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Cucurbits (Cucurbitaceae; Cucumis spp., Cucurbita spp., Citrullus spp.)

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CHAPTER 8

Cucurbits (Cucurbitaceae; Cucumis spp., Cucurbita spp., Citrullus spp.)


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8.1 INTRODUCTION

The Cucurbitaceae is a remarkable plant family, deserving of attention because of its economic, aesthetic, cultural, medicinal, and botanical significance. In the Old and New Worlds, cucurbits have been associated with human nutrition and culture for more than 12,000 years (Brothwell and Brothwell 1969; Lira-Saade 1995). Thus, the Cucurbitaceae, along with the Brassicaceae and Asteraceae, can be considered families of extraordinary importance to humans, and they follow cereals and legumes in their economic significance to human economy (Whitaker and Davis 1962; Nayar and More 1998).

From a lay perspective, the fruits of cultivated cucurbits characterize the family. The remarkable variation in size, shape, and color patterns are notable familial features. Equally impressive is the prodigious growth of the herbaceous, vining stems of some cucurbit species (Bates, Robinson, and Jeffrey 1990). The flesh of the fruits of many species is eaten as fruits or vegetables. Many cucurbits are important components of traditional medicines, and some may have modern pharmaceutical applications (Ng 1993). In certain cultures, the seeds, leaves, or shoots, roots, and flowers are also consumed. Moreover, edible and industrial oils can be extracted from the seeds. The name of the family, Cucurbitaceae, is likely derived from Latin, where the word “corbis” means “basket”
or “bottle” (Pražák, Novotný, and Sedláček 1941), reflecting one of the ways that fruits are used. Mature fruits can also be strikingly ornamental or can serve as containers or as musical instruments. In addition, the fibers of the *Luffa* fruits can be used as sponges or in the production of shoes (Moravec, Lebeda, and Křístková 2004).

A striking feature of cultivated cucurbits is their adaptation to a wide variety of agricultural environments. Although they are often grown in extensive monoculture typical of crop production in developed economies, they are also grown in traditional small gardens typified by low external inputs. Many cucurbits are adapted to environments considered marginal for agriculture where some are gathered as food sources or for medicinal purposes (Bates, Robinson, and Jeffrey 1990).

Within this family, the genera *Cucumis*, *Cucurbita*, and *Citrullus* are considered to have high economic importance. Of about 3000 plant species used for human consumption, only 150 species are cultivated extensively, and of these, thirty provide the bulk of human food. Among these elite species, *Citrullus* is ranked twenty-fourth (Raven, Berg, and Johnson 1993).

China, India, Iran, Turkey, Egypt, and the U.S. are among the world’s largest producers of cucurbits. China remains the world’s leading producer of the major cucurbits, exporting fresh fruits, watermelon, and squash seeds (Maynard 2001). Production of these crops has dramatically increased during recent agricultural history. The cultivated area of *Cucumis*, *Cucurbita*, and *Citrullus*, as reported by the United Nations' Food and Agriculture Organization (FAO 2004), was $8.5 \times 10^6$ ha in the year 2004. More specifically, overall production reached $4 \times 10^7$ metric tons of cucumbers and gherkins; $2.7 \times 10^7$ metric tons of cantaloupes and melons; $1.9 \times 10^7$ metric tons of pumpkins, squashes, and gourds; and $9.3 \times 10^7$ metric tons of watermelons.

There are many cucumber market classes worldwide (e.g., U.S. processing (pickling), U.S. fresh market, European glasshouse, Mediterranean, Asian glasshouse). As noted above, world cucumber production ranks second among all cucurbits. Cucumber production is centered in Asia, where in 2004, almost 82% of world production occurred. China, Iran, Turkey, and the United States represented about 74% of the world’s production (FAO 2004) with China accounting for almost 64%. European production followed well behind, representing about 10% of world production (Table 8.1). Extremely high yields of $7.1 \times 10^6$ kg/ha in the Netherlands, $4 \times 10^6$ kg/ha in Denmark, and $3.15 \times 10^6$ kg/ha in the United Kingdom are the result of intensive cultivation in glasshouses (FAO 2004). Other countries such as Japan, Spain, and Korea, also produce a significant volume of cucumbers in glasshouses and other protective structures (Rubatzky and Yamaguchi 1997). Much of this production utilizes parthenocarpic cultivars (seedless) for which cultivation is highly specialized (Rubatzky and Yamaguchi 1997).

### Table 8.1 World Cucumber and Gherkin Production in 2004

<table>
<thead>
<tr>
<th>Location</th>
<th>Production (metric tons)</th>
<th>Area Harvested (ha)</th>
<th>Mean Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>40,190,104</td>
<td>2,395,125</td>
<td>167,800</td>
</tr>
<tr>
<td>By Continent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>1,073,727</td>
<td>145,670</td>
<td>73,710</td>
</tr>
<tr>
<td>Asia</td>
<td>33,037,780</td>
<td>1,915,554</td>
<td>172,471</td>
</tr>
<tr>
<td>Australia</td>
<td>17,000</td>
<td>1,100</td>
<td>154,545</td>
</tr>
<tr>
<td>Europe</td>
<td>3,923,230</td>
<td>200,839</td>
<td>195,342</td>
</tr>
<tr>
<td>North and Central America</td>
<td>2,059,441</td>
<td>126,540</td>
<td>162,750</td>
</tr>
<tr>
<td>South America</td>
<td>76,430</td>
<td>5,237</td>
<td>145,342</td>
</tr>
<tr>
<td>By Nation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>25,558,000</td>
<td>1,502,900</td>
<td>170,058</td>
</tr>
<tr>
<td>Turkey</td>
<td>1,750,000</td>
<td>60,000</td>
<td>291,667</td>
</tr>
<tr>
<td>Iran</td>
<td>1,350,000</td>
<td>65,000</td>
<td>207,692</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>1,046,960</td>
<td>72,000</td>
<td>145,411</td>
</tr>
<tr>
<td>Russian Federation</td>
<td>715,000</td>
<td>60,000</td>
<td>119,167</td>
</tr>
</tbody>
</table>

Asia dominates the world production of cantaloupe and other melons by producing more than 71% of the total tonnage (Table 8.2) (FAO 2004). Europe, North America, and Central America follow, accounting for more than 20% of the global production. Among leading countries, China’s production is the highest (52%) followed by Turkey, the United States, Iran, Romania, and Spain (Table 8.2). Extremely high yields have also been recorded in Puerto Rico \((2.0 \times 10^6 \text{ kg/ha})\) and Canada \((1.2 \times 10^6 \text{ kg/ha})\). Developed countries produce less than one-third of total production, suggesting that the potential for export to these countries is high and that international trade could increase. However, if one considers the large volume of home-grown melons, such statistics may significantly underestimate actual production (Rubatzky and Yamaguchi 1997).

As with cucumber, there are many melon market classes. For instance, the horticultural groups, Cantalupensis (i.e., cantaloupe) and Inodorus (i.e., honeydew), are of particular commercial importance in the United States, Europe, and Asia (McCreight, Nerson, and Grumet 1993). The importance of other melon groups depends on the region of production and usage (Rubatzky and Yamaguchi 1997).

The cultivated \textit{Cucurbita} species, commonly referred to as squashes, pumpkins, and gourds, represent a very important source of nutrition, not only in Latin American countries, but also in many other regions worldwide. About 63% of the world’s 2004 production was in Asia, which was led by China (29%) and India (18%) (Table 8.3). Extremely high yields have been reported from Netherlands \((6 \times 10^5 \text{ kg/ha})\), Spain \((4.3 \times 10^5 \text{ kg/ha})\), Israel \((4.1 \times 10^5 \text{ kg/ha})\), and France \((4.1 \times 10^5 \text{ kg/ha})\) (FAO 2004). Overall, production in developing countries is nearly four times that of the developed countries, which likely reflects the use of \textit{Cucurbita} as a staple food in developing countries (Rubatzky and Yamaguchi 1997).

Watermelons are grown throughout the world in areas where a long, warm growing season prevails. In 2004, Asia was by far the most important watermelon production region with 79% of the world area in cultivation, resulting in 87% of global production (Table 8.4). China dominated Asian watermelon production with more than 75% of that continent’s production area and more than 84% of the Asian harvest (Table 8.4).

Between three and four million ha are collectively devoted to watermelon production in Africa, North America, Central America, and Europe with a smaller area (and lower yields) found in South America (Table 8.4). Production yields in Europe are typically below the world average, which is likely due to less favorable growing conditions (Maynard 2001). Nevertheless, exceptionally high yields have been recorded in Cyprus \((5.4 \times 10^5 \text{ kg/ha})\) and Spain \((4.1 \times 10^5 \text{ kg/ha})\) (FAO 2004).

