasserre et al. 1996; Miki et al. 1995) (Fig. 1). The expression patterns and regulatory mechanisms of some of the melon ACS genes have been investigated in detail by molecular biological studies. *CmACS1*, for example, was first identified as a wound-responsive gene and is known to be transcribed in the mesocarp of ripening fruits, as well (Yamamoto et al. 1995). *CmACS1* expression level increases in climacteric fruit after the burst of ethylene, suggesting that the positive-feedback mechanism works on the regulation of *CmACS1* expression (Owino et al. 2007; Sato-Nara et al. 1999; Yamamoto et al. 1995). In contrast, *CmACS2* was isolated from etiolated seedlings that had been treated with the growth-promoting phytohormone auxin (Ishiki et al. 2000). The expression of *CmACS2* is up-regulated transiently at the pre-climacteric stage and then declines at the time of harvest. The up-regulation of *CmACS2* expression coincides with rapid fruit development, which further suggests its induction by auxin (Ishiki et al. 2000). Putative auxin-responsive elements (AuxRDs), as well as a dehydration responsive element/C-repeat (DRE/CRT) element (‘GCCGAC’), have also been found in the promoter region of *CmACS2* (Mizuno et al. 2006), and yeast one-hybrid screening further identified the DRE binding factor (DREB) CmDREB1 and the ERF proteins CmERF1 and CmERF2 as candidate transcription factors for binding to the DRE/CRT element. *CmACS3* has also been shown to respond to auxin; the expression pattern of *CmACS3* is similar to *CmACS2*, although the *CmACS3* expression levels are lower than *CmACS2*. Another gene, *CmACS5,* has been reported as highly expressed in ripening fruit, and its expression is regulated in an ethylene-independent manner (Li et al. 2006). This gene has been recently shown to be a functional orthologue of cucumber *androecy* gene, *CsACS11*, that controls sex determination (Boualem et al. 2015). Therefore, *CmACS5* gene is also called as *CmACS11*. In addition to the *CmACS5/CmACS11*, *CmACS7* has been identified as the causal gene for *andromonoecious* (*a*) locus in melon (Boualem et al. 2008). In melon, sex determination of flowers can be genetically controlled by the *a* and *gynoecious* (*g*) loci, and the interplay of these two genes results in a range of sexual types (Martin et al. 2009). Among the ACO genes, *CmACO1* expression is induced during fruit ripening, as well as in response to wounding and ethylene treatment of leaves. *CmACO2* gene expression is detected at low levels in etiolated hypocotyls, and *CmACO3* is expressed in flowers (Balague et al. 1993; Lasserre et al. 1997; Yamamoto et al. 1995).

Shiomi et al. (1999) compared the ethylene biosynthetic activity as well as expression patterns of ACS and ACO genes in two different netted melon cultivars, 'Andes' and 'Earl's Favourite'. The 'Andes' fruits produce a considerable amount of ethylene and tend to have a shorter shelf-life than the 'Earl's Favourite' fruits, which produce a lesser amount of ethylene. *CmACS1* transcripts accumulate in the mesocarp and placenta of 'Andes' fruits at commercial harvest maturity, but not in any tissues of 'Earl's Favourite'. Shiomi et al. (1999) found that *CmACO1* transcripts accumulate in the mesocarp and placenta of both cultivars, whereas *CmACO2* was constitutively expressed. The expression of *CmACS1* was induced by exogenous ethylene in pre-climacteric 'Andes' fruits, but not in 'Earl's Favourite' fruits. Furthermore, ethylene perception inhibitor 1-MCP (1-methylcyclopropene) treatment inhibited the accumulation of *CmACS1*, *CmACO1*, and *CmACO2* in the 'Andes' cultivar. The authors concluded that the 'Earl's Favourite' fruit behaves like a non-climacteric fruit and that the main difference between the ethylene-forming capability of the two cultivars results from the expression of *CmACS1* during ripening. Another study has shown that *CmACO1* expression is induced by both ethylene treatment and wounding stimuli (Bouquin et al. 1997), and analysis of the gene's promoter sequence suggests that two independent mechanisms are responsible.

Recently, Saladie et al. (2015) reported a comparative transcriptome analysis using an oligo-based microarray with 75 K probes in two climacteric melon varieties, 'Védrantais' (subsp. *melo, cantalupensis* group) and 'Dulce' (subsp. *melo, reticulatus* group), and two non-climacteric varieties, 'PI 161375' (subsp. *agrestis, conomon* group) and 'Piel de sapo' (subsp. *melo, inodorus* group). Among the differentially expressed genes (DEGs), 450 genes were identified as common DEGs, whose expression levels were variable during fruit development and ripening in all genotypes, while 983 and 177 DEGs were specific to climacteric and non-climacteric cultivars, respectively. Among five *CmACS* genes represented on microarray, the expression levels of *CmACS1* were found to increase up to 10,000-fold at ripening stage, when compared with the corresponding levels at the developing stage, in the two climacteric varieties. Up-regulation of *CmACS1* expression was also observed in the non-climacteric 'PI 161375', although its extent was lesser than climacteric varieties. Similar to *CmACS1*, expression levels of *CmACS5* increased during ripening, but this up-regulation was restricted to the two climacteric varieties. Among four ACO genes represented on the microarray, expression levels of *CmACO1* significantly increased during ripening in both the climacteric varieties. 'PI 161375' also exhibited moderate up-regulation of *CmACO1,* like *CmACS1*. Expression of both *CmACS1* and *CmACO1* is constant and remains lower in 'Piel de sapo' than in the two climacteric varieties throughout fruit development and ripening, which is consistent with the non-climacteric behavior of this cultivar. Interestingly, the expression patterns of some other genes in 'PI 161375' were either intermediate between 'Piel de sapo' and the climacteric varieties or were more similar to the latter, as with *CmACS1* and *CmACO1*, indicating that classification of melon varieties into just two categories, climacteric and non-climacteric, is an over-simplification (Saladie et al. 2015).

Ethylene Perception and Signaling in Melon

As introduced above, the molecular mechanism of ethylene perception and signal transduction has been intensively studied in the model plant *Arabidopsis* (Ju and Chang 2015). Based on distinguishing structural features and overall sequence similarity, the members of the ethylene receptor family in *Arabidopsis* (*AtETR1*, *AtERS1*, *AtETR2*, *AtEIN4*, and *AtERS2*) can be divided into two subfamilies: subfamily I and II. Subfamily I ethylene receptors, *AtETR1* and *AtERS1*, have three transmembrane domains and a conserved histidine kinase domain, while subfamily II receptors, *AtETR2*, *AtEIN4*, and *AtERS2*, contain a putative signal peptide in addition to the three conserved transmembrane domains and a histidine kinase domain that lacks one or more elements necessary for catalytic activity. Although dominant negative mutations in a single receptor gene are known to confer ethylene insensitivity in both *Arabidopsis* and tomato (Chang et al. 1993; Okabe et al. 2011; Wilkinson et al. 1995; Yen et al. 1995), there are differences in the receptor system between the two plants (Klee and Giovannoni 2011). In *Arabidopsis*, no single loss-of-function mutations are known to result in a major effect on ethylene responses, indicating a substantial degree of functional redundancy among the members (Hua and Meyerowitz 1998). However, in tomato, subfamily II members seem to play a more important role than subfamily I members, because reduction (loss-of-function) of either SlETR4 or SlETR6 protein levels can confer ethylene-hypersensitive phenotype, in terms of fruit ripening, which can be restored by overexpression of *NR* (*SlETR3*) (Kevany et al. 2007; Tieman and Klee 1999).

In the melon genome, there are at least three ethylene receptor genes. *CmERS1* and *CmETR1* belong to subfamily I whereas *CmETR2* belongs to subfamily II ethylene receptors (Owino et al 2007; Sato-Nara et al. 1999). Additional components of the ethylene signal transduction pathway have also been reported (Saladie et al. 2015) (Fig. 2). In muskmelon from the Japanese *reticulatus* group, expression levels of *CmERS1* were shown to increase during enlargement of young fruits but decrease at the end of enlargement, while those of *CmETR1* are higher in the seed and placenta of developing and fully enlarged fruit than in other tissues. *CmETR1* expression levels also increase concurrent with the beginning of ethylene production during ripening. Expression of the subfamily II ethylene receptor *CmETR2* is induced earlier than those of *CmETR1* during ripening, but is down-regulated in parallel with reduction in ethylene production. *CmETR2* expression is also induced by ethylene treatment and suppressed by 1-MCP, indicating that *CmETR2* expression is regulated by a positive-feedback mechanism (Owino et al. 2007). CmERS1 protein is detected in the early stage of fruit development but not at the ripening stage, even though *CmERS1* transcripts can be detected in the same stage (Takahashi et al. 2002), suggesting that CmERS1 protein level is post-transcriptionally regulated. Similar regulation has also been reported in tomato, in which expression levels of *Nr*, *SlETR4*, and *SlETR6* do not correlate with their protein levels during fruit ripening (Kevany et al. 2007). CmERS1 protein accumulates at high levels in the pericarp of both cultivars at the early stages of fruit development, where frequent

cell division occurs. Membrane fractionation and GFP imaging analyses further reveal that the CmERS1 protein is localized at the endoplasmic reticulum (Ma et al. 2006). An N-glycosylation mutagenesis strategy further demonstrated that CmERS1 has three membrane-spanning domains, with its N-terminus facing the luminal space and the large C-terminal portion being located on the cytosolic side of the ER membrane.

A recent transcriptomics analysis that compared the expression profiles of climacteric and non-climacteric melon varieties indicates constant expression patterns of ethylene receptor genes and down-stream *EIN3-like* genes during fruit development and ripening (Saladie et al. 2015). However, differential expression of some ethylene response-related genes has been reported (Saladie et al. 2015). A putative *N-ACETYLTRANSFERASE HOOKLESS* (*CmATH*) gene and five members of putative EREBP (Ethylene-responsive element binding protein) transcription factor genes were identified as differentially expressed genes with higher expression levels in climacteric 'Védrantais' and 'Dulce' and intermediate in 'PI 161375' than the non-climacteric 'Piel de sapo'. By contrast, two other EREBP transcription factor genes were constantly expressed in 'Piel de sapo' and more highly than in other varieties.

Ethylene-Dependent and -Independent Biochemical Processes in Melon Ripening

Fruit ripening accompanies many biological changes, including accumulation of carotenoids, sugars, and volatiles. Involvement of ethylene-dependent and -independent pathways in the regulation of each process has been known in melon. Fruits of the climacteric 'Charentais' variety (*C. melo* var. *reticulatus* F_1 Alpha) undergo a rapid decrease in fruit firmness concurrent with a rise of ethylene production (Rose et al. 1998). Treatment of fruits with ethylene antagonist 1-MCP completely inhibits the softening process, indicating that the overall process of cell wall disassembly in the ripening melons is regulated by ethylene (Nishiyama et al. 2007). Transgenic melon plants with suppressed ethylene biosynthesis have also provided genetic evidence for ethylene-dependent and -independent regulation of fruit ripening. One of the pioneer studies was antisense-suppression of the *CmACO1* gene in the Charentais'-type cultivar 'Védrantais' (Ayub et al. 1996). This results in 97–99 % reduction in ethylene production relative to the wild type, even at the late stage of fruit ripening, as well as inhibition of various ripening processes. In this ACO-suppressed melon, continuously applied exogenous ethylene is required to restore fruit ripening in terms of rind yellowing, fruit softening, and activation of the peduncle abscission zone (Ayub et al. 1996; Guis et al. 1997). By contrast, coloration of the flesh and accumulation of sugars and organic acids are not affected in the transgenic melon. Subsequent biochemical analysis of cell wall polysaccharides in the ACO-suppressed transgenic melon, with or without exogenous ethylene, indicated that depolymerization of both pectins and xyloglucans are also strongly affected by

ethylene (Nishiyama et al. 2007). Similar results were also reported in transgenic 'Védrantais' melon in which an apple ACO gene was overexpressed in antisense orientation (Silva et al. 2004). The loss of chlorophyll in the rind, softening of the flesh, reduction of acidity, and maturation of the peduncular abscission zone were inhibited in the transgenic line, whereas the accumulation of carotenoids and soluble solids were not affected, indicating that the former processes are influenced by ethylene but the latter ones are not. The male parental line ('Krimka') of 'Galia' muskmelon has also been transformed with the *CmACO1* antisense gene in an attempt to delay fruit ripening (Nunez-Palenius et al. 2006; Nuñez-Palenius et al. 2006). Similar to other ACO-suppressed melon, yellowing of the rind, ripening index, decrease in titratable acidity and fruit softening were affected in the transgenic melon, while fruit size, seed development and mesocarp total soluble solids and pH were not affected. More recently, EMS-induced mutant alleles of *CmACO1* gene were isolated by TILLING in the inbred climacteric 'Charentais'-type cultivar 'CharMono' (*cantalupensis* group) (Dahmani-Mardas et al. 2010). The G194D mutation, which occurs in a highly conserved amino acid position of ACO, was shown to alter shelf-life, firmness, and rind color of the mutant fruits but did not affect fruit shape, sugar accumulation, or flesh color. In addition to the approaches described above, reduced ethylene production has also been achieved in transgenic melon that overexpress the T_3 bacteriophage *S*-AdoMet hydrolase (SAMase) gene under the control of a fruit specific promoter (chimeric ethylene-responsive E8/E4 promoter) (Clendennen et al. 1999). The SAMase enzyme catalyzes the degradation of *S*-AdoMet, a precursor of ethylene synthesis. Although 75 % less ethylene production was observed in transgenic fruits compared to wild type, the onset of maturity, measured on four different dates, was not significantly delayed, suggesting that the residual ethylene production was enough to induce ripening (Ayub et al. 1996). Taken together, it is considered that ripening processes such as climacteric respiration, aroma production, formation of the peduncular abscission zone, and yellowing, are largely affected by ethylene-dependent regulatory mechanisms, while sugar accumulation, titratable acidity, organic acid metabolism, total soluble solids, fruit weight and size, seed number, mesocarp size, and carotenoid accumulation in the flesh are affected by ethylene-independent ones (Pech et al. 2008). However, this does not exclude the possibility that distinct mechanisms converge to regulate the same process. Actually, expression of some cell wall metabolism genes has been reported to be regulated in an ethylene-independent manner during ripening of melon fruit (Nishiyama et al. 2007).

Dissection of Melon Ripening by Metabolite and Gene Expression Profiles

With advances in analytical equipment, it has become much easier to obtain comprehensive datasets of metabolites, transcriptomes, and genomic information in a large population. It is an interesting idea to statistically decipher the

ripening behavior and the quality of melon fruit based on such datasets, because it is often difficult for farmers or producers to judge the best timing for fruit harvest. For this purpose, extensive metabolic profiling has been conducted in the fruits of several melon genotypes (Allwood et al. 2014; Bernillon et al. 2013; Lee et al. 2014; Moing et al. 2011; Vallone et al. 2013). Among them, volatile profiling has been proven as useful as it enables evaluation of the fruit maturity without inflicting damage. Vallone et al. (2013) has demonstrated that a portable volatile-sensing system, ultra-fast gaschromatograph coupled with a surface acoustic wave sensor (UFGC–SAW), is useful to evaluate the maturity stage of muskmelon fruit (*C. melo* var. *reticulatus*). The abundance information of six measured peaks obtained by UFGC–SAW analysis was shown to positively correlate with sensory attributes in different maturity stages of melons. Allwood et al. (2014) also demonstrated that volatile profiling by thermal desorption gas chromatography mass spectrometry (TD-GC-MS) is an effective approach to distinguish melon shelf-life. A principal component analysis using the profiles of 58 volatiles successfully distinguished the shelf-life of four *cantalupensis* and one *inodurus* melons.

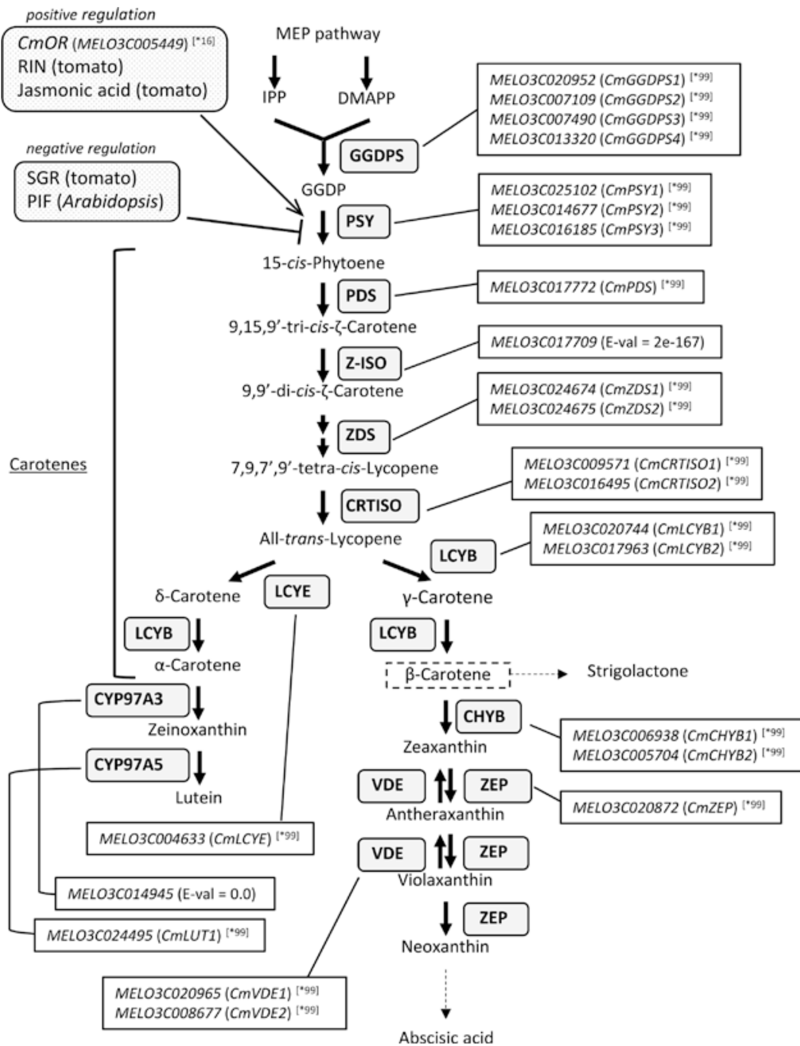
An integrative multi-omics analysis that employs metabolite-metabolite and metabolite-transcriptome association was recently reported by Freilich et al. (2015) to obtain insight into the intricate association of distinct biochemical processes in melon fruit. In this study, the endogenous levels of 77 metabolites that included ethylene, sugars, β-carotene, and aromatic compounds, as well as the expression levels of ~27,000 unigenes, were obtained individually in a RIL population of 96 individuals that was derived from a cross between 'Dulce' (*reticulatus* group) and 'PI 414723-S5' (*C. melo* var. *momordica*). Fruits of the 'Dulce' are sweet and aromatic with orange flesh, while those of the 'PI 414723' are sour and have an undesirable sulfurous aroma with light orange flesh. There is significant and continuous variation in both metabolite levels and gene expression levels in the 96 RIL populations with some progenies showing transgressive segregation. A hierarchical clustering based on metabolite-metabolite association shows that the quantified metabolites are classified into three groups (Clusters I, II and III). Metabolites in Cluster I include sucrose and β-carotene and are not associated with ethylene. In contrast, those of Clusters II and III mainly include the components of desirable and undesirable aromas, respectively, and are associated with ethylene. Subsequent metabolite-transcriptome clustering, combined with MAPMAN ontology analysis (Thimm et al. 2004), further demonstrated that the non-ethylene-associated Cluster I metabolites are associated with genes involved in plastid formation and activity rather than those involved in the synthesis of Cluster I metabolites. On the other hand, ethylene-associated Cluster II and III metabolites are characterized by enhanced activity of enzymes involved in the tricarboxylic acid (TCA) cycle and sulfur metabolism. These results agree with the previous notion that sweetness and carotenoid accumulation in the flesh are ethylene-independent, whereas volatile production and respiration burst are ethylene-dependent (Ayub et al. 1996).

Regulation of Carotenoid Accumulation in Melon

Chromoplasts are a specialized from of plastid that accumulates large quantities of carotenoids in the form of crystal structures (Egea et al. 2010; Klee and Giovannoni 2011; Nisar et al. 2015). In fruit tissues, chloroplasts differentiate from preexisting chloroplasts. During the differentiation, the photosynthetic capacity of affected chloroplasts is lost as the thylakoid structures disassemble, and instead, plastoglobules accumulate at the site of carotenoid accumulation. Carotenoids are a group of C_{40} isoprenoids that serve as components of photosynthetic machinery, precursors of phytohormones, and important contributors to both fruit nutrition and flower color (Cazzonelli and Pogson 2010; Yuan et al. 2015). The carotenoid biosynthetic pathway has been well defined in higher plants, and whole genome sequencing of melon identified possible genes involved in the pathway (Fig. 4). Phytoene synthase (PSY) catalyzes the first committed step in carotenoid biosynthesis and controls carbon flux into the subsequent pathway. Phytoene desaturase (PDS), ζ-carotene desaturase (ZDS), ζ-carotene isomerase (Z-ISO), and carotenoid isomerase (CRTISO) are responsible for *trans*-lycopene production, which is then used as the substrate for biosynthesis of β-carotene, lutein, and phytohormones, such as abscisic acid and strigolactone (Nisar et al. 2015). Alteration of PSY expression is known to significantly affect carotenoid content, not only in *Arabidopsis,* but also in crop plants (Cao et al. 2012; Diretto et al. 2007; Ducreux et al. 2005; Fraser et al. 2007; Maass et al. 2009; Welsch et al. 2010). In tomato fruits, for example, the MADS-Box transcription factor RIN binds to the promoter of *PSY1* and activates its expression during fruit ripening in a CNR-dependent manner (Martel et al. 2011). In addition, the phytohormone jasmonic acid, rather than ethylene, has been shown to be involved in the regulation of *PSY1* gene expression (Liu et al. 2012), and the tomato STAY-GREEN (SlSGR) protein has been shown to directly interact with PSY by inhibiting its enzymatic activity (Barry et al. 2008; Luo et al. 2013). The function of SGR is not restricted to the regulation of PSY enzyme and actually affects the regulation of more global processes, such as chloroplast-chromoplast differentiation. The tomato mutant of *SlSGR*, traditionally known as *green-flesh* (distinct from melon *gf* described below), exhibits a pleiotropic phenotype that includes lycopene accumulation, as well as a lack of chlorophyll degradation.

In melon, flesh color is determined by a balance of chlorophyll and carotenoid pigments, resulting in white, green, and orange colors (Burger et al. 2010). β-carotene is the main carotenoid molecule accumulated in orange-flesh melon varieties (Nunez-Palenius et al. 2008). Genetic studies have shown that there are two major genes responsible for the determination of flesh color; *green flesh* (*gf*) and *white flesh* (*wf*) (Clayberg et al. 1992; Hughes 1948). *Gf* is a dominant allele that confers orange flesh to the melon fruit irrespective of the *Wf* allele. When a melon plant carries a homozygous '*gf gf*' genotype, the flesh color becomes white (*Wf*-) or green (*wfwf*), dependent on the *Wf* allele. Recently, Tzuri et al. (2015) identified the *Gf* gene by genetic linkage analysis using a population derived from a cross between the orange-fleshed 'Dulce' (*reticulatus*

group) and the light-green-fleshed 'Tam Dew' (*inodorus* group). The gene was named *CmOr* according to its orthologous relationship with the cauliflower *Orange* (*Or*) gene, which encodes a DnaJ cysteine-rich, domain-containing protein that was previously suggested to regulate chloroplast-chromoplast differentiation (Lu et al. 2006). All orange-flesh melon varieties, including 'Védrantais', 'PI 414723', and 'Cezanne', carry a dominant *CmOR*^{orange} allele, whose translated protein contains His[108], while green or white-flesh varieties, such as 'Noy Yizre'el', 'PI 161375', 'Noy Amid', and 'Piel de sapo', carry a *CmOR*^{green} allele



whose translated protein contains Arg^{108}. In addition, transient expression of *CmOR*^{orange} is able to induce β-carotene accumulation in *Arabidopsis* callus, thus indicating the conserved role of the OR protein. In *Arabidopsis*, *AtOR* was recently shown to regulate the protein stability of PSY, but not its gene expression (Zhou et al. 2015). Another important insight into the *OR*-dependent regulation of carotenoid accumulation and chloroplast-chromoplast differentiation has also been obtained in melon by a study that used a bulked segregant RNA-seq (BSR-seq) approach (Chayut et al. 2015). Consistent with the result in *Arabidopsis*, expression levels of a series of carotenoid biosynthesis genes were not affected by *CmOr* allelic variation. Instead, an ontology analysis of DEGs between orange-flesh and green-flesh bulked segregants identified enrichment of specific functional groups in the DEGs, such as photosynthesis, RNA and protein regulation, and stress response. These DEGs may represent a part of the regulatory network that controls chloroplast-chromoplast differentiation.

Perspectives

Recent publication of the whole melon genome has greatly facilitated genome-scale studies of fruit ripening in the species (Argyris et al. 2015; Garcia-Mas et al. 2012). Comprehensive transcriptome analysis, as well as integrated

Fig. 4 *C. melo* genes relating to carotenoid biosynthesis. The carotenoid metabolic pathways in horticultural crops generally start with geranylgeranyl diphosphate (GGDP) that is synthesized from isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Phytoene synthase (PSY) catalyzes the first committed condensation step from GGDP to produce the first C_{40} carotene phytoene. Following several desaturation and isomerization steps, lycopene is produced, and subsequent cyclization reactions yield the α-carotene and β-carotene branches. β-carotene is a major compound that confers orange color to the flesh in melon. The melon genes previously annotated to each enzyme reaction are described in the figure, along with the biosynthesis pathway (Saladie et al. 2015) (indicated by asterisked numbers). The best possible matches (lowest E-value in BLAST search) for *Z-ISO* and *CYP93A3* were identified by BLAST search with software version 2.2.29+ using corresponding *Arabidopsis* genes as queries and the *C. melo* whole genome protein sequence dataset (version 3.5 obtained from the "Melonomics" website, https://melonomics.net/, (Garcia-Mas et al. 2012)) as the database. *CmOR* (*MELO3C005449*) is a positive regulator of the PSY enzyme that was identified by a genetic study (Tzuri et al. 2015). In tomato, RIPENING-INHIBITOR (RIN) and the phytohormone jasmonic acid are involved in the positive regulation of *PSY* gene expression (Fujisawa et al. 2013; Liu et al. 2012; Martel et al. 2011), while STAY-GREEN (SGR) inhibits PSY activity through direct protein-protein interaction (Luo et al. 2013). In *Arabidopsis*, phytochrome-interacting factor (PIF) is involved in the light-dependent negative regulation of *PSY* expression in etiolated seedlings (Toledo-Ortiz et al. 2010). *GGDPS* GGDP synthase, *PDS* phytoene desaturase, *Z-ISO* ζ-carotene isomerase, *ZDS* ζ-carotene desaturase, *CRTISO* carotenoid isomerase, *LCYE* lycopene ε-cyclase, *LCYB* lycopene β-cyclase, *CHYB* β-carotene hydroxylase, *CYP97C* cytochrome P450-type monooxygenase 97C, *ZEP* zeaxanthin epoxidase, *VDE* violaxanthin de-epoxidase

approaches of transcriptome, metabolite, and gene ontology analysis, have enabled robust bioinformatics approaches to dissect the complex regulatory mechanisms underlying melon fruit ripening, especially in a systems biology approach (Chayut et al. 2015; Freilich et al. 2015; Saladie et al. 2015). With respect to metabolite analysis, volatile information has been demonstrated as useful for evaluating fruit maturity in several melon genotypes (Allwood et al. 2014; Vallone et al. 2013). Further multivariate data analysis that integrates RNA-seq and multi-platform non-targeted metabolomics is likely to uncover a more comprehensive picture of the molecular network that governs fruit ripening, and the analysis will identify useful biomarkers that will also enable evaluation of fruit quality before harvest. In addition to these strategies, a resequencing approach that employs high-throughput next-generation DNA sequencing (e.g., Illumina Hiseq) is now proceeding and uncovering millions of SNPs and indels in the genomes of several representative climacteric and non-climacteric melon genotypes. In the future, newer technology, such as single-molecule DNA sequencing, may further clarify structural variation, such as copy-number variation, in addition to SNPs and indels. Together with the characterization of QTLs previously identified and reported by (Moreno et al. 2008; Perin et al. 2002; Vegas et al. 2013), comparative genomic studies that use comprehensive datasets will contribute greatly to elucidating the molecular basis of diversity in fruit ripening behavior, as well as other fruit quality traits, in melon varieties and wild relatives. By combining genotype, transcriptome, and metabolome datasets, it will also become possible to conduct *in silico* identification of key genes that regulate melon ripening. On the other hand, molecular genetic studies in tomato have already identified several key regulators involved in fruit ripening, such as *RIN, CNR, NR,* and *GR*, and epigenetic regulation through the DNA demethylase *SlDMR2* has also been shown to play a role in fruit ripening. Although the melon genome carries no apparent orthologues, but only partially conserved homologues for tomato genes (Table 1; E-value $\geq$ 2.0E-126, similarity $<$ 69 %), it is of particular interest to investigate whether similar molecular networks of transcription factors also play a role in melon fruit ripening. For this purpose, reverse genetics studies that employ TILLING and genome editing technologies are required to obtain the genetic evidence for the role of each candidate gene. This approach is also necessary to examine the role of DEGs or 'hub' genes, the expression of which is associated with ripening behavior (climacteric vs. non-climacteric) and accumulation of important metabolites, such as sugar and carotenoids. Together with systems biology and comparative genomics studies, establishment of TILLING and genome editing methodologies in several representative melon varieties will be key to investigating gene functions, as well as to developing new elite cultivars based on scientific knowledge.