<table>
<thead>
<tr>
<th>Location</th>
<th>Production (metric tons)</th>
<th>Area Harvested (ha)</th>
<th>Mean Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>27,371,268</td>
<td>1,313,727</td>
<td>208,348</td>
</tr>
<tr>
<td>By Continent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>1,601,325</td>
<td>79,250</td>
<td>202,060</td>
</tr>
<tr>
<td>Asia</td>
<td>19,537,759</td>
<td>902,582</td>
<td>216,465</td>
</tr>
<tr>
<td>Australia</td>
<td>64,150</td>
<td>2,635</td>
<td>243,454</td>
</tr>
<tr>
<td>Europe</td>
<td>3,160,000</td>
<td>161,210</td>
<td>196,018</td>
</tr>
<tr>
<td>North and Central America</td>
<td>2,440,099</td>
<td>125,081</td>
<td>195,082</td>
</tr>
<tr>
<td>South America</td>
<td>565,400</td>
<td>42,800</td>
<td>132,103</td>
</tr>
<tr>
<td>By Nation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>14,338,000</td>
<td>558,500</td>
<td>256,723</td>
</tr>
<tr>
<td>Turkey</td>
<td>1,700,000</td>
<td>115,000</td>
<td>147,826</td>
</tr>
<tr>
<td>U.S.A</td>
<td>1,240,000</td>
<td>46,000</td>
<td>269,565</td>
</tr>
<tr>
<td>Iran, Romania, Spain</td>
<td>1,000,000</td>
<td>70,000</td>
<td>142,857</td>
</tr>
<tr>
<td>Romania</td>
<td>1,000,000</td>
<td>52,000</td>
<td>192,308</td>
</tr>
<tr>
<td>Spain</td>
<td>1,000,000</td>
<td>39,100</td>
<td>255,754</td>
</tr>
</tbody>
</table>

In addition to the three genera noted above that are cultivated and traded worldwide, there are several other notable cucurbit genera of local or regional economic importance, including Benincasa, Lagenaria, Luffa, Momordica, and Sechium. General overviews of these secondary cucurbit crop species can be found in reports by Ng (1993), Bates, Merrick, and Robinson (1995), and Robinson and Decker-Walters (1997).

Extensive world production of cucurbits is based upon a broad array of genetic diversity, ranging from traditional landraces to elite F₁ hybrid cultivars. This diversity has evolved as a result of long periods of domestication designed to meet a wide range of human needs (e.g., various foods, medicines, processed, and other products) under vastly different environmental conditions and selection pressures. Taken collectively, this biodiversity comprises the body of cucurbit genetic resources. Many researchers have recognized the importance of the conservation, description, and effective use of these genetic resources, resulting in an extensive body of published research.

### Table 8.3 World Pumpkin, Squash, and Gourd (Cucurbita spp.) Production in 2004

<table>
<thead>
<tr>
<th>Location</th>
<th>Production (metric tons)</th>
<th>Area Harvested (ha)</th>
<th>Mean Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>19,015,901</td>
<td>1,468,434</td>
<td>129,498</td>
</tr>
<tr>
<td>By Continent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>1,782,414</td>
<td>226,010</td>
<td>78,864</td>
</tr>
<tr>
<td>Asia</td>
<td>12,121,615</td>
<td>890,753</td>
<td>136,083</td>
</tr>
<tr>
<td>Australia</td>
<td>93,226</td>
<td>6,584</td>
<td>141,595</td>
</tr>
<tr>
<td>Europe</td>
<td>2,177,300</td>
<td>118,510</td>
<td>183,723</td>
</tr>
<tr>
<td>North and Central America</td>
<td>120,811</td>
<td>160,710</td>
<td>120,811</td>
</tr>
<tr>
<td>South America</td>
<td>721,500</td>
<td>56,930</td>
<td>126,735</td>
</tr>
<tr>
<td>By Nation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>5,674,200</td>
<td>303,505</td>
<td>201,923</td>
</tr>
<tr>
<td>India</td>
<td>3,500,000</td>
<td>360,000</td>
<td>97,222</td>
</tr>
<tr>
<td>Ukraine</td>
<td>900,000</td>
<td>50,000</td>
<td>180,000</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>740,000</td>
<td>35,010</td>
<td>211,368</td>
</tr>
<tr>
<td>Egypt</td>
<td>710,000</td>
<td>39,200</td>
<td>181,122</td>
</tr>
</tbody>
</table>


### Table 8.4 World Watermelon Production in 2004

<table>
<thead>
<tr>
<th>Location</th>
<th>Production (metric tons)</th>
<th>Area Harvested (ha)</th>
<th>Mean Yield (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World</td>
<td>93,481,266</td>
<td>3,461,023</td>
<td>270,097</td>
</tr>
<tr>
<td>By Continent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Africa</td>
<td>3,799,605</td>
<td>182,604</td>
<td>208,079</td>
</tr>
<tr>
<td>Asia</td>
<td>81,156,597</td>
<td>2,658,635</td>
<td>305,257</td>
</tr>
<tr>
<td>Australia</td>
<td>110,955</td>
<td>4,335</td>
<td>255,952</td>
</tr>
<tr>
<td>Europe</td>
<td>3,954,683</td>
<td>351,392</td>
<td>112,543</td>
</tr>
<tr>
<td>North and Central America</td>
<td>3,135,114</td>
<td>127,181</td>
<td>246,508</td>
</tr>
<tr>
<td>South America</td>
<td>1,314,662</td>
<td>136,242</td>
<td>96,495</td>
</tr>
<tr>
<td>By Nation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>68,300,000</td>
<td>2,015,500</td>
<td>338,874</td>
</tr>
<tr>
<td>Turkey</td>
<td>4,000,000</td>
<td>140,000</td>
<td>285,714</td>
</tr>
<tr>
<td>Iran</td>
<td>1,900,000</td>
<td>90,000</td>
<td>211,111</td>
</tr>
<tr>
<td>U.S.A.</td>
<td>1,750,000</td>
<td>61,000</td>
<td>286,885</td>
</tr>
<tr>
<td>Egypt</td>
<td>1,600,000</td>
<td>60,000</td>
<td>266,667</td>
</tr>
</tbody>
</table>

8.2 GERMPLASM COLLECTION, MAINTENANCE, CHARACTERIZATION, AND DISTRIBUTION

The efficient utilization of cucurbit germplasm for crop improvement relies upon the ability of the world’s genebanks to conserve the breadth of genetic diversity present in cucurbitaceous crops and their wild and weedy relatives, to adequately characterize that diversity, and to make genetic resources and accurate information readily available to researchers. The following section summarizes the current status of cucurbit germplasm conservation and characterization, along with a brief examination of studies that investigate demand for, and usage of, cucurbit germplasm.

8.2.1 Gene Banks and Other Significant Germplasm Collections

The first global report of Cucurbitaceae genetic resources was compiled by Esquiná-Alcázar and Gulick (1983). The most recent compendium of cucurbit germplasm collections was prepared by Bettencourt and Konopka (1990). It provides general information about the holdings, maintenance conditions, availability, evaluation, and documentation of 68 of the world’s collections, emphasizing national genebanks and citing important breeding collections. More recently, information about the holdings of the world’s largest collections of *Citrullus*, *Cucumis*, and *Cucurbita* germplasm was summarized as part of the Food and Agriculture Organization’s effort to present “The State of the World’s Plant Genetic Resources for Food and Agriculture” (FAO 1998). In addition to these overviews, numerous articles have been published describing the current status of various genebank collections of cucurbit germplasm (Table 8.5).

In Europe, international cooperation among institutions holding germplasm collections of cucurbitaceous vegetables is coordinated by the International Plant Genetic Resources Institute (IPGRI), an autonomous international scientific organization, supported by the Consultative Group on International Agricultural Research (CGIAR). IPGRI’s formal status is conferred under an establishment agreement which, by January 2002, had been signed and ratified by the governments of nearly 50 nations (http://www.ipgri.org).

<table>
<thead>
<tr>
<th>Nation</th>
<th>Genera</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>Citrullus</td>
<td>DeQueiroz et al. (1996)</td>
</tr>
<tr>
<td>China</td>
<td>Cucumis</td>
<td>Hou and Ma (2000)</td>
</tr>
<tr>
<td>India</td>
<td>Citrullus, Cucumis, Cucurbita, Lagenaria, Luffa, Momordica</td>
<td>Mal et al. (1996), Seshadri (1988), and Sharma and Hore (1996)</td>
</tr>
<tr>
<td>Latvia</td>
<td>Cucumis</td>
<td>Lepse et al. (2000)</td>
</tr>
<tr>
<td>Poland</td>
<td>Citrullus, Cucumis, Cucurbita</td>
<td>Kotlinska (1994)</td>
</tr>
<tr>
<td>Portugal</td>
<td>Citrullus, Cucumis, Cucurbita</td>
<td>Carnide (2002)</td>
</tr>
<tr>
<td>Russia</td>
<td>Fourteen genera</td>
<td>Piskunova (2002)</td>
</tr>
<tr>
<td>Spain</td>
<td>Citrullus, Cucumis, Cucurbita, Lagenaria, Luffa, Momordica</td>
<td>de la Cuadra and Varela (1996), Nuez et al. (2000a), and Picó et al. (2002)</td>
</tr>
<tr>
<td>United States</td>
<td>Cucumis, Cucurbita</td>
<td>Clark et al. (1991)</td>
</tr>
</tbody>
</table>
In most European countries, research aimed at facilitating the long-term conservation and increased utilization of plant genetic resources operates under the aegis of The European Coopera­tive Programme for Crop Genetic Resources Networks (ECP/GR). The ECP/GR is entirely financed by the member countries, and it is coordinated by IPGRI. Within this program, working groups for various crop species have been established. The first ad-hoc meeting of the ECP/GR Informal Group on Cucurbits took place in January 2002 in Adana, Turkey (Díez, Picó, and Nuez 2002). The Cucurbits Working Group ECP/GR was officially approved in 2003 (ECP/GR 2005; Thomas et al. 2005).

### 8.2.2 Acquisition and Exploration

Since the 1980s, considerable effort has been directed toward expanding the diversity of ex situ germplasm collections by collecting wild, weedy, and landrace populations of cucurbits from their centers of diversity and from the refuges of traditional agroecosystems throughout the world. Andres (2000) has published an excellent summary of exploration efforts for *Cucurbita* with special insights on the logistics of collection. No analogous overview has been published for other cucurbit genera, but many interesting and important exploration reports can be found in the literature (Table 8.6). Many more acquisition efforts have been described in less detail, often in reports on the evaluation of cucurbit landraces for their adaptive and horticultural characteristics (discussed in Section 8.6.2). In addition to the extensive collection and exploration programs developed in the United States (Williams 2005) at the beginning of the twenty-first century, similar activities are being undertaken in some European countries to help conserve landraces used in traditional farming systems [e.g., Poland (Kotlinska 1994) and Romania (Strajeru and Constantinovici 2004)].