Table 1 *C. melo* genes homologous to the known fruit ripening regulators of tomato

Tomato gene code	Tomato gene name	Hit with lowest E-value	E-value	Score	Identity (%)
Solyc10g006880.2.1	*NON-RIPENING* [38, 43]	*MELO3C016540*	3.0E-119	352	54.99
Solyc05g012020.2.1	*RIPENING-INHIBITOR* [41]	*MELO3C026300*	5.0E-98	290	59.11
Solyc02g077920.2.1	*COLORLESS-NONRIPENING* [42]	*MELO3C025597*	2.0E-43	144	60.18
Solyc03g044300.2.1	*APETALA2a* [65]	*MELO3C007572*	1.0E-119	355	62.12
Solyc03g114840.2.1	*SlMADS1* [63]	*MELO3C026300*	5.0E-105	308	63.45
Solyc07g055920.2.1	*TOMATO AGAMOURS-LIKE 1* [47, 61]	*MELO3C002691*	4.0E-93	278	64.63
Solyc06g069430.2.1	*FRUITFULL 1* [62, 69, 70]	*MELO3C002050*	2.0E-107	314	64.94
Solyc03g114830.2.1	*FRUITFULL 2* [62, 69, 70]	*MELO3C002050*	5.0E-114	330	68.42
Solyc02g086930.2.1	*HB-1* [64]	*MELO3C021534*	7.0E-94	282	57.99
Solyc11g017470.1.1	*NAC4* [66]	*MELO3C010632*	2.0E-126	366	62.46
Solyc08g080090.2.1	*STAY-GREEN/GREEN FLESH* [156]	*MELO3C005616*	6.0E-119	345	68.15

The best possible matches from the melon genome (lowest E-value in BLAST search) for *NON-RIPENING, RIPENING-INHIBITOR, COLORLESS-NONRIPENING, APETALA2a, SlMADS1, TOMATO AGAMOUS-LIKE 1, FRUITFULL 1* and *2, HB-1, NAC4,* and *STAY-GREEN/GREEN FLESH* were searched using BLAST software (version 2.2.29+) with each tomato gene used as a query and the *Cn melo* whole genome protein sequence dataset (version 3.5 obtained from the "Melonomics" website, https://melonomics.net/, (Garcia-Mas et al. 2012)) as the database

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Genomic Aspects of Melon Fruit Quality

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Abstract Fruit quality in melon (*Cucumis melo*) and in other cucurbit species is primarily determined by sweetness, acidity, aroma, color and shelf-life. During ripening, the mesocarp (fruit flesh), the consumed tissue, generally softens due to degradation of cell walls, and accumulates soluble sugars, organic acids, volatiles and additional secondary metabolites. Flesh and rind color undergo developmental changes, the most noticeable of which are changes in pigmentation. This chapter reviews the current knowledge of genes that regulate, or participate in, the major metabolic pathways affecting sugar and acid metabolism (sweetness), volatile organic compounds (aroma) and pigments (color) of the melon fruit.

Keywords Transcriptome • Sugar metabolism • Carotenoids • Flavonoids • Aroma • Volatile organic compounds (VOCs)

Introduction

Fruit ripening is a genetically programmed event, characterized by a number of biochemical and physiological processes that alter fruit firmness, color, flavor, aroma, and texture (Giovannoni 2001, 2004, 2007). Fruit quality in melon (*Cucumis melo* L.) and other cucurbit species is primarily determined by the three major components of taste, i.e. sugars, aroma and volatile flavor compounds, together

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with color and length of storage life. Melon fruits display an extreme diversity of traits including size, shape, flesh and rind color, netting, aroma, sugar content, acidity and presence of different volatile organic compounds (Burger et al. 2006, 2009; Grumet et al. 2007; Nuñez-Palenius et al. 2008). We here review the current knowledge of genes that regulate, or participate in, the major metabolic pathways affecting sugar and acid metabolism (sweetness), volatiles (aroma) and color of the melon fruit.

Historical Perspective

The first gene cloned in melon, over 20 years ago, was *ACC oxidase CmACO1*, (MEL1, Balague et al. 1993). This gene was cloned from the first cDNA library of melon, using a tomato probe termed at the time "ethylene forming enzyme". The silencing of this gene resulted in a major reduction of ethylene production in a Charentais-type melon (expressing an antisense *CmACO1* gene), and these experiments had a major impact on the understanding of ethylene-dependent and ethylene-independent metabolic pathways in the fruit (Ayub et al. 1996). The major findings of that study were that the synthesis of aroma volatiles, respiratory climacteric and degreening of the fruit rind were all ethylene-dependent processes while the initiation of climacteric ripening, sugar accumulation, coloration, and loss of acidity of the fruit flesh were ethylene-independent (Ayub et al. 1996; Pech et al. 2008). This was the first demonstration that melon differs from the iconic model species of fleshy fruit, tomato, in its regulation of certain metabolic pathways that are associated with fruit development, including the accumulation of sugar and carotenoids, and the loss of acidity. The silenced *CmACO1* melon line was the subject of further studies, leading to a deeper insight into the effects of ethylene-dependent and -independent processes on melon fruit biology (Guis et al. 1997; Bauchot et al. 1998; Hadfield et al. 2000; Flores et al. 2001a, b). Additional silencing studies of ethylene-related genes were reported, including a *CmACO1* gene in a different background (Nuñez-Palenius et al. 2006; Hao et al. 2011).

This first melon fruit cDNA library (Balague et al. 1993) enabled the cloning of additional important melon fruit genes such as *phenylalanine ammonia lyase* [*PAL*, (Diallinas and Kanellis 1994), *phytoene synthase* (Karvouni et al. 1995), *CmAC02* and *CmAC03* (Lasserre et al. 1996), Mel2 and Mel7 (a homologue of a *major latex protein* gene in opium poppy), both cloned based on differential expression by Aggelis et al. (1997). Mel2 was later functionally identified as an *alcohol acetyltrasferase* (*CmAAT1*) by Yahyaoui et al. (2002) (see below). The functions of several of these genes were analyzed (e.g. *CmAAT*, (Yahyaoui et al. 2002; El-Sharkawy et al. 2005) while the functions of others are still unknown (e.g. MEL7). Additional fruit cDNA libraries were developed soon after, leading to deeper understanding of cell wall metabolism during fruit ripening (Hadfield et al. 1998, 2000; Rose et al. 1998) and of ethylene synthesis and perception (Miki et al. 1995; Yamamoto et al. 1995; Ishiki et al. 2000), and reviewed in the "Fruit ripening in melon" (Chapter "Landscape Genomics of Angiosperm Trees: From Historic Roots to Discovering New Branches of Adaptive Evolution").

By 2004, approximately 100 melon gene sequences were available through the NCBI (National Center for Biotechnology Information) databases. Subsequently, with the advances in classical sequencing technologies, the number of ESTs constructed and sequenced increased significantly. Over 5000 cDNA clones from melon fruit (Ibdah et al. 2006) together with 1800 from melon phloem tissue (Omid et al. 2007), combined with the publically available NCBI genes, were assembled into 3269 unigenes deposited in the first melon database (http://melon.bti.cornell.edu/), later superceded by the Cucurbit Genomics Database (http://www.icugi.org, see "Database" Chapter "Phylogeny and Evolution of the Cucurbitaceae"). The information accumulated in the database enabled the cloning and functional analysis of five novel genes involved in the biosynthesis of fruit volatiles, *CmCCD1* (Ibdah et al. 2006), *CmTpsDul* and *CmTpsNY* (Portnoy et al. 2008), and *CmArAT1* and *CmBCAT1* (Gonda et al. 2010). Shortly thereafter, the Spanish melon consortium released nearly 30,000 melon ESTs, resulting in an additional 13,000 unigenes (http://www.melogen.upv.es/) (Gonzalez-Ibeas et al. 2007). An International Cucurbit Genomics initiative, funded by the industry, led to the development of over 100,000 melon ESTs, one quarter of which were fruit ESTs (http://www.icugi.org) (Clepet et al. 2011). In addition to gene discovery, these ESTs enabled the construction of saturated maps (Cuevas et al. 2008, 2009; Harel-Beja et al. 2010), mapping of candidate genes and a map merging project (Diaz et al. 2011) and "Genetic mapping of complex traits in cucurbits" (Chapter "Genetic Resources of Cucumber"). Clearly, the significant progress in fruit quality research was attained through the long term scientific collaboration between research teams and amalgamation of data from various sources.

The end of the past decade marked the transition to the application of Next Generation Sequencing (NGS) technologies, leading to comprehensive genomic and transcriptomic studies. The melon genome, published first in 2012 [(Garcia-Mas et al. 2012; Argyris et al. 2015) and "Melon Genome" (Chapter "Genetic Mapping of Complex Traits in Cucurbits")], was a breakthrough in melon research and greatly enhanced fruit quality research. Gene expression levels, derived from transcriptome analyses, further enhanced gene discovery, including regulatory genes that could not be cloned based on homology or predictions of metabolic pathways.

The first NGS-based transciptome analysis (454-pyrosequencing) of melon fruit (flesh and rind at four developmental stages) was described by Portnoy et al. (2011). In addition to a more than tenfold increase of the number of reads, the report provided an insight into the high accuracy of gene sequencing and of gene expression data obtained by 454-pyrosequencing, compared with classical methods of sequencing or qPCR. Expression patterns of 40 selected fruit-expressed genes were compared with expression profiles obtained by qPCR. Significant correlations were obtained between 454 pyrosequencing and qPCR data, except for genes having very low levels of expression. Shortly thereafter, another 454-based melon transcriptome analysis was reported, leading to the isolation of large collections of simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) used to evaluate the diversity among three genotypes (Blanca et al. 2011). A year later, Blanca et al. (2012) published the resequencing of the transcriptome of 67 accessions, grouped into eight pools that represent all of the cultivar-groups (also referred to as botanical

varieties) of the species. In this study, the sequencing technology was SOLiD™ and the reads were aligned to the newly published melon genome (Garcia-Mas et al. 2012), allowing for mining of single nucleotide variants (SNV) on a genomic scale. In a recent study of phenotypic variability (Leida et al. 2015), a collection of 175 accessions was phenotyped for fruit characteristics and genotyped using 251 SNPs from the collection identified (Blanca et al. 2012). The SNPs were selected from reference and candidate genes associated with sugar metabolism and ripening behavior. A significant amount of variability at both the phenotypic and candidate gene levels was observed, and an association between allelic diversity and several of the candidate genes for sugar metabolism and ripening behavior was detected (Leida et al. 2015).

RNA-Seq by Illumina advanced technology followed the 454-pyrosequencing and SOLiD™ methodologies to become the recent technology of choice. It is now commonly accepted that digital expression (based on reads count) is at least as accurate as qPCR, provided that the coverage of reads is sufficiently high (Wang et al. 2009; Hoen et al. 2013). With the fast improvements of the NGS technologies, the number of reads is not a limiting factor anymore, and sophisticated approaches are applied to obtain a better insight into the data obtained.

An oligo-based microarray, using 17,510 unigenes derived from ESTs, enabled the analysis of pathogen resistance and fruit quality traits in melon (Mascarell-Creus et al. 2009). An advanced microarray was later applied for transcriptome analyses of developing melon fruits of two climacteric and two non-climacteric genotypes (Saladié et al. 2015). One main conclusion was that there exists a continuous spectrum of melon fruit ripening behavior rather than two strict patterns of climacteric versus non-climacteric. In addition, differential expression patterns of genes related to ethylene metabolism, carotenoid accumulation, sugar and cell wall metabolism and transcriptional regulation were observed. This indicates a coordinated reprogramming of gene expression during fruit development and ripening (Argyris et al. 2015; Saladié et al. 2015).

Intricate associations between sweetness, color and aroma in melon fruits were explored using a population of recombinant inbred lines (RIL) as a source of phenotypic and genotypic variations (Freilich et al. 2015). Ripe fruits were analyzed for both the quantified level of 77 metabolic traits (sugars, carotenoids and volatiles) and for RNA-Seq based expression profiles. Inter-metabolite association patterns and metabolites versus gene association patterns enabled the division of the metabolites into two major groups: (i) the first included ethylene and aroma determining volatiles, the accumulation patterns of which were correlated with the expression of genes involved in the glycolysis and TCA cycle pathways and (ii) the second included sucrose and carotenoids, the accumulation levels of which were correlated with the expression of genes associated with plastid formation. These findings were in good agreement with those of Ayub et al. (1996) and, due to the high resolution, contributed further insight into specific biosynthetic pathways associated with the ethylene cluster or with the sucrose-carotenoids-pH cluster.