An important research topic related to the collection of cucurbits from traditional agroecosystems is the investigation of in situ gene flow and delimitation of populations. Research on the extent

<table>
<thead>
<tr>
<th>Nation</th>
<th>Genera</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>Cucurbita</td>
<td>Tasaki (1993)</td>
</tr>
<tr>
<td>Brazil</td>
<td>Citrullus, Cucumis, Cucurbita</td>
<td>Duarte and de Queiroz (2002)</td>
</tr>
<tr>
<td>China</td>
<td>Citrullus, Cucumis, Cucurbita, Luffa</td>
<td>Wehner et al. (1996)</td>
</tr>
<tr>
<td>Cuba</td>
<td>Citrullus, Cucumis, Cucurbita, Sechium</td>
<td>Hammer et al. (1991)</td>
</tr>
<tr>
<td>Ecuador</td>
<td>Cucurbita</td>
<td>Andres (2000)</td>
</tr>
<tr>
<td>India</td>
<td>Cucumis</td>
<td>Anonymous (1993)</td>
</tr>
<tr>
<td>India</td>
<td>Citrullus, Cucumis</td>
<td>Pareek et al. (1999)</td>
</tr>
<tr>
<td>India</td>
<td>Cucumis</td>
<td>Pitchaimuthu and Dutta (1999)</td>
</tr>
<tr>
<td>Indonesia</td>
<td>Lagenaria</td>
<td>Piferré (1995)</td>
</tr>
<tr>
<td>Italy</td>
<td>Citrullus, Cucumis, Cucurbita</td>
<td>Laghetti et al. (1998)</td>
</tr>
<tr>
<td>Macedonia</td>
<td>Cucurbita</td>
<td>Ivanovska and Posimonova (2004)</td>
</tr>
<tr>
<td>Malawi</td>
<td>Cucurbita</td>
<td>Chigwe and Saka (1994)</td>
</tr>
<tr>
<td>Mexico</td>
<td>Cucurbita</td>
<td>Andres (2000)</td>
</tr>
<tr>
<td>Morocco</td>
<td>Citrullus</td>
<td>Prendergast et al. (1992)</td>
</tr>
<tr>
<td>Panama</td>
<td>Cucurbita</td>
<td>Andres (2000)</td>
</tr>
<tr>
<td>Romania</td>
<td>Cucurbita</td>
<td>Strajeru and Constantinovici (2004)</td>
</tr>
<tr>
<td>Spain</td>
<td>Citrullus, Cucumis, Cucurbita</td>
<td>Nuez et al. (1986a, 1986b, 1987, 1992)</td>
</tr>
<tr>
<td>Sudan</td>
<td>Cucumis</td>
<td>Mohamed and Taha (2004)</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Citrullus, Cucumis, Cucurbita, Lagenaria</td>
<td>Pistrick et al. (1994)</td>
</tr>
<tr>
<td>United States</td>
<td>Cucurbita</td>
<td>Andres (2000)</td>
</tr>
<tr>
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<td>Cucurbita</td>
<td>Segovia et al. (2000)</td>
</tr>
<tr>
<td>Zambia</td>
<td>Cucurbiteaeae</td>
<td>Whitaker (1984)</td>
</tr>
<tr>
<td>Zimbabwe</td>
<td>Citrullus, Cucumis</td>
<td>Toll and Gwarazimba (1983)</td>
</tr>
</tbody>
</table>
of in situ gene flow has employed isozyme, morphological, and secondary chemical markers to
document hybridization between cultivated populations and neighboring wild or weedy popula­
tions of Cucurbita, primarily in Mexico and Texas (Merrick and Nabhan 1984; Nabhan 1984;  
Kirkpatrick and Wilson 1988; Wilson 1990). Controlled experiments under more intensive, field­
production conditions have also used genetic markers to document the frequency and distance of 
gene flow through pollen transfer between Citrullus (Rhodes, Adamson, and Bridges 1987) and  
Cucumis (Handel 1983; Handel and Mishkin 1984) populations in the absence of physical isolation  
other than distance.

8.2.3 Collection Regeneration and Maintenance

The ability of genebanks to conserve the genetic profiles of cucurbit germplasm relies on protocols  
that ensure the production of high-quality seed and minimize outcross contamination, genetic drift,  
and changes brought about through selective regeneration environments. Once produced, the seeds  
should be held under moisture and temperature conditions that prolong their longevity to increase  
the interval between regeneration events and reduce potential changes to genetic profiles.  
Most cultivated cucurbits have large flowers that are attractive to pollinating insects. The  
ability to preserve their genetic identity relies upon the use of tents, cages, greenhouse isolation  
chambers (Figure 8.1 and Figure 8.2), and similar systems to ensure isolation and the manipu­
lare of insects and/or human labor to effect pollination. Descriptions of the field cages and  
greenhouse chambers used to isolate cucurbit germplasm for seed production can be found in  
reports by Grewal and Sidhu (1979), Ellis et al. (1981), Ruszkowski and Bilifiski (1984), and Cox,  
Able, and Gustafson (1996).

Figure 8.1 Regeneration of wild Cucumis species in greenhouse cages. (Photo courtesy of L. Clark.)
Another key component in the regeneration system is to have effective, easily manipulated insect pollinators. The effective use of queenright nucleus hives of honeybees (Apis mellifera) in field cages of cucurbits was first described by Ellis et al. (1981) and was refined by Neykov (1997). Other bee genera, i.e., Bombus, Megachile, and Pithitis (Grewal and Sidhu 1979; Ruszkowski and Biliński 1984), and fly genera, i.e., Lucillia, Musca, and Phomita (Neykov 1997), have been used in field cages as alternatives to honeybees or hand pollination. Under greenhouse conditions, Bombus ruderarius and B. terrestris have been shown to be effective pollinators of Cucumis (Ruszkowski and Biliński 1984; Fisher and Pomeroy 1989). The pollination of Cucumis by Megachile rotundata in greenhouses has also been reported (Szabo and Smith 1970), but Ruszkowski and Biliński (1984) found Megachile to be somewhat inferior to Bombus as pollinators.

Widrlechner et al. (1992) used isozyme markers to compare genetic profiles between 157 pairs of genebank seedlots of C. sativus with one lot produced in a field cage with honeybees and the other being an older, open-pollinated sample of the same accession that the first lot replaced. As expected, the cage-pollinated samples were generally more homogeneous than the open-pollinated samples they replaced. In addition, certain rare isozyme alleles were found in the cage-pollinated samples, but not in the open-pollinated samples.

In addition to the preservation of genetic profiles, the production of disease-free, highly viable seeds with appropriate longevity for long-term storage must be an important consideration in any seed regeneration protocol (Sackville Hamilton and Chorlton 1997). Pertinent research reports on the quality of cucurbit seed production include investigations on optimal fruit maturity (Edwards, Lower, and Staub 1986; Oluoch and Welbaum 1996), the duration of seed fermentation (Nienhuis and Lower 1981) and washing (Oluoch and Welbaum 1996), and seed-drying methods (Zhang and Tao 1988; Ji, Guo, and Ye 1996; Kong and Zhang 1998; Shen and Qi 1998).

Once high-quality seeds are produced, they should be conserved under optimal storage conditions. Fortunately, typical storage conditions for germplasm conservation in base collections (−15 to −18°C) can preserve the viability of cucurbit seeds for many years. Roos and Davidson (1992) reported median viability periods (P50) for C. melo and C. sativus of 32–79 years and for C. lanatus of 34–48 years. Based on 11 years of storage at −18°C, Stoyanova (2001) calculated even more impressive P50 values of 297 years for Cucurbita, 506 years for Cucumis melo, and 785
years for *C. sativus*. Mean moisture contents for the samples in that study were typically in the range of 4–6%. Likewise, after 15 or more years of storage at −15°C, Specht et al. (1998) reported that all *C. sativus* and most *C. melo* samples tested were in the 71–100% germination class. Similar results were reported for *C. pepo* samples stored between 12 and 14 years.

Extended longevity for cucurbit seeds has also been noted in samples stored at above-freezing temperatures. Fan et al. (1989) reported that about half of 44 seed samples of *C. sativus* that had been stored for 23 years in a storehouse under low ambient humidity in western China retained at least 50% germination after storage. And, recently, Reitsma and Clark (2005) reported that the oldest *Cucumis* and *Cucurbita* seedlots of sufficient quality to distribute from the North Central Regional Plant Introduction Station’s genebank were now more than 40 years old. Those seedlots have generally been held at 4°C and between 25 and 40% RH. These favorable results from using typical cold storage may help explain the overall lack of information about cryogenic seed storage of cucurbits. Only Chernova (1990) has reported on the successful use of liquid nitrogen vapor in storing *Citrullus* seeds.

Tissue culture and pollen storage can be valuable conservation tools as supplements or alternatives to seed storage, especially in cases where valuable germplasm accessions produce no viable seeds. Of the two methods, pollen storage may be the more intractable. An early report (Griggs, Vansell, and Iwakiri 1953) indicated that storage of *C. melo* pollen should be feasible at −18°C, but evidently did not lead to additional research or the refinement of protocols. Islam and Khan (1998) subsequently reported storage of *M. dioica* pollen up to 45 days at 0°C, but their determination of viability was based upon acetocarmine staining, which is not a vital stain. Wang and Robinson (1983) reported that *Cucurbita* pollen was extremely short-lived (typically living only a few hours) and noted that a variety of treatments, including the use of rapid freezing, a nitrogen atmosphere, and storage in organic solvents, could not extend its viability. On a more positive note, considerable progress has been made in Japan in the development of cucurbit pollen-storage methods, primarily for *Citrullus* (reviewed by Sugiyama, Morishita, and Nishino 2002b), with the best long-term results obtained by storing pollen in ethyl acetate at −20°C (Sugiyama, Morishita, and Nishino 2002b).

In contrast to pollen storage, tissue-culture methods for cucurbits are relatively well-developed, although organogenesis and regeneration success can be strongly influenced by genotype (see Molina and Nuez 1996). Protocols for embryo and ovule culture were recently reviewed by Skálová, Lebeda, and Navrátilová (2004) and those for protoplast culture by Gajdová, Lebeda, and Navrátilová (2004). Somatic embryos of *C. melo* generated from callus culture have been successfully cryopreserved after desiccation (Shimonishi et al. 1991, 2000). From a germplasm conservation perspective, however, the most valuable culture system is one that produces and preserves apical and/or axillary meristems because intact meristems are generally more stable genetically than are many other cultured tissues (Withers 1989). A method to establish shoot-primordia cultures for *C. melo* was reported (Nagai, Nomura, and Oosawa 1989), but it is labor intensive, requiring repeated subculture. Ogawa et al. (1997) developed a slow prefreezing procedure to cryopreserve shoot primordia of a *C. melo* cultivar. However, to date, there are no reports of the application of this promising procedure to germplasm conservation.

### 8.2.4 Germplasm Characterization

It is crucial that genebank managers correctly establish the taxonomic identity of the collections they conserve (see Section 8.3 for an overview of taxonomic studies). Accurate characterization of germplasm should support its proper identification at both the species and cultivar levels, which facilitates both maintenance and utilization and reduces the frequency of unintended duplication or near-duplication (maintenance of closely related populations). A broad range of techniques has
been employed to apply morphological, protein, and DNA markers to characterize germplasm collections (reviewed by Bretting and Widrlechner 1995), including those of cucurbits.

Studies solely employing traditional, morphological methods to characterize cucurbit germplasm are typically focused on revealing valuable horticultural traits and are discussed within that context in a review of research on screening for useful traits (see Section 8.6.2).

Polymorphic proteins have been widely used to characterize patterns of genetic diversity within, and among, cucurbit germplasm collections. Nearly all pertinent reports focus on enzyme or isozyme variation. The only notable exceptions are those made by Indian researchers who examined electrophoretic (SDS PAGE) variation in seed-storage proteins in *C. melo* (Singh, Shukla, and Tewari 1999; Sawant and More 2002) and in *C. sativus* (Singh and Ram 2001) and by Navot and Zamir (1987) who analyzed variation in seed-storage proteins in *Citrullus*.

Many of the initial studies of cucurbits that establish protocols for isozyme detection and analysis describe the genetic control of polymorphic banding patterns and examine evolutionary relationships were reviewed by Dane (1983), Doebley (1989), Puchalski and Robinson (1990), and Weeden and Robinson (1990). Two other key papers describing genetic control of and linkage relationships among loci responsible for isozyme variation are those of Knerr and Staub (1992) for *C. sativus* and Staub, Meglic, and McCreight (1998) for *C. melo*.

With well-developed arrays of isozyme polymorphisms available for *Citrullus, Cucumis, Cucurbita,* and *Momordica,* extensive surveys of isozyme variation have been used to elucidate genetic relationships among germplasm collections of those four genera. The most comprehensive of such surveys have been conducted for hundreds of accessions of *C. sativus* from throughout the world, emphasizing those conserved by the U.S. National Plant Germplasm System (NPGS). Results from this long-term survey were published as a series of papers with two overall assessments (Knerr et al. 1989; Meglic, Serquen, and Staub 1996) and more specialized studies of historic cultivars (Meglic and Staub 1996b) and collections from India (Staub, Serquen, and McCreight 1997b) and China (Staub et al. 1999). Similar surveys have been conducted on extensive samples of *Citrullus* (Navot and Zamir 1987) and *C. melo* germplasm (Meglic, Horejsi, and Staub 1994; McCreight et al. 2004) and more limited sets of *C. colocynthis* populations from Israel and Sinai (Zamir, Navot, and Rudich 1984); of landraces of *C. melo* from Spain (Esquinas-Alcazar 1981), India (Sujatha et al. 1991), and eastern and southern Asia (Akashi et al. 2002b; Kato et al. 2002); of wild *Cucumis* species (Frederick, and Marty 1987; Puchalski and Robinson 1990; Staub, Staub et al. 1992); of wild and domesticated *Momordica charantia* L. (Marr, Mei, and Bhattarai 2004); and of six genera of the Benincaseae (Walters et al. 1991). Peroxidase banding patterns were also used as one line of evidence in the correct assignment of the newly discovered Xishuangbanna cucumber to the species *C. sativus* (Qi, Yuan, and Li 1983).

Isozyme analyses have been used to evaluate traditional, morphologically defined taxa in *Cucurbita* with varying results. In an initial survey with three enzyme staining systems, Puchalski and Robinson (1978) reported general agreement with an earlier taxonomic treatment of relationships among *Cucurbita* species (Bemis et al. 1970). More extensive surveys of isozyme variation among *Cucurbita* species (Puchalski and Robinson 1990) and, more specifically, in wild, weedy, domesticated Mexican *Cucurbita* presented more complex views of genetic differentiation and gene flow among taxa (Wilson 1989). Isozyme analyses by Decker-Walters et al. (1990) did not support traditional infraspecific classifications for *C. maxima, C. argyrosperma,* or *C. moschata.* The most thorough isozyme analyses, however, have been devoted to *C. pepo* in its wild, weedy, and domesticated forms (Ignart and Weeden 1984; Decker and Wilson 1987; Decker-Walters et al. 1993) and have been instrumental in clarifying patterns of variation and domestication within this species.

A recent analysis of *M. charantia* germplasm (Marr, Mei, and Bhattarai 2004) examined both morphometric and isozyme variation to describe patterns of crop evolution and overall diversity. Isozyme variation supported a single domestication event with extremely low levels of variation among domesticated accessions. However, this analysis could not identify the wild populations most closely related to the original domesticate.
The discovery of a tight linkage between an acid phosphatase locus and a locus controlling resistance to nematode infestation in *Lycopersicon* (Rick and Fobes 1974) encouraged other researchers to investigate possible linkages between enzyme loci and those controlling disease resistance and other useful, but difficult to evaluate, traits. In cucurbits, this led to the observation that peroxidase banding patterns could be used as a marker for resistance to *Pseudoperonospora cubensis* in *C. melo* (Reuveni, Shimoni, and Karchi 1990, 1992). In contrast, Lebeda and Doležal (1995) found no general relationship between peroxidase banding patterns and reaction to *P. cubensis* in *C. sativus*. Similarly, Kennard et al. (1994), Meglic and Staub (1996a), and Bradeen et al. (2001) found no useful linkage relationships between enzyme and disease resistance loci in *C. sativus*. Similarly, Kennard et al. (1994), Meglic and Staub (1996a), and Bradeen et al. (2001) found no useful linkage relationships between enzyme and disease resistance loci in *C. sativus*. In *Citrullus lanatus*, Benscher and Provvidenti (1991) conducted an initial study that suggested a possible linkage between a phosphoglucoisomerase locus and resistance to *Zucchini yellow mosaic virus* (ZYMV), but omission of this research from a later review of ZYMV resistance in *C. lanatus* by Provvidenti (1993) suggests that the proposed linkage could not be confirmed.

Many different classes of DNA markers have been used to characterize cucurbit germplasm, including both plastid and nuclear markers. Plastid markers are typically highly conserved, making them especially valuable for revealing phylogenetic relationships at or above the species level (Decker-Walters, Chung, and Staub 2004a). The first report investigating restriction endonuclease site polymorphisms in cucurbit chloroplast DNA was presented by Juvik and Palmer (1984) who conducted an initial survey of 12 accessions representing four genera: *Citrullus*, *Cucumis*, *Cucurbita*, and *Lagenaria*. A more extensive analysis of chloroplast DNA focusing on *Cucurbita* (Wilson, Doebley, and Duvall 1992) is valuable in elucidating relationships between domesticated taxa and their wild progenitors. More recently, mitochondrial DNA sequence analysis was conducted upon a similar set of *Cucurbita* accessions (Sanjur et al. 2002) built upon Wilson, Doebley, and Duvall (1992) findings and suggested at least six independent domestication events. Less-conserved, hypervariable regions within chloroplast DNA have also recently been evaluated as a source of markers, called consensus chloroplast simple sequence repeats (or cc SSRs), for the characterization of cucurbits by Chung and Staub (2003, 2004), but results of broad-based screening germplasm collections with cc SSRs have not been reported.

Of the many nuclear-marker classes available, the internal transcribed spacer (ITS) regions of nuclear ribosomal RNA genes evolve at a rate that gives them similar utility to many plastid gene markers in revealing phylogenetic relationships at or above the species level. For example, Jobst, King, and Hemleben (1998) and Garcia-Mas et al. (2004) conducted a phylogenetic analysis of the Cucurbitacae based on ITS markers that clarified evolutionary relationships at the tribal level. Jarret and Newman (2000) evaluated ITS sequence variation to help construct a phylogeny of all known species of *Citrullus*.

Many other nuclear marker classes such as random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSRs) are considerably more variable and are most useful for clarifying relationships at or below the species level. RAPD markers have been the most widely used to characterize intraspecific patterns of diversity among cucurbit germplasm collections. There are more than twenty publications reporting results of RAPD analyses of cucurbit germplasm, including analyses of *Citrullus*, *Cucumis*, *Cucurbita*, and *Lagenaria*. Key papers are summarized in Table 8.7. Notably, none of these studies (Table 8.7), either individually or collectively, provides a comprehensive analysis of diversity within any cucurbit crop in a manner analogous to the isozyme analyses of *C. sativus* described above.