A targeted approach combining RNA-Seq and bulked segregant analysis (BSR-Seq) applied by Chayut et al. (2015) revealed metabolic and cellular processes

associated with allelic variation in *CmOR* (Tzuri et al. 2015) and β-carotene accumulation in melon fruit. Differentially expressed genes, identified by this approach, were clustered into functional groups. The relatively enriched functional groups were the ones involved in photosynthesis, RNA and protein regulation, and response to stress. These genes are likely to be part of the regulatory network of *CmOr* and may therefore help in better understanding the mode of action of the *CmOr* gene in the mediation of carotenoid accumulation in the fruit mesocarp. BSR-Seq combined with fine mapping further enabled the identification of a gene, *CmKFB*, encoding for a protein that negatively regulates flavonoid accumulation in muskmelon (Feder et al. 2015 and Flavonoids section below). This is a clear demonstration of the feasibility of identifying and cloning of a novel regulatory gene with no *a priori* knowledge or candidate gene availability.

Recently, high resolution NGS-based QTL analyses were performed using either RNA-Seq or genotyping by sequencing (GBS). Novel genes associated with fruit quality were identified using both methodologies (Galpaz et al., Kenigswald et al., and Gonda et al., unpublished).

Sugar and Organic Acids

Sugar Accumulation in Melon

It has long been established that the quality of melon fruit is determined primarily by its sugar content (Yamaguchi et al. 1977). Sugar levels are determined by a complex of source-sink relationships in the melon plant as a whole. This complex includes the metabolism of assimilates and partitioning within the source leaf, subsequent translocation and partitioning among the various sinks, and finally, the metabolic processes in the developing fruit sink itself, which plays a strong role in controlling the fate of imported assimilates (Schaffer et al. 1996).

The sugar content in the mature sweet fruit is comprised of the disaccharide sucrose and its two hydrolysis products, the hexoses glucose and fructose. Significantly, the increase in total sugar content during fruit maturation is particularly due to the accumulation of sucrose during the final stages of fruit development (Rosa 1928; Schaffer et al. 1987, 1996; Burger and Schaffer 2007). Developmentally, the increase in sugar content during ripening is primarily a function of the accumulation of sucrose, while glucose and fructose levels are modulated to a much lesser extent. Most importantly, variation in sucrose levels also accounts for the genetic differences in total sugar contents within *Cucumis melo* (Stepansky et al. 1999; Burger et al. 2000, 2009).

The genetic variation for sugar content in *Cucumis melo* is extremely broad, ranging from low sugar accessions with Brix values of 3–5, to high values of 15–18 (Stepansky et al. 1999; Burger et al. 2000, 2009; Burger and Schaffer 2007). Although most of the high-sugar accessions are from subsp. *melo*, due to selection for sweetness over the past millennium (Paris et al. 2012), nevertheless there are some high-sugar accessions of subsp. *agrestis* (Burger et al. 2006).

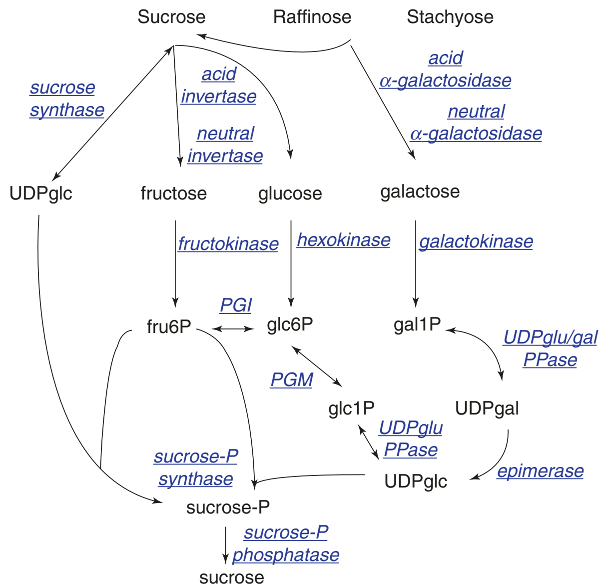

Fig. 1 Metabolic pathway of sugar metabolism in melon fruit, indicating enzyme reactions (After Dai et al. 2011)

The metabolic pathway leading to sucrose accumulation in melon has been largely identified. The accumulation of sucrose during development is determined by the metabolism that takes place within the fruit sink itself, and this metabolism is developmentally controlled. Sweet fruits are characterized by a metabolic transition during their development that leads to extensive accumulation of sucrose. The complete metabolic pathway involves nearly 20 enzyme reactions and the main enzyme activities that affect the sucrose levels in the fruit have been identified (Gao et al. 2004; Burger and Schaffer 2007; Dai et al. 2011). The complex pathway of sugar metabolism in melons and other cucurbit fruit begins with translocated raffinose oligosaccharides and continues through multiple pathways of hydrolysis, hexose phosphorylations, transglycosylations, nucleotide sugar metabolism, sucrose cleavage and synthesis, as well as rearrangements of epimerization, isomerization and mutase reactions (Dai et al. (2011) and Fig. 1).

The *Cucurbitaceae* are unique in their sugar metabolism pathway [Schaffer et al. (1996) and "Phloem Biology of the Cucurbitaceae" (Chapter "Comparative and Evolutionary Genomics of Angiosperm Trees")] and therefore serve as a model for fleshy fruit sink metabolism research, alongside tomato and grape. While tomato and grape are primarily sucrose translocators and hexose sugar accumulators, cucurbit fruits are unique in being galactosyl-sucrose translocators in which fruit sugar content is controlled by sucrose accumulation. For example, in all of our experience with measuring sugar levels in genetic variants and segregating populations, we have never observed a high sugar melon genotype characterized by predominantly hexose accumulation. Thus, the cucurbit fruit is novel for fleshy fruit sugar accumulation and metabolism.

Early inquiries (Schaffer et al. 1987; Hubbard et al. 1989; Burger and Schaffer 2007) focused on the major enzymes of sucrose metabolism, invertases (INV),

sucrose synthase (SS) and sucrose phosphate synthase (SPS) and emphasized particularly the balance between sucrose breakdown (INV and SS) and sucrose synthesis (SPS) activities in determining net sucrose accumulation, and the contribution of the length of the sucrose accumulation period (Burger and Schaffer 2007). Further studies (Gao and Schaffer 1999; Carmi et al. 2003; Gao et al. 2004; Dai et al. 2006, 2011) broadened the repertoire of involved metabolic enzymes and supported the conclusion that numerous enzymes of the pathway, including multiple isoforms of α-galactosidase (AGAL), INV, SPS, SS, among others, showed interesting correlations between their activities and the fruit-flesh sugar content dynamic during fruit development. These combined results led to the striking conclusion that the metabolic transition to sucrose accumulation in melon is not due to merely a single enzyme and that therefore a biochemical approach to account for the genetic variability is a Sysiphian task, unlikely to lead to further progress.

Following the metabolic characterization at the biochemical level, the developmental transition to sucrose accumulation was characterized at the transcriptomic level (Dai et al. 2011). A comprehensive analyses of the complete pathway controlled by approximately 50 genes, at the level of gene transcription, allowed for a global view of sugar metabolism during melon fruit sink development (Dai et al. 2011), (Fig. 2a, b). Recently, the transcriptome of sugar accumulating melon fruit was reported (Zhang et al. 2016), suggesting a metabolic transition of complex global pathways. The results of the developing transcriptome point to the same conclusion as the enzymatic studies, that there is a coordinated transition of multiple components of the complex pathway responsible for the metabolic transition from sugar utilization for growth to sugar storage.

As mentioned, there is a fortuitously broad genetic variability for sugar content in *Cucumis melo* (Stepansky et al. 1999; Burger et al. 2009), and this can be utilized as a resource for identifying the molecular-genetic control of sugar accumulation, for crop improvement as well as for elucidating the evolutionary processes leading to the domestication of sweet fruit. Over the past decade research has focused on determining the genetic components of the variability in the species at the molecular genetics level, based on QTL and map-based cloning strategies. Disappointingly, as will be described, to date no single gene controlling melon fruit sugar content has been functionally identified.

There has been some progress in studying the genetic control of the sugar accumulation trait based on the genetic variability for sugar content. We reported six highly significant QTLs for sugar content based on the RILs mapping population derived from the cross of ‘Dulce’ (high sugar, Reticulatus Group) and PI414723 (low sugar, Momordica Group) (Harel-Beja et al. 2010). On the other hand, data from the cross of ‘Noy Yizre’el’ (high sugar, Cantalupensis Group) × ‘Faqqous’ (low sugar, Flexuosus Group) indicated that a single major gene controls sucrose accumulation (Burger et al. 2002). Additional QTL studies based on various high and low sugar segregating populations were reported. Diaz et al. (2011) collected this data and developed a consensus linkage map of melon and combined it with QTL studies from 18 mapping experiments, reporting more than 10 QTLs for Brix,

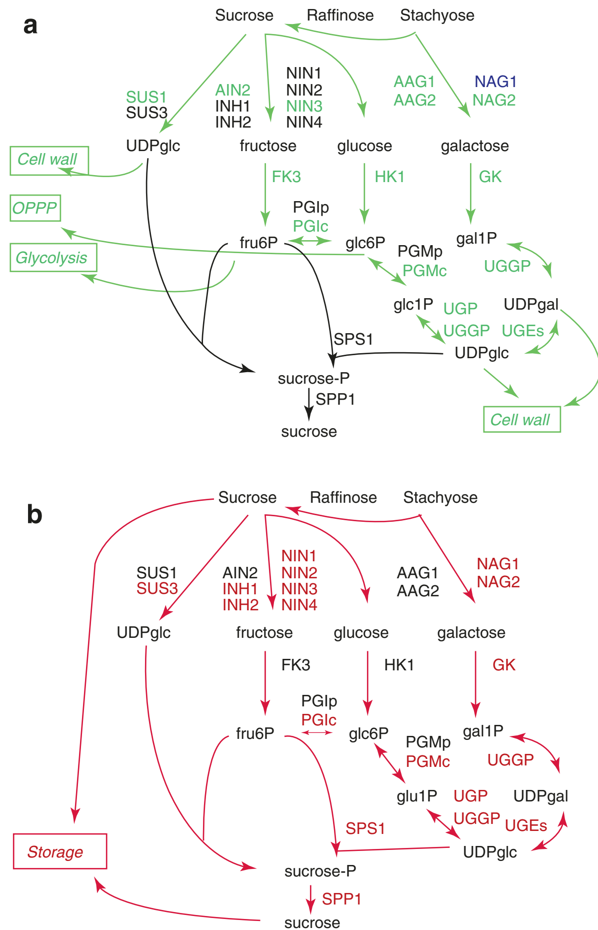

Fig. 2 Proposed model of flux of sugar metabolism in young (**a**) and mature (**b**) melon fruit, as suggested by transcriptomic data. Genes displaying either upregulated or stable gene expression, at the respective stages, are indicated in *color*. *Broad arrows* indicate the proposed flux directions at each stage. Metabolic pathway of sugar metabolism in melon fruit, indicating enzyme reactions (After Dai et al. 2011)

sugars and sucrose, some overlapping between studies and some peculiar to individual studies.

These differences in putative QTLs based on population structure are likely not artifactual, but due to the complex genetic control of sugar accumulation in *Cucumis melo*, combined with different sources of genetic variability among the different accessions. Most recent phylogenetic analyses of the species (Deleu et al. 2009; Serres-Giardi and Dogimont 2012; Leida et al. 2015) supported its division into two subspecies, *agrestis* and *melo*, which together comprise16 cultivar-groups (Pitrat et al. 2000; Burger et al. 2009; Esteras et al. 2012). The difference in the number of genes determining sugar levels derived from different crosses is very likely a reflection of the genetic distance and evolutionary history of the different groups. Thus, ‘Dulce’ (subsp. *melo*, Reticulatus Group) and PI414723 (subsp. *agrestis*, Momordica Group) are more distantly related to each other than are the two subsp. *melo* accessions, ‘Noy Yizre‘el’ (Cantalupensis Group) and ‘Faqqous’ (Flexuosus Group).

Based on this, we have identified pairs of low and high sugar varieties which represent both genetically close and genetically distant genotypes (unpublished). We expect that large differences in sugar content between very closely related genotypes will be governed by a small number of genes with large effects (major genes) while distantly related genotypes may differ in a larger number of QTLs with smaller, additive effects.

Most significantly, none of the QTLs for sugar content in melon fruit listed by Diaz et al. (2011) co-localize with any of the more than 50 candidate genes coding for the sugar metabolism pathway (Harel-Beja et al. 2010). This conclusion was reached based on co-localization of polymorphisms for each of the genes with the QTL map position. This points to the likely contribution of either major regulatory genes (i.e., transcription factors) or genes coding for uncharacterized transporters that may play a role in sugar transport and accumulation.

Recently, Leida et al. (2015) described the genetic structure of *Cucumis melo* based on 175 accessions representing the broad phynotypic variation of this species. In addition to supporting the accepted subspecies and group classification, they were able to perform association analyses between sugar content and polymorphisms, particularly among candidate genes for sugar metabolism. The results point to the relative strength of GWAS analyses for the identification of candidate genes in correlation with phenotypic and genetic variability. However, it also emphasizes that final functional gene identification will likely be dependent on a mixture of research strategies, combining candidate gene analyses together with recombination-based fine mapping using appropriate population structures.

Acidity in Melon

Acid levels are a major determinant of the taste and quality of most fruits, in combination with sugars and flavor volatiles (Sweeney et al. 1970; Ulrich 1971). Most edible fruit have pH values in the acidic range of three to five but the molecular-genetic control of acid accumulation is still largely undeciphered.