The degree of repeatability and variation in genetic control of RAPD banding patterns can be a concern and has led some cucurbit researchers to employ other types of nuclear DNA markers. These marker classes are typically thought to be more repeatable and often display codominant product expression, theoretically supplying more genetic information. Of the codominant markers, early studies often used restriction fragment length polymorphisms (RFLPs) (see Dijkhuizen et al. 1996; Garcia-Mas et al. 2000), but once SSR loci and appropriate primers were established in
### Table 8.7 Articles Describing Random Amplified Polymorphic DNA (RAPD) Variation among Cucurbit Germplasm Accessions

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Number and Type of Accessions</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td><em>Citrullus colocynthis</em> and <em>lanatus</em></td>
<td>Forty-two germplasm accessions and five cultivars</td>
<td>Levi et al. (2001a)</td>
</tr>
<tr>
<td><em>Citrullus lanatus</em></td>
<td>Thirty-nine diverse cultivars</td>
<td>Lee et al. (1996)</td>
</tr>
<tr>
<td><em>Cucumis hystrix</em> × <em>hytivus, melo, metuliferus,</em> and <em>sativus</em></td>
<td>Forty-six American cultivars and twelve germplasm accessions</td>
<td>Levi et al. (2001b)</td>
</tr>
<tr>
<td><em>Cucumis melo</em></td>
<td>Thirty-two diverse breeding lines</td>
<td>Garcia et al. (1998)</td>
</tr>
<tr>
<td><em>Cucumis melo</em></td>
<td>Fifty-four germplasm accessions and breeding lines</td>
<td>Perl-Treves et al. (1998), Stepansky et al. (1999)</td>
</tr>
<tr>
<td><em>Cucumis melo</em></td>
<td>Six diverse accessions</td>
<td>Garcia-Mas et al. (2000)</td>
</tr>
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<td><em>Cucumis melo</em></td>
<td>Forty-six germplasm accessions</td>
<td>Staub et al. (2000)</td>
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<tr>
<td><em>Cucumis melo</em></td>
<td>108 African germplasm accessions and eighteen reference lines</td>
<td>Milki et al. (2001)</td>
</tr>
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<td><em>Cucumis melo</em></td>
<td>Fifteen Spanish landraces</td>
<td>López-Sesé et al. (2002)</td>
</tr>
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<td><em>Cucumis melo</em></td>
<td>114 East and South Asian landraces</td>
<td>Kato et al. (2002)</td>
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<td><em>Cucumis melo</em></td>
<td>Forty-two New World wild populations, ten Old World wild populations, and fourteen cultivated accessions</td>
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<td>One Turkish and ten Portuguese landraces</td>
<td>Carnide et al. (2004)</td>
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<td>Nine Portuguese landraces and one French cultivar</td>
<td>Barroso et al. (2004)</td>
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<td>Seventeen Greece landraces and inbred lines</td>
<td>Staub, Fanourakis, and López-Sesé (2004)</td>
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<td><em>Cucumis melo</em></td>
<td>Sixty-seven Japanese cultivars</td>
<td>Nakata et al. (2005)</td>
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<td><em>Cucumis sativus</em></td>
<td>Forty-four cultivars, thirty-nine breeding lines, and thirty-five germplasm accessions</td>
<td>Horejsi and Staub (1999)</td>
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<tr>
<td><em>Cucumis sativus</em></td>
<td>Fifty germplasm accessions</td>
<td>Zhang et al. (2002)</td>
</tr>
<tr>
<td><em>Cucumis sativus</em></td>
<td>Twenty-six African germplasm accessions and twenty-one reference lines</td>
<td>Milki et al. (2003)</td>
</tr>
<tr>
<td><em>Cucurbita ficifolia, maxima, moschata,</em> and <em>pepo</em> and <em>Lagenaria siceraria</em></td>
<td>Eight Greek landraces</td>
<td>Pavlikal et al. (2004)</td>
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<td><em>Cucurbita moschata</em></td>
<td>Nineteen germplasm accessions of <em>C. maxima</em> and eight related cucurbit accessions</td>
<td>Ferriol et al. (2003b)</td>
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<td><em>Cucurbita pepo</em></td>
<td>Thirty-one landraces and forty-three cultivars of <em>L. siceraria</em> and one accession of <em>L. sphaerica</em></td>
<td>Gwanama et al. (2000)</td>
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<tr>
<td><em>Lagenaria siceraria</em> and <em>L. sphaerica</em></td>
<td>Thirty-one landraces and forty-three cultivars of <em>L. siceraria</em> and one accession of <em>L. sphaerica</em></td>
<td>Decker-Walters et al. (2002b)</td>
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<td><em>Lagenaria breviflora,</em> <em>L. siceraria</em> and <em>L. sphaerica</em></td>
<td>One accession of <em>L. breviflora,</em> one wild and three domesticated accessions of <em>L. siceraria,</em> and six accessions of <em>L. sphaerica</em></td>
<td>Decker-Walters et al. (2004b)</td>
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</table>
exhibited lower CVs than did either isozymes or SSRs in classes, both in relation to each other and in relation to morphological observations. In addition, RAPDs were superior to quantitative analysis of morphological traits in resemblance to polymorphisms (AFLPs) (Garcia-Mas et al. 2004) and SSRs among taxa. It should not be surprising that considerable knowledge has been gained about the comparative utility of marker classes. Fortunately, studies have been conducted that compare multiple marker classes, both in relation to each other and in relation to morphological observations. One of the first comparative analyses was conducted by Dijkhuizen et al. (1996) who determined that RFLP marker data revealed differences between C. sativus var. sativus and var. hardwickii when isozyme data were not as conclusive.

Since then, many molecular studies in cucurbits have been conducted via RAPD analysis, so it should not be surprising that considerable knowledge has been gained about the comparative utility of marker classes. In terms of coefficients of variation (CV) per band, RAPDs exhibited lower CVs than did either isozymes or SSRs in Cucumis (Staub et al. 1997a, 2000) with moderate congruence between results generated by each marker class. In contrast, Zhuang et al. (2004) reported a high degree of congruence between genetic relationships revealed by RAPDs and SSRs among Cucumis taxa.

In a three-way comparison, measurements of genetic distance from RAPDs more closely resembled those from AFLPs than those from isozymes for C. sativus (Horejsi and Staub 1999). In addition, RAPDs were superior to quantitative analysis of morphological traits in C. melo in calculating genetic distances in comparison to known pedigree data (Garcia et al. 1998). A three-way comparison of AFLP, RAPD, and RFLP markers to describe patterns of genetic variation among six diverse accessions of C. melo gave similar results for each marker class (Garcia-Mas et al. 2000), but the authors indicated that AFLPs were most efficient in detecting polymorphisms. In addition, Ferriol, Picó, and Nuez (2003b) reported that SBAPs were superior to RAPDs in revealing patterns of genetic diversity in C. maxima, noting that the results of RAPD analyses conformed neither to groupings by morphology nor geographic origin, and, similarly, there was no correlation between RAPD analyses and morphological or geographical groupings of C. melo landraces from Portugal (Camide et al. 2004) or Spain (López-Sesé, Staub, and Gomez-Guillamon 2003). Ferriol, Picó, and Nuez (2003a) and Ferriol et al. (2004a) also evaluated other Cucurbita species with SRAPs and AFLPs and noted some interesting distinctions. SRAPs were generally more concordant with morphological variation and agronomic traits, consistent with this marker class’s connection to open reading frames; however, AFLPs grouped C. maxima accessions by geographic origin, reflecting the bottleneck that occurred with the introduction of this species to the Old World (Ferriol, Picó, and Nuez 2004b).

Katzir et al. (2000) noted that SSR data supported a dendrogram of C. pepo germplasm that had been developed by ISSR marker analysis, and in a more comprehensive study of C. pepo germplasm, Paris et al. (2003) confirmed a high degree of congruence among AFLP, ISSR, and SSR marker data that they then used in support of existing botanical and horticultural classifications.

Ideally, characterization based on multiple lines of evidence, including molecular markers and morphological, phenological, and biochemical information such as that reported by Paris et al.
CUCURBITS (CUCURBITACEAE; CUCUMIS SPP., CUCURBITA SPP., CITRULLUS SPP.)

(2003) for *C. pepo*, should shed the greatest light upon relationships among germplasm accessions. In *C. sativus*, Horejsi and Staub (1999) reported that patterns of variation revealed by RAPD marker analysis were consistent with geographic origin and morphological characters. Shortly thereafter, Staub and Ivančić (2000) reported on a combined analysis of genetic variation in *C. sativus* including both isozyme and RAPD markers. For *C. melo*, Perl-Treves et al. (1998), Stepiński, Kovalski, and Perl-Treves (1999a) presented results of a comprehensive analysis of morphological markers, sugar composition, and RAPD and ISSR markers to test an infraspecific classification scheme proposed by Munger and Robinson (1991). From a more limited analysis of fifteen Spanish landraces of *C. melo*, López-Sese et al. (2002) reported that patterns of genetic variation revealed by RAPDs and SSRs were generally congruent, and bulk sampling within an accession was appropriate for the assessment of large collections. Another recent study combining multiple lines of evidence, in this case, from AFLP data and morphological types, was published by Che et al. (2003) for *C. lanatus*.

Beyond simple characterization, molecular-marker data can be analyzed to help create core collections, as defined by Frankel (1984), that can serve as a management tool for the efficient sampling of genetic diversity from large germplasm collections. The most progress in the establishment of core collections for cucurbit germplasm has been made following the suggestion of Staub (1994a, 1994b), which ultimately led to the selection of a core collection for *C. sativus* from the holdings of the U.S. NPGS based on past isozyme analyses, historical and geographical information, and disease-evaluation data (Staub et al. 2002).

From a practical perspective, just as work has been conducted to search for close associations between isozyme loci and those that control useful traits (reviewed above), researchers have searched for such associations between DNA markers and useful-trait loci. These efforts can be facilitated through the development of comprehensive linkage maps (see Kennard et al. 1994; Périn et al. 2002b; Levi et al. 2004b; Zraidi and Lelley 2004), but also can emerge from germplasm characterization studies. Examples linking RAPD banding patterns with useful trait loci in *Citrullus* have been reported by Lee et al. (1996) for high levels of free fruit sugars and by Levi et al. (2001a) for resistance to gummy stem blight (*Didymella bryoniae*) and Fusarium wilt. And the development of a linkage map for *C. melo* by Périn et al. (2002b) has been used to locate loci controlling tolerance to sulfur application (Perchepied et al. 2004) and to map two disease-resistance loci (Brotman et al. 2004). A second linkage map for *C. melo* has been used to locate loci controlling resistance to cotton aphid (*Aphis gossypii*) and powdery mildew caused by *P. xanthii* (syn. *S. fuliginea*) (Fukino et al. 2002). Additional examples of exploitable linkage relationships are discussed in Section 8.5.2 and Section 8.5.3.

8.2.5 Germplasm Descriptors

Standardized lists of evaluation descriptors and protocols are often used to provide structure and comparability for germplasm characterization and evaluation. Internationally recognized descriptor lists have typically been published by the International Board for Plant Genetic Resources (IBPGR) and its successor organization, IPGRI. However, only a single international list for cucurbits has been published, one for *C. melo* (IPGRI 2003). Development of international descriptor lists for other important cucurbits was identified as a crucial task by the newly developed Working Group on Cucurbiteaceous Vegetables in Adana, Turkey (Diez, Pico, and Nuez 2002). As a preliminary step, sets of the most significant descriptors, called minimum descriptor lists, were elaborated (Thomas et al. 2004). Descriptor lists for *Cucumis* (Křístková et al. 2003) and cultivated *Cucurbita* species (Křístková et al. 2005) have been developed for the national gene bank collections of the Czech Republic (Table 8.8 and Figure 8.3). A recent discussion of the development of new descriptor systems for *Cucumis* and *Cucurbita* was recently presented by Vinter et al. (2004).
Table 8.8 Morphological Descriptors for Cultivated Cucurbita Species

<table>
<thead>
<tr>
<th>Number</th>
<th>Descriptor Name</th>
<th>Scale</th>
<th>Descriptor State</th>
<th>Explanation</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1; Morphological descriptors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4; Leaf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4.1.4; * S (I)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf blade—</td>
<td>1</td>
<td>acute</td>
<td></td>
<td>Figure 8.3</td>
<td>Fully developed leaf from the middle part of plant at botanical maturity</td>
</tr>
<tr>
<td>shape of</td>
<td>2</td>
<td>subacute</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>apex of</td>
<td>3</td>
<td>obtuse</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>terminal lobe</td>
<td>4</td>
<td>mucronate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>rounded</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>truncate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>sinuate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *, Highly discriminating descriptor; S = descriptor characterizing species; I = descriptor discriminating infraspecific variation; ( ), letter in parentheses is on a secondary significance.


Although descriptor lists, whether from international standards or individual genebanks, can be used to guide morphological characterization per se (the verification of botanical and horticultural identity and clarification of genetic relationships among collections), as exemplified in Cucumis melo by Costa et al. (1989) and in Cucurbita by Nuez et al. (2000b) and by Křištková, Křištková, and Vinter (2004), they are more often applied to the evaluation of germplasm to uncover valuable traits (see Section 8.6.2 for an in-depth treatment of this topic). In addition to the reports cited above, an extensive project to characterize germplasm accessions of C. pepo, primarily to elucidate patterns of variation, has been conducted by researchers at the Newe Ya‘ar Research Center in Israel (Nerson, Paris, and Paris 2000; Paris 2001a; Paris and Nerson 2003).

8.2.6 Germplasm Distribution Analysis and Its Incorporation into Collection Management

Knowledge of the patterns of distribution of germplasm accessions from genebanks, especially as related to the extent and intended uses of distributions, is central to efficient genebank management and can help curators meet future demand (Widrlechner and Burke 2003). Unfortunately, to date, the body of published research on patterns of cucurbit germplasm distribution or how well it meets users’ needs is extremely limited. Without such information, it is difficult for curators to apply international guidelines for seed regeneration (Sackville Hamilton and Chorlton 1997) that require good projections of future germplasm demand.

Figure 8.3 Descriptor 1.4.1.4. Leaf blade of Cucurbita species—shape of apex of terminal lobe (example. From Křištková, E., Křištková, A., Vinter, V., and Lebeda, A., Hort Sci., Prague, in press.) Morphotypes of C. pepo (According to Paris, H. S., Econ. Bot., 43, 13–43, 1989): AC = acorn; PU = pumpkin; ZU = zucchini. Origin of accessions: PI 285611, PI 512225 (Plant Introduction Station, Iowa State University, Ames, U.S.A.); 09H4200137, 09H4200616 (Research Institute of Crop Production, Gene Bank Workplace in Olomouc, Czech Republic); C. moschata cv. Butternut = Stokes, U.S.A.; C. pepo cv. Table Queen = Ball Seeds, Canada; C. pepo cv. Zelena = Nohel, Czech Republic.
Clark et al. (1991) presented a simple distribution analysis, classifying the types of uses that were made of the cucurbit germplasm conserved by the North Central Regional Plant Introduction Station (NCRPIS) in Iowa. Gao et al. (2000, 2001) conducted an extensive user survey to classify important characteristics of researchers requesting samples and of the germplasm that had been distributed, how those germplasm accessions were being used, and factors limiting utilization. *C. sativus* was included among the ten major crops in their analysis, and, as part of that study, Gao et al. (2001) presented a summary of how *C. sativus* germplasm accessions were important in the development of new cultivars in China. In a somewhat less formal fashion, Queiroz et al. (2004) described how local *Citrullus* germplasm has been successfully integrated into varietal development in Brazil.

Widrlechner and Burke (2003) analyzed distribution patterns for *C. melo* and *sativus* and *C. pepo* germplasm accessions from the NCRPIS over a 12-year period. They noted an overall decline in distribution rate for all three species, which they attributed to three possible causes: (1) declines experienced after the completion of large-scale evaluation projects (which was true for *C. sativus*); (2) those caused by the gradual incorporation of valuable germplasm into users' research and crop improvement programs; and (3) those resulting from an increase in targeting of germplasm distributions as increasing amounts of evaluation data are made available to users. Circumstantial evidence was presented in support of increased targeting as average shipment size declined over time. The distributions of cucurbit germplasm on a per-accession basis were normally distributed, simplifying the prediction of demand among individual accessions, which can then be applied to diverse managerial tasks (Sackville Hamilton and Chorlton 1997; Widrlechner and Burke 2003).

### 8.3 TAXONOMY

#### 8.3.1 Introduction

Taxonomy is both the master and servant of biology: *master* in that the results of all other fields of biological research contribute to its database, and *servant* in that it provides name assignment opportunities for all practitioners of biology, without which repeatability and, therefore, application of the scientific method in biology would be possible. This dichotomy is also reflected by differences in philosophical and methodological approaches: one termed *empiricist*, which explores phylogenetic methods and cladistics often with the goal of accurately depicting evolutionary history, and the other, *instrumentalist* that is more closely connected to phenetic methods of analysis whose goal is the production of practical taxonomic treatments (Jeffrey 1990).

Organisms, as a result of evolutionary processes, possess basic genetic information about ordered and evolved biological patterns. Thus, a key task for systematics is to characterize and apply such information to plant classification. There is, however, little certainty in taxonomy. Scientists generally choose among competing taxonomic treatments solely on the basis of their ability to explain observations of organismal properties under consideration.

Issues that complicate the adoption of taxonomic treatments arise from attempts to adhere to the dynamic nomenclatural provisions of the International Code of Botanical Nomenclature (ICBN) (Greuter et al. 2000). Such immediate procedural issues, when combined with a legacy of complex taxonomic history, often result in valid taxonomic treatments that may or may not be functional in the long term. Such adherence to taxonomic precedents is, nevertheless, important because it ensures that for any given taxonomic circumscription, a taxon will have only one name by which it is known (Jeffrey 1990).

The assignment of a unique name is particularly important for cucurbits because their long history of cultivation in diverse regions has resulted in an abundance of common names where identical names have been applied to different species (Rubatzky and Yamaguchi 1997). However,
just as International Code of Nomenclature for Cultivated Plants (ICNCP) establishes the rules for botanical names (Brickell et al. 2004), defines rules for the proper description and nomenclature of cultivated plants at the cultivar and group levels. These rules have been increasingly applied to the description and classification of cultivated cucurbits (Jeffrey 2001).

### 8.3.2 Taxonomic Relationships at the Familial Level

Cucurbitaceae is taxonomically isolated from other genetically related families and is best referred to a monotypic order, the Cucurbitales (Jeffrey 1990). Although, to date, its sole putative relatives are members of the Begoniaceae and Datiscaceae of the order Begoniales, wider genetic affinities among three families are somewhat obscure (Jeffrey 1990).

Members of the Cucurbitaceae are predominantly tropical with 90% of the species found in three main areas: Africa and Madagascar, Central and South America, and Southeast Asia and Malaysia. There are about 118 extant genera and 825 species (Jeffrey 1990). Geographic distribution of cucurbit diversity is illustrated in Figure 8.4 that maps the cumulative natural ranges of 120 cucurbit genera with patterns of generic diversity corresponding well to those at the specific level.