The sweet melons, *Cucumis melo*, are fairly unique among fleshy fruit in that they have very low acidity, and all cultivated sweet melons have pH values in the near neutral range of ~6–7 (Kubicki 1962; Pitrat et al. 2000; Burger et al. 2003, 2009; Harel-Beja et al. 2010). But sweet melons comprise only a minority of the designated cultivar-groups (referred to by some as botanical varieties) of *C. melo* (Pitrat et al. 2000; Burger et al. 2009), of which the most widely cultivated are the muskmelons (Reticulatus Group), cantaloupes (Cantalupensis Group), and casabas (Inodorous Group). The majority of the melon cultivar-groups, however, do not become sweet, but instead become acidic and sour-tasting upon ripening. Among these are the snake melons (Flexuosus Group), chate melons (Adzhur Group) and Indian phut or snapmelons (Momordica Group), which are grown across much of northern Africa

and central-southern Asia and are consumed when immature, much like cucumbers (*Cucumis sativus* L.), before becoming acidic. Thus, genetic variability for fruit acidity in melon is unique, given the extreme difference of nearly two pH units, reflecting [H+] differences of 10^2, between acidic and non-acidic genotypes.

Sourness, or acidity, of mature melon fruit flesh was reported over 50 years ago to be conferred by a single dominant locus termed *So* (Sour) or *PH* (Kubicki 1962) and the presence of a single major gene controlling the trait has been subsequently confirmed in different genetic population structures (Harel-Beja et al. 2010; Diaz et al. 2011). As all sweet melon accessions are non-acidic, they are homozygous for the recessive mutant, *ph/ph*. Therefore, the *PH* gene is intriguing, not only for understanding fruit physiology but also for clarifying the sequence of molecular events contributing to plant domestication and crop species diversification.

The historical development of research leading to the identification of the *PH* gene in melon reflects the development of research strategies taken over the past decades. The trait was initially mapped to a 10 cm region (Danin-Poleg et al. 2002), then further limited to a ~2 cm region on the distal portion of chromosome 8 (Harel-Beja et al. 2010). An extensive co-localization screen of nearly 60 genes encoding fruit organic acid metabolism and the major H+ transporters did not support the identity of any of these logical candidates as the *PH* gene (Cohen et al. 2014), pointing again to the limitations of candidate gene strategies.

We finally identified the PH gene by a map-based cloning strategy combining fine mapping based on two intraspecific segregating populations: a RIL population derived from a cross of the high pH muskmelon (Reticulatus Group) 'Dulce' and the low pH Indian phut snap-melon (Momordica Group) PI 414723 (Harel-Beja et al. 2010), and a near-isogenic pair of melon lines segregating for the pH trait, derived from crossing the high pH cantaloupe (Cantalupensis Group) 'Noy Yizre'el' and the low pH snake melon (Flexuosus Group) 'Faqqous' (Burger et al. 2003). The melon genome allowed us to narrow the major QTL to a 40 Kb region harboring four ORFs and only one of the four, coding for a membrane transporter, was found to be expressed in melon fruit (MELO3C025264). Subsequent research confirmed the function of the transporter and uncovered the molecular evolutionary event that led to non-acidic melon fruit, as a four-amino acid duplication in one of the transmembrane domains of the transporter (Cohen et al. 2014). Most significantly, the results of the project revealed that orthologs of this major gene are ubiquitous and responsible for fruit acidity in numerous plant families. The unique genetic variability for acidity in the melon species, and particularly the mutation that caused low acidity, was crucial for the functional identification of this important transporter throughout the plant world.

There yet remains to functionally identify additional genes contributing to fruit acidity and numerous QTLs have been reported [see Diaz et al. (2011) for list]. Even among the low acid sweet lines there is variability (e.g., pH 6.2 compared to 7.2) and the identification of these "minor" genes is the upcoming challenge.

Pigments of Melon

Carotenoids

Carotenoids are indispensable to human nutrition and health but they are synthesized only by photosynthetic organisms (Fraser and Bramley 2004). In addition to serving as antioxidants, carotenoids with beta rings are the primary dietary source of vitamin A, whose deficiency is a significant public health problem in many parts of the world. Moreover, carotenoids serve as major pigments that contribute to the color of many red, orange and yellow fruits, flowers and animals.

Fruit-flesh color, a major attribute of melon fruit quality, is largely determined by carotenoid content. Carotenoids accumulated in melon fruits include mostly β-carotene, which has a dual importance: it is a pro-vitamin A compound, thus increasing the health attributes of melon fruit, and it is an orange pigment that colors the fruit flesh. Understanding the genes and the mechanisms that underlie melon fruit-flesh carotenoid accumulation is essential for efficient and marker assisted breeding of quality melons.

Substantial advances have been made in our understanding of carotenoid metabolism in plants (Giuliano et al. 2008; Cazzonelli and Pogson 2010; Farré et al. 2010; Shumskaya et al. 2012; Li and Yuan 2013; Nisar et al. 2015; Zhou et al. 2015). The metabolic pathway leading to the accumulation of carotenoids in plants has been well elucidated and extensively reviewed by many authors, including recently by Nisar et al. (2015). Briefly, carotenoid content is a net result of biosynthesis, degradation, and stable storage capacity (Nisar et al. 2015; Yuan et al. 2015b). Thus, all genes and factors that regulate these processes affect the final levels of carotenoid accumulation. A scheme of the carotenoid biosynthetic pathway is illustrated in Fig. 3.

Melon fruit-flesh color is determined by a combination of chlorophyll and carotenoid pigments, resulting in white, green, and orange fruit flesh colors (Fig. 4). The main carotenoid accumulated in orange-flesh cultivars is β-carotene; quantitative differences in β-carotene are responsible for most of the variation in flesh-color intensity (Burger et al. 2009). Qualitatively, fruit flesh color is largely governed by two major genes, green flesh (*gf*) and white flesh (*wf*), acting epistatically. Orange flesh is determined by *Gf* and is dominant to green flesh (*gf*). When a melon fruit carries *gfgf,* it has either white (*Wf-*) or green flesh (*wfwf*) (Hughes 1948; Clayberg 1992; Monforte et al. 2004). With the aid of genomic tools, the nature of *gf* has recently been explored and the *wf* gene is close to be identified. Quantitatively, several studies have identified different quantitative trait loci (QTL) controlling melon fruit-flesh color in various genetic backgrounds (Monforte et al. 2004; Paris et al. 2008; Cuevas et al. 2009; Harel-Beja et al. 2010; Diaz et al. 2011), however the genes involved quantitatively in melon fruit carotenoid accumulation are still unclear.

The two major *loci* that control melon fruit flesh color, *gf* and *wf*, have been mapped using linkage analysis. *Green-flesh* (*gf*) was mapped to chromosome 9 (Perin et al. 2002; Cuevas et al. 2009) and white-flesh (*wf*) was mapped to

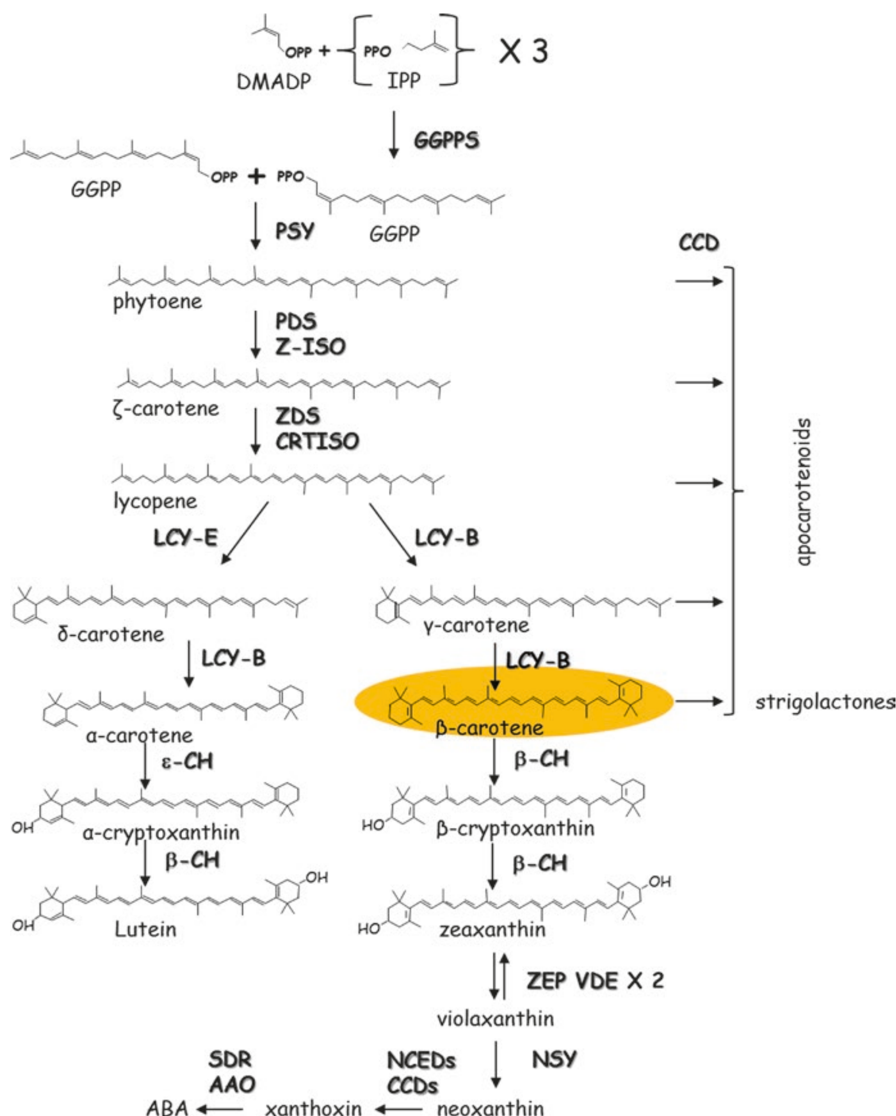

Fig. 3 Schematic diagram of the carotenoid biosynthetic pathway: Arrows represent enzymes, names are bolded. Two C20 GGPP are condensed to form C40 phytoene in the first committed step of carotenogenesis, catalyzed by PSY. Phytoene is desaturated by PDS and ZDS. The desaturated molecules are isomerized to form all-trans-lycopene by Z-ISO and CRTISO. All-trans-lycopene can either be cyclized twice by β-LCY to yield β-carotene or once by β-LCY and once by ε-LCY to yield α-carotene. β-carotene accumulation results in orange plant tissue. β-carotene is hydroxylated to form zeaxanthin which after epoxidation by ZEP is metabolized into ABA in a series of reactions. Violaxanthin de-epoxidase (VDE) may reversely form zeaxanthin. α-carotene is hydroxylated to form lutein. CCDs may degrade carotenoids to yield apocarotenoids

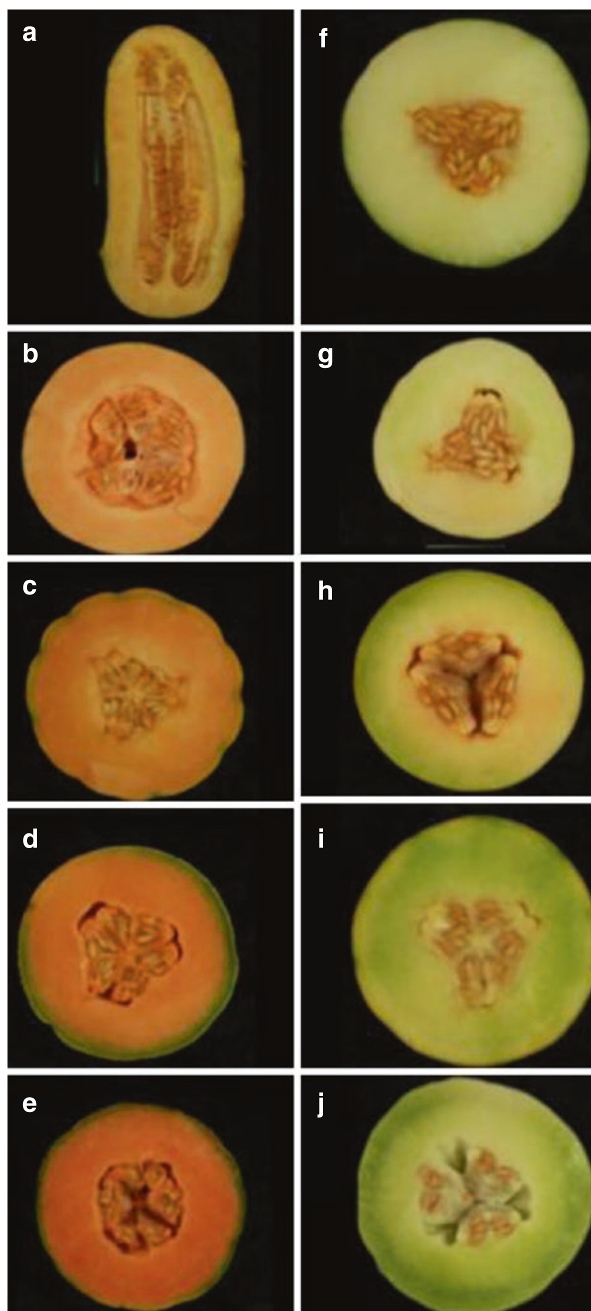

Fig. 4 Representative fruit of ten inbred lines cut open showing various flesh color phenotypes. (**a**–**e**): *Orange flesh* phenotype. (**f**–**j**): *White and green flesh* phenotypes. Accession names and taxonomic groups: (**a**) PI 414723 (subspecies *agrestis*); (**b**) Indian Best, Chandalak; (**c**) CEZ, Cantalupensis (Charentais type); (**d**) 'Dulce', Reticulatus; (**e**) HP, Cantalupensis (Magenta type); (**f**) 'Piel de Sapo', Inodorus; (**g**) 'Noy 'Amid', Inodorous (Yellow Canary type); (**h**) 'En Dor', Ameri; (**i**) Noy Yizre'el, Cantalupensis; (**j**) 'Tam Dew', Inodorous (Honey Dew type). Photographs from Chayut et al. 2015

chromosome 8 (Cuevas et al. 2009). Additional minor QTLs affecting flesh color have also been described (Monforte et al. 2004; Cuevas et al. 2009; Harel-Beja et al. 2010; Diaz et al. 2011; Leida et al. 2015), however, only when advanced genomic tools became available, the major gene governing carotenoid accumulation in melon fruit was functionally identified, enabling better understanding of this trait.