The family is divided into two unequally sized subfamilies. The Zanonioideae, with only 18 genera and 80 species of little economic value, is characterized by small, tricolporate, striate pollen grains; two or three free styles; and bifid tendrils spiralling both below and above the branching point. The subfamily Cucurbitoideae includes about 100 genera with styles united into a single column, branched tendrils with a non-spiralling basal part, and various, but not striate, pollen grains. The following tribes (and genera) of Cucurbitoideae are of major economic importance: Benincaseae (*Benincasa, Citrullus, Coccinia, Lagenaria, Luffa*), Melothrieae (*Cucumeropsis, Cucumis*), Cucurbitae (*Cucurbita*), Cyclanthereae (*Cyclanthera*), Joliffieae (*Momordica, Telfairia*), Sicyoeae (*Sechium*), Schizopeponeae (*Schizopepon*), and Trichosantheae (*Hodgsonia, Trichosanthes*) (Jeffrey 1990; Rubatzky and Yamaguchi 1997).
8.3.3 General Characterization

The Cucurbitaceae is represented by annual and perennial herbs, semi-shrubs, aculeateous shrubs, and a few rare succulent trees. Trailing or climbing vine growth with nodal branching is typical of many species. However, primary stem growth of some Cucurbita and Cucumis cultivars is substantially reduced, giving plants a “bush” habit (Rubatzky and Yamaguchi 1997). In Cucurbita, plants generally develop a strong, fairly long taproot (1-2 m long) and a highly branched network of shallow, secondary roots. Some perennial species develop large storage roots, and in moist environments after a period of drought, adventitious roots develop at the nodes of some Cucurbita species. In Cucumis species, plant roots are diffuse and reside in the upper 23 cm of the soil horizon.

Cucurbit leaves have bi-collateral vein fascicles and generally contain phytoliths (Piperno et al. 2002). Leaves of most cucurbits are alternate, and their blade is entire, toothed, or palmately lobed. Among species, foliage differs in size, texture, and shape such as the differences in the orientation and depth of lobes. Tendrils are borne in leaf axils and may be simple, highly spiralled, or branched. They are usually absent in bush-type Cucurbita.

Floral morphology is often poorly differentiated within a genus, but flowers vary widely among the various genera. Commonly, plants are monoecious, but other forms of sex expression occur or can be induced (Kalloo 1988). In contrast, sex expression in cucumber ranges from androecious to gynoecious to include hermaphroditic flower types. Most cucurbit fruits are fleshy berries or hard-rinded pepos, but extreme variation occurs in fruit shape, color, and size. Cucurbit seeds do not contain endosperm, but their large cotyledons contain amounts of carbohydrates and lipids sufficient for embryo development (Rubatzky and Yamaguchi 1997). A detailed morphological description of the family is given by Robinson and Decker-Walters (1997).

Any comprehensive discussion of taxonomic characterization must go beyond an examination of gross morphology and its comparative analysis. Modern taxonomic treatments of Cucurbitaceae also incorporate evidence from biochemistry such as the production of toxic cucurbitacins from terpene metabolism, cytology, cytogenetics, molecular genetics, taxon crossing ability, and coevolution with insects and pathogens in delimiting taxa (Bates, Robinson, and Jeffrey 1990; Kirkbride 1993). Data obtained by classical means (e.g., anatomical studies of phytoliths) are also considered (Piperno et al. 2002). The following sections briefly summarize the current state of taxonomic literature for economically important cucurbit genera.

8.3.4 Cucumis

In the most recent comprehensive biosystematic monograph of the genus Cucumis, Kirkbride (1993) recognized 32 species (Table 8.9, Figure 8.5). Of these, in addition to the two economically important species, cucumber (C. sativus L.) and melon (C. melo L.), two wild species C. anguria L. (West Indian gherkin), and C. metuliferus E. Meyer ex Naudin (African horned cucumber or jelly melon) are also commercially exploited for fruit production (Morton 1987; Baird and Thieret 1988). Other wild species originating mostly from arid and/or semi-arid regions of Africa are cultivated as ornamental plants, e.g., C. dipsaceus Ehrenberg ex Spach (hedgehog gourd) and C. myriocarpus Naudin (gooseberry gourd) (Kirkbride 1993).

Based on their geographic distributions, morphological characteristics, chromosome numbers, isozyme banding patterns, chloroplast DNA profiles, and patterns of interspecific hybridization, the species of genus Cucumis fall into two principal groups, generally recognized as subgenera (Jeffrey 1980; Raamsdonk, den Nijs, and Jongerius 1989; Kirkbride 1993). Sometimes these groups have been treated as distinct genera (as Cucumis and Melo) (Pangalo 1951), but that classification has not been commonly accepted.

Subgenus Cucumis represents a compact and isolated group of two species, C. sativus (2n = 2x = 14; Indian subcontinent origin) and C. hystrix Chakr. (2n = 2x = 24; Chinese origin)
Table 8.9 Organization of the Genus *Cucumis*

<table>
<thead>
<tr>
<th>Cucumis spp.</th>
<th>Chromosome Number (n)</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Subgenus Cucumis</strong></td>
<td></td>
<td>Asia</td>
</tr>
<tr>
<td>C. sativus L.</td>
<td>7</td>
<td>India, Sri Lanka, Burma, China,</td>
</tr>
<tr>
<td>C. sativus L. var. hardwickii (Royle) Alef.</td>
<td>7</td>
<td>India</td>
</tr>
<tr>
<td>C. hystrix Chakravarty</td>
<td>12 (Chen et al. 1997b)</td>
<td>India, Burma, China, Thailand</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Subgenus Melo (Miller)</th>
<th>C. Jeffrey</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Section Aculeatosi Kirkbridge</strong></td>
<td></td>
</tr>
<tr>
<td>C. myriocarpus Naudin</td>
<td>12</td>
</tr>
<tr>
<td>subsp. myriocarpus</td>
<td></td>
</tr>
<tr>
<td>subsp. leptodermis (Schweickerdt) Jeffrey &amp; Halliday</td>
<td>12</td>
</tr>
<tr>
<td>C. africanus L.</td>
<td>12</td>
</tr>
<tr>
<td>C. heptadactylus Naudin</td>
<td>24</td>
</tr>
<tr>
<td>C. calahariensis Meuse</td>
<td>?</td>
</tr>
</tbody>
</table>

| **Series Anguroidei Kirkbridge** | |
| C. anguria L. var. anguria | 12 | both vars: Angola, Botswana, Cape Verde Islands, Malawi, Mozambique, Namibia, S. Africa, Sierra Leone, Swaziland, Tanzania, Zaire, Zambia |
| var. longaculeatus Kirkbridge | 12 | Zimbabwe |
| C. sacleuxii Paillieux & Bois | 12 | Kenya, Madagascar, Tanzania, Uganda, Zaire |
| C. carolinus Kirkbridge | ? | Ethiopia, Kenya |
| C. dipsaceus Ehrenberg ex Spach | 12 | Ethiopia, Kenya, Somalia, Tanzania, Uganda |
| C. prophetarum L. subsp. prophetarum | 12 | Egypt, Mali, Mauritania, Nigeria, Senegal, Somalia, Sudan, Iran, Iraq, Israel, Oman, Qatar, Saudi Arabia, Yemen, Socotra, Syria, United Arab Emirates, Jordan |
| C. prophetarum L. subsp. dissectus (Naud.) C. Jeffrey | 12 | Chad, Egypt, Ethiopia, Kenya, Mauritania, Niger, Rwanda, Somalia, Tanzania, Uganda, Saudi Arabia, Yemen |
| C. pubituberculatus Thulin | ? | Somalia |
| C. zeyheri Sonder | 12 (24) | Lesotho, Mozambique, S. Africa, Swaziland, Zambia, Zimbabwe |

| C. prolatis Kirkbridge | ? | Kenya |
| C. insignis C. Jeffrey | 12? | Ethiopia |
| C. globosus C. Jeffrey | 12? | Tanzania |
| C. thulinianus Kirkbridge | ? | Somalia |
| C. filifolius A. Richard | 12 (24) | Ethiopia, Kenya, Rwanda, Tanzania, Uganda, Zaire |
| C. aculeatus Cogniaux | 24 | Ethiopia, Kenya, Rwanda, Tanzania, Uganda, Zaire |
| C. pustulatus Naudin ex Hooker | 12, 48, 72 | Chad, Kenya, Niger, Nigeria, Sudan, Tanzania, Uganda, Saudi Arabia, Yemen |
| C. meeusei C. Jeffrey | 24 | S. Africa, Botswana, Namibia |

*continued*
<table>
<thead>
<tr>
<th>Cucumis spp.</th>
<th>Chromosome Number (n)</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. jeffreyanus Thulin</td>
<td>?</td>
<td>Ethiopia, Kenya, Somalia</td>
</tr>
<tr>
<td>C. hastatus Thulin</td>
<td>?</td>
<td>Somalia</td>
</tr>
<tr>
<td>C. rigidus E. Meyer ex Sonder</td>
<td>?</td>
<td>S. Africa, Namibia</td>
</tr>
<tr>
<td>C. baladiensis Thulin</td>
<td>?</td>
<td>Somalia</td>
</tr>
<tr>
<td><strong>Series Metuliferi Kirkbride</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. rostratus Kirkbride</td>
<td>?</td>
<td>Ivory Coast, Nigeria</td>
</tr>
<tr>
<td><strong>Section Melo (Miller) Kirkbride</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Series Hirsuti Kirkbride</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. hirsutus Sonder</td>
<td>12</td>
<td>S. Africa, Angola, Botswana, Burundi, Congo, Kenya, Malawi, Mozambique, Sudan, Swaziland, Tanzania, Zaire, Zambia, Zimbabwe</td>
</tr>
<tr>
<td><strong>Series Humifructosi Kirkbride</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Series Melo (Miller) Kirkbride</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. melo L. subsp. melo</td>
<td>12</td>
<td>Africa, Iran, Afghanistan, Burma, China, India, Japan, Pakistan, Malesia, New Guinea, Australia, Fiji Islands, Papua New Guinea</td>
</tr>
<tr>
<td>subsp. agristis (Naudin) Pangalo</td>
<td>12</td>
<td>Africa, Saudi Arabia, Yemen, China, Burma, India, Japan, Korea, Nepal, Pakistan, Sri Lanka, Thailand, Malesia, Indonesia, New Guinea, Philippines, Australia, Guam, Papua New Guinea, Samoa, Solomon Islands, Tonga Islands</td>
</tr>
<tr>
<td>C. sagittatus Peyritsch</td>
<td>12</td>
<td>S. Africa, Angola, Namibia</td>
</tr>
</tbody>
</table>


(Kirkbride 1993; Chen et al. 1997b). Molecular-marker analyses have recently confirmed that these two species form a coherent group in relation to subgenus *Melo* (Zhuang et al. 2004). This is also reflected in the development of a stable amphidiploid hybrid, *C. ×hytivus* Chen & Kirkbride, derived from the artificial hybridization of these two species (Chen and Kirkbride 2000).