Tomato has become the primary model for fleshy fruit ripening due to its agronomic and horticultural economic importance, and its wide natural phenotypic variation in color and corresponding altered carotenoid content and composition. Tomato fruit carotenoid accumulation and composition is largely regulated at the transcriptional level; transcription of *PSY* differentiates between carotenoid accumulating and non-accumulating fruit while transcription of other carotenoid metabolism genes affect fruit carotenoid composition (Hirschberg 2001; Liu et al. 2015). Regulation of fruit carotenoid accumulation at the transcriptional level has been shown to be the basic regulating mechanism in many other crops (Yuan et al. 2015b). In melon, interestingly, comparison of developing fruit transcriptomes from green and orange bulks, derived from a cross between the green-fruited 'Tam Dew' (Fig. 4j) and the orange-fruited 'Dulce' (Fig. 4d), revealed no difference in the expression of carotenoid metabolism genes (Chayut et al. 2015). Altering carotenoid metabolic flux towards β-carotene accumulation in later fruit developmental stages was associated with changes in the transcript level of structural genes; LCY-E expression was downregulated and LCY-B was upregulated, possibly shifting the metabolic flux from the α-carotene branch; leading to lutein, to the β-carotene branch. At the same time, the expression of β-OH was downregulated, decreasing further metabolism of β-carotene (for genes/enzymes activities see Fig. 3). However, these transcriptional changes occurred in both the orange and the green bulks, indicating that melon is unique in the way fruit carotenoid accumulation is regulated; melon fruit carotenoid quantitative accumulation is not regulated at the transcriptional level of carotenoid metabolism structural genes while carotenoid qualitative composition is probably regulated at this level.

A detailed mapping approach identified the *Orange* gene, *CmOr*, the melon homolog of the cauliflower *BoOr* gene (Lu et al. 2006), as the previously-described *gf* locus in melon. Sequence analysis of various orange and non-orange fruited melon accessions and segregants identified the 'golden SNP' in *CmOr,* which changes the highly conserved arginine^{108} of green and white fruited CmOR protein to histidine (Tzuri et al. 2015). The validity of the 'golden SNP' was assayed by site directed mutagenesis of *CmOr* and functional analysis in dark grown *Arabidopsis* callus (Tzuri et al. 2015). Additionally, dark-grown *Arabidopsis* calli, transformed with site-directed arginine to histidine, mutagenized *Arabidopsis Or* gene (*AtOr*) and accumulated large amounts of β-carotene, while the non-mutagenized transgenic calli remained white (Yuan et al. 2015a), indicating the biotechnological potential of the 'golden SNP'.

Global food security demands pro-vitamin A bio-fortified staple crops (Graham et al. 2001; Fraser and Bramley 2004). The *Or* gene is a molecular switch that induces carotenoid accumulation in non-photosynthetic tissues and thus serves as a promising tool for this purpose. The discovery of the 'golden SNP' in the melon

CmOr gene (Tzuri et al. 2015) and the confirmation of this SNP potency in other plant species (Yuan et al. 2015a) defined a precise site for DNA editing as a possible new path toward β-carotene biofortification of crops via a non-transgenic approach.

CmOr (*gf*) and *wf* are key players in the determination of melon fruit carotenoid accumulation. The *CmOr* haplotype explained most of the flesh-color variation in a core collection of more than 170 melon accessions. The *wf* gene identity has not been published yet and *CmOr* is involved in the regulation of chromoplasts, and their carotenoid storage capacities and formation. The exact mechanism by which *CmOr* operates is not completely clear. A green and an orange developing melon fruit comparative bulk segregant RNA-seq analysis identified differentially expressed genes (DEG) that were clustered into functional groups (Chayut et al. 2015). This DEG list includes participants of the network of transcriptional events that associate induced fruit β-carotene accumulation with *CmOr* allelic variation. Studying the effect of allelic variation in these genes could create invaluable molecular targets for 'finer tuning' of fruit β-carotene accumulation through breeding and biotechnology.

Genome Wide Association Studies (GWASs) have proven powerful in revealing the complex genetic bases of many phenotypes in various plant species (Ogura and Busch 2015). A GWAS on a wide collection of melon accessions could associate *Cucumis melo* genetic particular genetic factors with variations in β-carotene accumulation. A major part of fruit β-carotene accumulation can be expected to be explained by the 'golden SNP' and a minor part of it by allelic variation in *wf*. Nevertheless, other loci that are associated with the 'fine tuning' of melon fruit β-carotene accumulation will likely be discovered. Co-localization of such loci with DEG could indicate and identify effective allelic variation in the OR-associated network of genes.

Flavonoids

Fruit rind color is one of the first attributes that influences consumers' choice and acceptability of the product and thus is considered an important parameter determining fruit quality. Moreover, fruit rind color often differentiates among market types (Pitrat et al. 2000). Melons exhibit large variations in morphological and physiological characters such as fruit size, taste, and internal and external color, and are economically important fruits cultivated in tropical and temperate regions of the world (Pitrat et al. 2000; Burger et al. 2009). Rind color of ripe melon fruits ranges in hue from green to yellow to orange, and in shading and intensity from almost black to almost white, and can also be striped or otherwise variegated in two or more colors. The pigments conferring this external fruit color variation have been reported to be carotenoids, mainly β-carotene, and chlorophylls (Burger et al. 2009).

Flavone derivatives are accumulated in leaves of cucumber and melon (Krauze-Baranowska and Cisowski 2001). Kaempferol and quercetin glycosides accumulate in the reproductive organs of some cucurbits (Imperato 1980). However, even

though flavonoids comprise a large pigment group in nature, until recently they have not been reported as being part of the melon or any other cucurbit fruit pigmentation system.

Flavonoids are polyphenolic secondary metabolites ubiquitous to plants. Thus far, about 9000 flavonoids have been characterized. Flavonoids are subgrouped into chalcones, flavanones, flavandiols, flavones, flavonols, anthocyanins, proanthocyanidins, and aurones, among others. The flavonoid biosynthetic pathway has been studied extensively in several plants, and is highly conserved. In plants, flavonoids have diverse functions, acting as powerful antioxidant agents, antimicrobial compounds, UV protectants, insect protectants, pollen germination stimulants, and visual attractors to pollinators. It has been well established that flavonoid biosynthesis genes are regulated by the interaction of different families of transcription factors (Falcone Ferreyra et al. 2012).

In 2010, a new pigmentation system that is based on the flavonoid family of pigments was reported; yellow canary melons (Inodorus Group) accumulate naringenin chalcone, a flavonoid, as their major fruit rind pigment (Tadmor et al. 2010). Naringenin chalcone (NarCh) is also prominent in the fruit rind of other melon cultivar-groups, occurring together with carotenoids (mainly β-carotene) and chlorophyll, in 'Eshkolit Ha'Amaqim' (Reticulatus Group), Noy Yizre'el (Cantalupensis Group) as well as 'Rochet', 'Tendral Verde Tardio' and 'Tam Dew' (Inodorus Group). NarCh is produced by chalcone synthase (CHS) from p-coumaroyl-CoA and malonyl-CoA, and subsequently converted into naringenin (Nar) by chalcone isomerase (CHI).

In wild-type tomato, NarCh accumulates to as much as 1 % of the dry weight of the tomato fruit cuticle. The *y* mutant does not accumulate NarCh due to downregulation of the metabolic pathway, by a MYB transcription factor (Adato et al. 2009; Ballester et al. 2009). It was assumed that NarCh accumulation is regulated in melon by a similar mechanism (Tadmor et al. 2010). As there are tens of MYB homologs in melon, an RNA-Seq of bulks of the tails of a segregating population was chosen as the strategy that will identify this gene.

F_3 families were developed and visually phenotyped for fruit rind color. From these families, 12 NarCh-accumulating families which did not segregate for the yellow rind phenotype and 7 F_3 families which did not segregate for white fruit (non-NarCh-accumulating homozygotes) were selected. These families represented the tails of the segregating population, were bulked according to their fruit rind colors and, accordingly, were assumed to be fixed for the 'yellow' or the 'white' allele, while arbitrarily segregating for all other traits (Feder et al. 2015).

Using RNA-Seq of bulks representing the tails of a population segregating for naringenin chalcone accumulation, followed by fine mapping, a Kelch domain-containing F-box protein coding gene, *CmKFB*, was identified that negatively regulates naringenin chalcone accumulation. *CmKFB* is highly transcribed in NarCh-non-accumulating tissue but is downregulated in NarCh-accumulating tissue, such as the yellow rind of canary-type casaba melons including 'Noy 'Amid'. Further metabolite analysis indicated that downstream flavonoids are accumulated together with naringenin chalcone, while *CmKFB* expression diverts the biochemical

flux towards coumarins and general phenylpropanoids. Thus, CmKFB functions as a regulator which diverts flavonoid metabolic flux.

To functionally analyze *CmKFB* in a heterologous system, a stable transformation of tomato MP-1 cultivars was used. Tomato plants accumulate anthocyanins in vegetative tissues in response to stress and accumulate NarCh, kaempferol and quercetin in their fruit peels (Mintz-Oron et al. 2008). Tomato stable *CmKFB* transgenic plants did not accumulate visible amounts of anthocyanins in any observed tissues: leaves, stems, flowers. Peels of ripe fruits from transformed plants had significantly reduced NarCh in the cuticle as compared with the MP-1 control. Seedlings from both transgenic plants were germinated and grown under cold stress conditions. After 15 days, a clear difference could be observed between anthocyanin accumulating seedlings and seedlings which did not accumulate anthocyanins. The plantlets with anthocyanins did not harbor the transgene, indicating overexpression of *CmKFB* in tomato represses flavonoid accumulation in a similar manner as observed in melon.

The identification of a new flavonoid-based pigmentation system in melon, together with advances in deciphering the genetic variability of carotenoid based pigmentation patterns, opens our imagination to the possibility of expanding the color palette of melon fruit to include such novelties as blue or purple anthocyanin-containing and red lycopene-containing melons with increased nutritional value. Future research to expand our understanding of the complex genetically controlled, metabolically driven phenomenon of fruit pigmentation, combined with continued mining of natural and induced genetic variability, should support our creative efforts.

Aroma

The full flavor and aroma of fruits is due to often complex mixtures of volatile organic compounds (VOCs). Different melon cultigens are characterized by different aromas (Shalit et al. 2001; Kourkoutas et al. 2006). Attempts to characterize a single or a few molecules that impart the unique aromas to melon fruits are complicated by this formidable variability and are reflected by different profiles of VOCs in chemical analyses. Although ripe melon fruits often display hundreds of VOCs, systematic analyses have allowed us to determine that only a fraction of them contribute to the noticeable aromas of the fruits (Gonda et al. 2016). In earlier studies, which included gas-chromatography olfactometry (GC-O), only 11 odorants were identified in melon (Schieberle et al. 1990), but a more recent study led to the description of as many as 33 volatile compounds impacting the aroma of melon fruit (Wang and Lin 2014). Often, these odorants are dependent on the studied genotype and it is difficult to extrapolate and generalize the information for all melon genotypes. These diverse aroma volatiles found in melons arise from various related and unrelated metabolic pathways and involve many genes and enzymes shaping the perceptible aroma of the melon fruit (Gonda et al. 2016). Among the most important

compounds detected in the aromas of different melon cultigens are many volatile esters, alcohols, aldehydes, sulfur compounds and terpenes. Pioneering works aimed at elucidating the pathways and key genes encoding structural biosynthetic genes of VOCs revealed enzymes that show promiscuity towards substrates and are often involved in the formation of different aroma volatiles from various biosynthetic pathways.

The first aroma-related gene was isolated from melon by screening for genes with enhanced expression in the ripe fruit (Aggelis et al. 1997). The gene was termed MEL2 and its biochemical function remained obscure until subsequent studies indicated that this gene encodes a protein with alcohol *acyl transferase activity* (*AAT*) (Yahyaoui et al. 2002). This enzyme catalyzes the condensation of alcohol and acyl-CoA substrates to generate volatile esters that often contribute fruity, sweet and floral aromas to the fruits (Jordan et al. 2001) and is a member of the *BAHD acytransferase* gene family (Table 1). By screening cDNA libraries with degenerative primers, melon was found to carry four copies of *AAT* genes (*CmAAT1-4*) but only three of them were expressed in the ripe fruit (Yahyaoui et al. 2002; El-Sharkawy et al. 2005). Among those, only two were shown to be active using *in vitro* assays towards various substrates in heterologous yeast (El-Sharkawy et al. 2005; Lucchetta et al. 2007). The preceding enzyme in the pathway to volatile esters belongs to the *alcohol dehydrogenase* (*ADH*) family. These enzymes reversibly convert aldehydes and ketones into alcohols. Melon *ADH* genes were found to be present in two copies and both expressed in the ripe fruit (*CmADH1* and *CmADH2*). Each gene product displayed similar enzymatic activities towards the different alcoholic substrates tested (Manríquez et al. 2006). Interestingly, the biosynthetic source of the volatile aldehyde substrate for ADH can be derived either from lipid or amino acid catabolism (see below). Moreover, both *AAT* and *ADH* genes expression show ethylene dependency (El-Sharkawy et al. 2005; Manríquez et al. 2006) and are mainly expressed in climacteric melons.

Amino-Acid Derived and Sulfur Compounds

Amino acids serve as precursors for many aroma volatiles of various functionalities in many fruits (Schwab et al. 2008; Dudareva et al. 2013). The main amino acids catabolized into volatiles are the essential amino acids which cannot be synthesized in the mammalian body. Thus, the amino-acid derived volatiles are postulated to act as nutritional cues for seed dispersers in the wild ancestors of our crops (Goff and Klee 2006). Extensive studies have indicated that essential amino acids are readily metabolized into aroma volatiles by ripe melon fruit cubes and they include the sulfur containing L-methionine, the aromatic amino acid L-phenylalanine as well as the branched-chain amino acids (BCAAs) L-isoleucine, L-leucine and L-valine (Gonda et al. 2010).

Exogenous L-phenylalanine gave rise to important aroma volatiles such as phenethyl alcohol and ethyl phenyl acetate in melon cubes, and the pathway was

Table 1 Genes functionally identified in melon fruit contributing to its unique aroma

Gene name	Enzymatic activity	Substrate	Product	Biosynthetic pathway	Representing volatiles	References	Gene number
CmAAT1-4	Alcohol acyl transferase	Alcohol + acyl CoA	Ester	Multiple pathways; ester biosynthesis	Ethyl butanoate	Yahyaoui et al. (2002), El-Sharkawy et al. (2005), Lucchetta et al. (2007)	MELO3C024771 MELO3C024766 MELO3C024762 MELO3C017688
CmADH1-2	Alcohol dehydrogenase	Aldehyde + NADH/ NADPH	Alcohol + NAD/ NADP	Multiple pathways; alcohol and ester biosynthesis	(*Z,Z*)-3,6-Nonadienol	Manríquez et al. (2006)	MELO3C023685 MELO3C014897
CmCCD1	Carotenoid cleavage dioxygenase	Multiple carotenoids	*apo*-carotenoids	Carotenoid catabolism	β-ionone	Ibdah et al. (2006)	MELO3C023555
CmTPSNY	Sesquiterpene synthase	Farnesyl diphosphate	Sesquiterpene	Terpenoid biosynthesis	δ-cadinene	Portnoy et al. (2008)	MELO3C016588
CmTPSDul	Sesquiterpene synthase	Farnesyl diphosphate	Sesquiterpene	Terpenoidbiosynthesis	*E-E*-α-farnesene	Portnoy et al. (2008)	MELO3C016595
CmArAT1	Aromatic amino acids aminotransferase	L-phenylanine	Phenyl pyruvic acid	Phenylalanine catabolism	2-phenethyl acetate	Gonda et al. (2010)	MELO3C025613
CmBCAT1	Branched-chain amino acids aminotransferase	L-valine, L-leucine, L-isoleucine	3-methyl-2-oxobutanoic acid, 4-methyl-2-oxopentanoic acid, 3-methyl-2-oxopentanoic acid	Branched-chain amino acids catabolism	Methyl-2-methylbutanoate	Gonda et al. (2010)	MELO3C010776
CmMGL	L-methionine-gamma-lyase	L-methionine	2-oxobutanoate + methanethiol + ammonia	Methionine catabolism	Ethyl (methylthio) acetate	Gonda et al. (2013)	MELO3C013774

shown to be initiated by the action of an aromatic amino-acid aminotransferase (ArAT) (Gonda et al. 2010). This pathway was discovered in melon cubes and was previously unknown to be operational in plants but is similar to the well-studied Erlich pathway prevalent in microorganisms (Dickinson et al. 2003). By screening expressed sequence tags (EST) libraries, Gonda et al. (2010) characterized a gene termed *CmArAT1* that encodes the initial enzyme involved in the catabolism of L-phenylalanine to its derived volatiles (Table 1). The enzyme releases phenylpyruvic acid as non-volatile intermediate. Moreover, exogenous phenylpyruvic acid (the product of this enzyme) also gave rise to the mentioned phenylalanine-derived compounds at even higher (up to 32-fold) levels than phenylalanine (Gonda et al. 2010).

Similarly to L-phenylalanine degradation by *CmArAT1*, the catabolism of the BCAAs was found to be initiated by a branched-chain amino acids aminotransferase (BCAT) enzyme that produced the corresponding α-keto acids of the given amino acid. The encoded gene was identified by functional expression in *E. coli* and termed *CmBCAT1* (Gonda et al. 2010). The expression of *CmBCAT1* is highly upregulated upon fruit ripening in climacteric varieties and it is one of the most highly expressed genes in the ripe fruit (Portnoy et al. 2011). Exogenous BCAA and derived α-keto acids, administered to ripe melon cubes, gave rise to VOC's structurally resembling the side-chain of the specific amino acid. The keto acids were by far more efficient precursors thus demonstrating the operability of this pathway in melon fruit cubes (Gonda et al. 2010).

The catabolism of L-methionine into aroma compounds in melon fruit is more complex than the catabolism of the BCAAs. L-methionine undergoes two major catabolic routes. The first one is characterized by transamination in a similar fashion to the metabolism of BCAA and it brings about the formation of many important C_3-thioether volatiles such as ethyl-3-(methylthio) propanoate (Gonda et al. 2010). However, it is still not clear if this step is catalyzed by CmArAT1, CmBCAT1, or if a specific L-methionine aminotransferase is present but still elusive. Nevertheless, other sulfur containing volatiles that originate from L-methionine are produced via the γ-cleavage of this amino acid into ammonia, methanethiol and α-ketobutyric acid (Gonda et al. 2013). The enzyme that catalyzes this reaction is termed L-methionine-γ-lyase (MGL) and was found to be encoded by the *CmMGL* gene (Table 1) (Gonda et al. 2013). As methanethiol is very reactive, it is further readily metabolized into other sulfur containing aroma volatiles such as C_2-thioehters, thioesters and sulfides. *CmMGL* was isolated by screening of EST libraries of ripe fruits and its functionality in the production of these volatiles was demonstrated by their co-segregation with *CmMGL* transcript levels in the aforementioned RILs population, as determined by RNA sequencing (Gonda et al. 2013). Sulfur volatiles are very important to the overall aroma of melons and their levels are crucial for consumer preference. At low and moderate levels, thioesters and thioethers impart important flavor notes to melon fruit, but when present at high concentrations they cause undesirable off-flavors that undermine quality and acceptability of the fruit (Mussinan and Keelan 1994; Jordan et al. 2001; Wang and Lin 2014).

Fatty-Acid Derived Compounds

Many aroma volatiles are derived from fatty acids and are major contributors to the acceptable aroma of many fruits including melons. Often, they occur as esters but the non-esterified alcohols and aldehydes are especially important in honeydew type melons, which possess an aroma resembling that of green cucumbers (Perry et al. 2009; Lignou et al. 2014). The non-esterified fatty acid derived volatiles include mainly short-chain aliphatic aldehydes and alcohols (Shalit et al. 2001; Gonda et al. 2016). Fatty-acid derived aldehydes, also known as green-leaf volatiles (GLV), contribute fresh and green notes to melon aroma and are the major volatile compounds present in unripe melon fruits as well as in cucumbers (Schieberle et al. 1990; Beaulieu and Grimm 2001; Shalit et al. 2001). Fatty-acids (mainly linoleic and linolenic acids) are the main precursors and are catabolized by two consecutive enzymes: lipoxygenase (LOX) and hydroperoxide lyase (HPL) (Schwab et al. 2008). These reactions have been demonstrated in the fruits of various cucumber cultivars and their corresponding encoding genes were isolated and functionally characterized (Galliard and Phillips 1976; Phillips and Galliard 1978; Wan et al. 2013; Chen et al. 2015). The expression of *CmLOX1* was reported to increase during the development of fruit mesocarp (Whitaker and Lester 2006), although young mesocarp and vegetative tissues also express this gene. The relative expression of 18 melon LOX genes was measured by qRT-PCR (Zhang et al. 2014) and LOX activity was extracted from fruit of five melon cultivars (Tang et al. 2015). These studies indicate that although further experimentation is still needed, it seems that *CmLOX03*, *CmLOX05*, *CmLOX11*, *CmLOX12*, *CmLOX16* and *CmLOX18* are important in the production of straight-chain aroma volatiles. Two additional melon LOX genes (*CmLOX10* and *CmLOX13*) were functionally expressed in *E. coli* and their biochemical roles were validated in-vitro, but their relation to melon aroma was not evaluated (Cao et al. 2016). To-date, no HPL coding gene has been characterized from melon to estimate its contribution to GLV production in the ripe fruit.

Terpenoids

Another group of volatile aroma compounds found in melon fruits are terpenoids. Terpenoids are one of the most diverse groups of specialized chemical compounds in nature and more than 40,000 different structures have been described (Schwab et al. 2008). They are composed of additions of 5-carbon isoprenoid backbone units normally assembled in a head-to-tail or a head-to-head manner (Dudareva et al. 2013). The common volatile terpenoids in melon are monoterpenes that are composed of ten carbons, and sesquiterpenes that are composed of 15 carbons. Norisoprenes, also named apocarotenoids, constitute another type of terpenes found in orange-flesh melons that are derived from the oxidative breakdown of carotenoids, non-volatile 40 carbon tetraterpenoids that are important pigments (see

Section on "Carotenoids"). Apocarotenoids are very intense odorants and can be detected by the human nose at concentrations of less than 0.1 ppb and contribute to melon aroma. Carotenoid oxidative cleavage is catalyzed by a group of enzymes termed carotenoid cleavage dioxygenase (CCD), that cleave a carotenoid molecule to produce the apo-carotenoid volatile (Mcquinn et al. 2015). Ibdah et al. (2006) isolated and functionally characterized *CmCCD1* by screening for the tomato orthologue (Simkin et al. 2004) in a cDNA library derived from ripe melon fruits. The recombinant CmCCD1 enzyme cleaved carotenoids in bacteria expressing various carotenoids, including β-carotene, δ-carotene, α-carotene and lycopene, and produced various apocarotenoid volatiles including α-and β-ionone and pseudoionone, according to the parent carotenoid cleaved. Some of these carotenoids are not normally found in melon fruit. Additionally, *CmCCD1* was expressed both in orange-flesh melons that accumulate apo-carotenoids, and in green-flesh melons that do not accumulate apo-carotenoids. These experiments demonstrated that CmCCD1 has the ability to produce more compounds than it normally produces *in vivo* and is an example of a silent metabolism phenomenon where the plant apparently maintains the ability to biosynthesize compounds, but the lack of precursor prevents their production (Lewinsohn and Gijzen 2009).

Sesquiterpenes tend to accumulate at higher levels in the melon fruit rinds as compared to melon flesh and different melons display different sesquiterpene profiles, or lack sesquiterpenes (Portnoy et al. 2008). Screening for terpene synthase homologue genes in two cDNA libraries followed by rapid amplification of cDNA ends (RACE) yielded two sesquiterpene synthase genes, *CmTps_NY* and *CmTps_Dul* that produced α-copaene and γ- and δ-cadinene (Dul) or (*E*,*E*)-α-farnesene (NY) *in vitro* (Table 1). The *in vitro* results were strikingly in accordance with the sesquiterpenes naturally produced by the genotype they were isolated from (Portnoy et al. 2008). Finally, melon fruits contain also monoterpenes such as linalool and 1,8-cineole (Gonda et al. 2016), but the corresponding structural genes have not been identified yet. Future research utilizing the genetic population and technologies described in this chapter should lead to their discovery, as well.

Future Directions and Strategies

The transition from the pre- to the post-genomic era is analogous to the comparison between navigation using a 1:500,000 road map, where one has general orientations and only major landmarks can be identified, and navigation using GoogleMap and Street-View, where physical resolution is achieved and all the details along the path are exposed. The availability of fully assembled and partly annotated (mostly protein-coding portion) genomes is therefore a fundamental factor in the navigation towards understanding of phenotypic variation and biological pathways and the ultimate identification of causative variants that are involved. The pre-genome era in melon, prior to the publication of the melon genome sequence in 2012 (Garcia-Mas et al. 2012), mostly involved the use of high-density genetic maps and comparative

expression data (transcriptomes) to link, using different approaches, between phenotypes and molecular attributes. As described above, genetic and genomic tools are already being used in melon to identify major genes that contribute to variation in fruit color, aroma and flavor. Availability of a high-quality reference genome for melon in conjunction with recent advances in multiple disciplines and research technologies can now further promote the genetic dissection of fruit quality in melon. Key directions, approaches and tools that are already implemented in other organisms and will potentially contribute to future genomic research in melon, are briefly described:

1. *High-capacity NGS-based genotyping* - Reduced representation methods for genome-wide high-density NGS-based genotyping are now available for cost-effective diversity screening and efficient genotyping of mapping populations (Elshire et al. 2011; Poland and Rife 2012; Sonah et al. 2013). This is now setting the stage for routine mapping of simple and complex traits to a genic resolution.
2. *Whole-genome resequencing of diversity panels* - The continuous decline in sequencing costs is turning the objective of whole-genome deep resequencing of diversity panels into a feasible objective for melon. As demonstrated in widely studied model organisms (The International Hapmap 2005; Gore et al. 2009; Weigel and Mott 2009; Mackay et al. 2012), such analyses provide insights into structural variation and allow the creation of detailed haplotype maps. These will serve as effective infrastructures for high resolution genomic analyses in melon.
3. *Advanced, high-throughput phenotyping methods* – The core of functional genomics is the ability to comprehensively generate high-quality phenotypes. Advances in remote-sensing and imaging technologies will most likely be adopted to collect fruit quality phenotypes in melon, as demonstrated for other crops (Brewer et al. 2006; Ignat et al. 2012; Tanabata et al. 2012; Araus and Cairns 2014; Granier and Vile 2014; Minervini et al. 2014; Schmilovitch et al. 2014). The ability to screen large populations using these approaches will complement the ultra-dense, cost-effective genotyping platforms and will facilitate high resolution mapping of simple and complex fruit quality traits.
4. *Multi-allelic mapping approaches* - The use of diversity panels and multi-allelic population structures such as the Nested-Association mapping (NAM) (Yu et al. 2008) or the Multiparent Advanced Generation Inter-Cross (MAGIC) (Beyer et al. 2008; Kover et al. 2009; Pascual et al. 2015) are becoming complementary common approaches to the traditional bi-parental designs in genetic studies in plants. The advantages are the ability to capture the large spectrum of allelic variation and represent wide phenotypic diversity in a single genetic study. Advances in genotyping alongside dedicated statistical tools and computational capabilities are promoting the shift towards these experimental designs.
5. *Zoom in on quantitative variation* - Fruit quality components of melon, including color, aroma and flavor, are quantitative polygenic traits. While major genes explaining the variation in these traits have recently been identified (Cohen et al. 2014; Feder et al. 2015; Tzuri et al. 2015), much of the remaining part of this

variation is explained by multiple genes, QTLs. The components that are described above will allow the shift in focus from discovery of major genes to the dissection of quantitative variation and identification of QTLs with smaller effects on fruit quality traits. The ability to map QTLs to the ~100 Kb scale or to a genic level will benefit from development and implementation of complementary methodologies for candidate-gene prioritization and functional validation. Integrative approaches combining comparative-mapping data with multi-experiment gene expression and functional annotations will likely to improve the identification of causative genes.

6. *Genome editing* – The recent revolution in genome editing using the CRISPR/CAS9 technology is already taking place in plant genetic research (Belhaj et al. 2015) and is expected to provide powerful capabilities for testing and validation of candidate genes for fruit quality traits in melon. This technology will also allow the non-transgenic modifications of alleles to improve fruit quality traits in elite breeding germplasm.

The improved capabilities to genotype, phenotype and map fruit-quality traits in melon will have a significant impact on melon breeding. The ability to identify causative genes and causative SNPs alongside the overall ability to explain, using genetic markers, significant portions of the phenotypic variation will allow effective implementation of marker-assisted selection for multiple fruit attributes and will lead to a more efficient development of innovative cultivars that are hard to create via conventional breeding.

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Cucurbit Genetics and Genomics: A Look to the Future

Rebecca Grumet, Jordi Garcia-Mas, and Nurit Katzir

Abstract The Cucurbitaceae family includes many high-value and flavorful crops consumed as vegetables, fruits, and seeds in diets throughout the world (Chap. 1, McCreight 2016). Crops such as squashes and pumpkins (*Cucurbita pepo*, *maxima* and *moschata*), cucumbers (*Cucumis sativus*), melons (*Cucumis melo*) and watermelons (*Citrullus lanatus*), make significant contributions to human nutrition. In the past decade we have seen tremendous progress with respect to genomic technologies, and cucurbit crops, with their small genome sizes [~367, 450, 385, and 400 Mbp for cucumber, melon, watermelon, and squash, respectively], have benefitted richly from these advances. The recent assemblies of draft genome sequences for the four major cucurbit species make it feasible to identify, characterize and utilize genes in ways that were not possible even a few years ago. This volume explored the genetic diversity of cucurbit crops, advances to unravel cucurbit genomes, and evolving applications of genomics to understand cucurbit growth, development and adaptation to their environments.

Keywords Cucumis • Citrullus • Cucurbita • Genetic resources • Genetic diversity • Genome structure • Genomic databases • Fruit development • Fruit quality • Phloem • Sex expression

Introduction

The Cucurbitaceae family includes many high-value and flavorful crops consumed as vegetables, fruits, and seeds in diets throughout the world (Chap. 1, McCreight 2016). Crops such as squashes and pumpkins (*Cucurbita pepo*, *maxima* and *moschata*),

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cucumbers (*Cucumis sativus*), melons (*Cucumis melo*) and watermelons (*Citrullus lanatus*), make significant contributions to human nutrition. In the past decade we have seen tremendous progress with respect to genomic technologies, and cucurbit crops, with their small genome sizes [~367, 450, 385, and 400 Mbp for cucumber, melon, watermelon, and squash, respectively], have benefitted richly from these advances. The recent assemblies of draft genome sequences for the four major cucurbit species make it feasible to identify, characterize and utilize genes in ways that were not possible even a few years ago. This volume explored the genetic diversity of cucurbit crops, advances to unravel cucurbit genomes, and evolving applications of genomics to understand cucurbit growth, development and adaptation to their environments.

Genetic Resources of Cucurbits

As richly illustrated in the preceding chapters, the Cucurbitaceae family is marked by extensive diversity. It is comprised of 96 genera collectively containing approximately 1000 species. The origins of cucurbit species are traced to tropical and subtropical regions in Asia. Subsequent radiation throughout central and southern latitudes resulted in broad distribution in Asia, Australia, Africa, and Meso- and South America. Studies described in Chap. 2 (Renner and Schaefer 2016) indicate that there are still more cucurbits to be discovered. Every sequencing project to date has uncovered new species; even new relatives of economically important crops have been found. As such, the authors state an urgent need to expand collections of wild material, and to sequence both new collections, and collections housed in herbaria throughout the world, so we may identify, classify, and characterize the rich diversity encompassed by cucurbit species.

These themes, the need for collection, and the need for characterization, were strongly echoed in each of the crop-based chapters describing genetic resources. Collections to survey unexplored regions or counter loss of habitat in the wild, serve both botanical and agronomic needs. Germplasm collections are critical to conserve and utilize genetic resources for crop improvement. The collection, conservation and evaluation of these resources are necessary to stem genetic erosion and provide a reservoir for future traits. Of particular importance for many breeding efforts are sources of resistance to diseases and pests, as well as traits influencing fruit quality and yield. Germplasm collections are especially critical for crops such as watermelon and cucumber which have a very narrow genetic base (Chap. 4, Naegele and Wehner 2016; Chap. 5, Levi et al. 2016). Efforts by national and international germplasm centers have resulted in collection, storage and evaluation of germplasm from primary, secondary and tertiary centers of diversity. An incomplete list of countries maintaining collections of various cucurbit species include: Bolivia, Brazil, China, Columbia, Czech Republic, Columbia, Costa Rica, India, Italy, Mexico, Philippines, Portugal, Russia, Spain, Thailand, Turkey, and U.S.A. The existence of multiple collections not only provides greater diversity of germplasm, but to the extent that there is redundancy among collections, there is also additional safety against possible losses.

The importance of germplasm characterization was also a recurring theme. While collections are the essential foundation, effective future use depends on characterization of the accessions, and knowledge about their relationships. Much progress has been made in describing phenotypic traits of crop species such as cucumber, melon, watermelon, and squashes. Looking forward, it is anticipated that molecular and genomic analyses will be of increasing value in this endeavor. They will allow for deeper understanding of diversity among accessions, population structure contained within collections, and the genetic basis for critical traits. Molecular characterization also can be particularly informative in cases where genetic relationship remain to be clarified, such as the relationship among the numerous diverse groups of melons (*Cucumis melo*) (Chap. 3, Pitrat 2016).

Finally, authors universally stressed the importance of investment in continued maintenance, preservation, and improvement of the cucurbit collections around the world. While current collections provide extremely valuable resources, there is need to expand these collections to provide representation of previously uncollected material. This is especially important for relatives of the more diverse, and less intensively cultivated squashes, pumpkins, and gourds (e.g., *Cucurbita* spp., *Momordica charantia*, *Lagenaria siceraria*, *Benincasa hispida*, *Luffa* spp., *Trichosanthes*) (Chap. 6, Paris 2016; Chap. 7, Dhillon et al. 2016). Furthermore, as landrace-derived and heirloom cultivars are increasingly replaced by modern cultivars, there is accelerated loss of genetic diversity within crops. Even comparatively recent cultivars and hybrids that are no longer commercially produced may be difficult to find, but may contain valuable traits that have not been incorporated into the newest cultivars. In addition, it was recommended that germplasm collections include important breeding materials. Type-lines, and pre-breeding materials, where key traits have been introgressed from unadapted backgrounds into cultivated inbred lines, can provide valuable starting material for future breeding efforts. Ultimately, germplasm collections must not only serve as repositories for diversity, but as a source for utilization and exchange. As we look to the future, there is need for sustained effort to maintain and facilitate access for to cucurbit germplasm throughout the world.

Cucurbit Genomes

One major breakthrough in cucurbit research has been the recent availability of the genome sequences of the three main species in the family, cucumber (Chap. 9, Weng 2016), melon (Chap. 8, Casacuberta et al. 2016) and watermelon (Chap. 10, Yong and Guo 2016), as well as unpublished genome sequences for several members of the *Cucurbita* genus (Chap. 11, Montero-Pau et al. 2016). The advent of next-generation sequencing (NGS) technologies allowed an early completion of the sequence of three cucurbit genomes, cucumber being the first plant genome that was published using mainly NGS. The relatively small size of the genomes of cucumber, melon and watermelon made it possible to obtain draft genome sequences of enough quality to be used

for addressing several fundamental biological questions and for their application in plant breeding. Sequencing technologies and bioinformatic pipelines are in continuous progress, and we expect to have improved assemblies and annotations in future releases of these three genomes. Despite its importance, genomic research in *Cucurbita* has started later than in other cucurbits, however unpublished draft genomes for *C. pepo* and *C. maxima* are already available. It is expected that in the following years the genomes of more cucurbits are sequenced, mainly due to the reduction of sequencing costs and availability of improved genome assembly pipelines.

The study of the genetic diversity in natural populations has also been addressed from a genomic perspective in cucumber, melon and watermelon, where 115, 8 and 20 representative accessions have been re-sequenced, respectively. This data offers a large source of variation with enormous potential for mining new alleles and for uncovering genes underlying traits that were domesticated and selected in these species. It is also expected that the number of re-sequenced accessions will grow exponentially during the next years, an issue that is related with the above-mentioned need for cucurbit germplasm characterization.

Genetic mapping of complex traits in cucurbits has also been reviewed (Chap. 15, Gonzalo and Monforte 2016). The genome sequences for several cucurbit species have facilitated the construction of saturated genetic maps, allowing the identification of QTLs involved in agronomically important traits such as fruit quality and morphology, yield, and disease resistance. The enormous amount of genomic tools is making possible to dissecting these complex traits and characterizing the underlying genes, which represent invaluable tools for breeding.

The availability of genome sequences has also been used for performing comparative analysis among cucurbits (Chap. 12, Nimmakayala et al. 2016) The genomes of cucumber, melon and watermelon showed few traces of ancient duplication events, however no recent whole-genome duplications were detected, which seems to be a common trend in this family. The comparison of melon and cucumber genomes suggests that five chromosome pairs from the 12-chromosome ancestor were fused in the cucumber lineage and several inter- and intra-chromosome rearrangements occurred. Also, the reference genomes of cucurbits can be used to identify major genes affecting agronomic characters in relative species without an available genome sequence.

There is urgent need for the development of databases for storing, managing and providing access to the huge amount of genomic data accumulated (Chap. 14, Bai et al. 2016). In this chapter, existing cucurbit databases and other databases useful for cucurbit genomic research are described. The common CuGenDB database and species-specific databases and their main features are discussed. Managing of big data is now a hot topic in genomics research, and future directions point to the construction of interactive databases that can be easily accessed and that contain and combine information acquired from different sources as genome sequences, genome variations, transcriptomes, metabolomes and phenotypes.

Finally, in Chap. 13 (Havey 2016) the organellar genomes of cucurbits are discussed. The cucurbit family contains some of the largest mitochondrial DNAs among plants, and the mitochondrial DNAs of *Cucumis* species are paternally

transmitted, a rare phenomenon among plants. This chapter discusses the evolutionary implications of the paternal transmission of mitochondria and the future possibilities to mutate mitochondrial genes.

Genomic Analysis of Cucurbit Biology

The genetic and genomic resources described above have already been used to address biologically important topics such as phloem biology, floral sex expression and fruit development, ripening and quality. Fruit biology is of major importance both from a fundamental viewpoint as well as from the agronomic perspective, especially for the major cucurbit crops. The Cucurbitaceae family is well known for its remarkable diversity in fruit morphology and quality traits. Chapter 18 (Grumet and Colle 2016) reviews the current knowledge of the genetic factors involved in the complex process of fruit growth and development in cucurbits and their implications to fruit morphology. Fruit ripening that characterizes the final stages of development is discussed in Chap. 19 (Yano and Ezura 2016). The focus of this chapter is melon, a species known to have both climacteric and non-climacteric cultivars. Many of the genes associated with ethylene synthesis, perception and signal transduction have been identified in melon and, in general, comply with the current knowledge in tomato, the model plant of fleshy fruit ripening. Less is known about the genetic factors that discern the non-climacteric from the climacteric melons. This chapter also addresses fruit quality traits that are developed as part of fruit ripening, including sweetness, aroma and color. A further and detailed coverage of these traits and the genetic factors associated with their synthesis are provided in Chap. 20 (Gur et al. 2016). The advancements in high throughput metabolomics, along with the genomic methodologies described here further enhanced research of important aspects of fruit quality traits. Notably, among the genetic factors that were deciphered in recent years are several that are unique to, or were first discovered in cucurbits. For example, the determination of fruit acidity by *CmPH* and the accumulation of carotenoids in the fruit flesh determined by *CmOR*.

The cucurbits are considered as model plants in studies of two important systems that are unique to the family, phloem biology (Chap. 16, Turgeon 2016) and floral sex determination (Chap. 17, Rodriguez et al. 2016). Due to the large sieve tubes and pores, an obvious advantage for light microscopy, phloem biology was initially studied in cucurbits prior to the genomic era. Recently, further aspects have been studied using molecular tools, for example, the unique array of extrafascicular sieve tubes and the exudes of the "phloem" that are easily collected and are therefore used in many metabolomic and proteomic analyses.

Cucurbit sex determination is intensively studied due to the highly diverse sexual systems in the family. Chapter 17 focuses on sex determination in *Cucumis* and the breakthrough research that has led to the identification of the major genetic factors affecting flower sexuality. This includes components involved in ethylene biosynthesis, transcription regulation and epigenetic processes. While major components

of the pathway were elucidated, the events occurring downstream to certain identified factors are still unknown. A TILLING platform developed for melon contributed especially to these studies of sex determination.

With the above described genetic and genomic resources and the rapid development of novel genomic methodologies, it is expected that marked advances will be made in the near future. An issue that requires special attention is the hindrance to cucurbit genomic research due to the lack of efficient transformation methodologies.

Conclusions and Look to the Future

The future holds promise, opportunities and challenges for our understanding, utilization and improvement of cucurbit crops. The continually increasing technological progress and knowledge base provides tremendous new potential to unravel genetic relationships, evolution, and biology of cucurbits, and to use these tools and information for continued advancement of these important crops. At the same time, if we are to be able to make optimal use of these tools and resources, it is critical that we prioritize conservation of critical germplasm; expand storage, management and access to immensely increasing genomic data; and develop improved functional genomic methodologies for phenotyping and genetic manipulation of cucurbit crops.

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