The foothills of the Himalaya Mountains in India are the mostly likely center of origin for the domestication of *C. sativus*, which fits well with levels of genetic diversity observed in Indian cucumber landraces (Staub, Serquen, and McCreight 1997b). Interpretation of the domestication of the cucumber depends on the status of *C. sativus var. hardwickii* (i.e., if this taxon is truly wild or feral or if it represents both wild and feral populations) (Bates and Robinson 1995; for more details, see Section 8.3.5). Cucumber has been cultivated for about 3,000 years in India, and soon after was
disseminated to the south and east of the Himalayas. From India, cucumber was brought to Greece and Italy, and later, China (Bisognin 2002). Historical records have confirmed cucumber cultivation in France and the Great Moravian Empire in central Europe in the ninth century (Moravec, Lebeda, and Kléstková 2004). The subgenus *Melo* is essentially African in its distribution and can be divided into three to six groups (Singh 1990; Kirkbride 1993). Most species are diploids with $2n = 2x = 24$, but some species have $2n = 48$ or even $2n = 72$ (Table 8.9) (den Nijs and Custers 1990; Singh 1990; Bates and Robinson 1995). The natural range of *C. melo* has yet to be conclusively determined. Prior to domestication, it may have been limited to Africa or may have reached the Near East or perhaps farther east to Asia (Bates and Robinson 1995). The center of diversity and perhaps of the origin of the principal melons of world commerce (i.e., the *C. melo* *Inodorus* and *Cantalupensis* Groups) is located in the Near East and adjacent central Asian regions (Jeffrey 1980). The Indian subcontinent may have been the original home of the *Conomon* Group and other local variants and may have been the scene of redomestication of feral forms (Bates and Robinson 1995). Melon was introduced to Central America in 1516 (Ware and McCollum 1980) and quickly expanded widely in the New World.

A recent provisionary infraspecific classification of cultivated *C. melo*, based on fruit morphology, plant biology, and geographic distribution, was proposed by Pitrat, Hanelt, and Hammer (2000). Decker-Walters et al. (2002a) conducted a complementary analysis of feral, New World *C. melo* populations in relation to those found in the Old World. Although extensive assessment of genetic variation at the protein and DNA level has occurred (Staub and Ivandic 2000), a similar comprehensive assessment of morphological characteristics within the species *C.
sativus remains to be completed. The description of cultivar groups by Křístková et al. (2003), based on fruit shape and geographic distribution, however, serves as a good starting point. As new cucumber cultivars are being developed with new combinations of traits, new groupings within this species may be needed.

8.3.5 Cucurbita

The genus Cucurbita is exclusively native to the New World (Figure 8.4 and Figure 8.6), but has been widely cultivated in the Old World since the 1500s (Paris 1989, 2001a, 2001b). It is not closely related to other cucurbit genera (Merrick 1995). The basic chromosome number of all Cucurbita species is $2n = 2x = 40$, and karyotypes suggest that these species are of allopolyploid origin (Singh 1979; Weeden and Robinson 1990). Results from electrophoretic analyses also helped confirm this genus’ polyploid (Kirkpatrick, Decker, and Wilson 1985) or, more specifically, allotetraploid (Weeden 1984) origin.

Whitaker and Bemis (1975) recognized 27 Cucurbita species. After careful analysis of type specimens, synonymy and additional taxonomic evidence (including coevolutionary studies involving the specialized pollinators, Peponapis and Xenoglossa; Hurd, Linsley, and Whitaker 1971), the genus is now thought to consist of between 12 and 15 species with five regularly cultivated (Lira-Saade 1995; Jeffrey 2001; Sanjur et al. 2002) (Table 8.10, Figure 8.7 through Figure 8.9). The genus can be divided into two groups based on ecological adaptation. The first group consists of mesophytic annuals or short-lived perennials with fibrous root systems. It includes all five major cultivated species. Wild taxa within this group occur from the southeastern United States south to central Argentina, typically below 1300 m above sea level. A recent sequence analysis of an intron from the mitochondrial gene, nad1, indicated that C. ficifolia Bouche was basal to all other taxa in this group (Sanjur et al. 2002).

The second group, the xerophytic, long-lived perennial species, are characterized by the presence of fleshy storage roots. They are adapted to arid zones or high-elevation regions from the southwestern United States to southern Mexico (Merrick 1995) and, based on chloroplast DNA

Figure 8.6 Cucurbita argyrosperma subsp. sororia in native habitat in Manzanillo, Colima, Mexico. November 1982. (Photo courtesy of L.C. Merrick.)
Table 8.10 Organization of the Genus *Cucurbita*

<table>
<thead>
<tr>
<th>Genus Argyrosperma</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. argyrosperma</em> C. Huber&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Mexico, Central America, southwestern U.S.A.</td>
</tr>
<tr>
<td>subsp. <em>argyrosperma</em></td>
<td></td>
</tr>
<tr>
<td>subsp. <em>sororia</em> (L.H. Bailey) L. Merrick &amp; D.M. Bates</td>
<td>Pacific Coast from Mexico to Nicaragua</td>
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</tbody>
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<thead>
<tr>
<th>Genus Ficifolia</th>
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<tbody>
<tr>
<td><em>C. ficifolia</em> Bouché&lt;sup&gt;a&lt;/sup&gt;</td>
<td>From Mexico highlands south to northern Chile and Argentina</td>
</tr>
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<tr>
<th>Genus Maxima</th>
<th></th>
</tr>
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<tbody>
<tr>
<td><em>C. maxima</em> Duchesne&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Argentina, Bolivia, Chile</td>
</tr>
<tr>
<td>subsp. <em>maxima</em></td>
<td></td>
</tr>
<tr>
<td>subsp. <em>andreaana</em> (Naudin) Filov</td>
<td>Argentina, Bolivia</td>
</tr>
<tr>
<td><em>C. moschata</em> Duchesne&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Lowlands of Mexico, Central America</td>
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<tr>
<th>Genus Pepo</th>
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<tbody>
<tr>
<td><em>C. pepo</em> L.&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Northern Mexico and southern U.S.A.</td>
</tr>
<tr>
<td>subsp. <em>fraterna</em> (L.H. Bailey) Filov</td>
<td>Northeastern Mexico</td>
</tr>
<tr>
<td>subsp. <em>ovifera</em> (L.) Harz</td>
<td>South-central U.S.A.</td>
</tr>
<tr>
<td>subsp. <em>pepo</em></td>
<td></td>
</tr>
<tr>
<td>subsp. <em>taxana</em> (Scheele) Filov</td>
<td>Texas and southeastern U.S.A.</td>
</tr>
<tr>
<td><em>C. ecuadorense</em> H.C. Cutler &amp; Whitaker</td>
<td>Pacific coast of Ecuador</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Genus Okeechobeensis</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. okeechobeensis</em> (J.K. Small) L.H. Bailey</td>
<td>Palm Beach County, Florida, U.S.A.</td>
</tr>
<tr>
<td>subsp. <em>okeechobeensis</em></td>
<td></td>
</tr>
<tr>
<td>subsp. <em>martinezii</em> (L.H. Bailey) T.W. Walters &amp; D.S. Decker</td>
<td>Veracruz, Mexico</td>
</tr>
<tr>
<td><em>C. lundelliana</em> L.H. Bailey</td>
<td>Mexico lowlands of Yucatan, Guatemala, Belize</td>
</tr>
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<tr>
<th>Genus Digitata</th>
<th></th>
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<tbody>
<tr>
<td><em>C. digitata</em> A. Gray&lt;sup&gt;b&lt;/sup&gt;</td>
<td>New Mexico and Arizona, U.S.A.</td>
</tr>
<tr>
<td><em>C. cylindrata</em> L.H. Bailey&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Baja California, Mexico</td>
</tr>
<tr>
<td><em>C. palmata</em> S. Watson&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Southern California and Arizona, U.S.A.</td>
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<thead>
<tr>
<th>Genus Foetidissima</th>
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<tbody>
<tr>
<td><em>C. foetidissima</em> Kunth&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Western U.S.A. and Mexico</td>
</tr>
<tr>
<td><em>C. pedatifolia</em> L.H. Bailey&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Central Mexico</td>
</tr>
<tr>
<td><em>C. × scabridifolia</em> L.H. Bailey&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Northeastern Mexico</td>
</tr>
<tr>
<td><em>C. radicans</em> Naudin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Mexico</td>
</tr>
</tbody>
</table>

<sup>a</sup> Cultivated species.
<sup>b</sup> Perennials.

analyses, are believed to be ancestral to members of the first group (Wilson, Doebley, and Duvall 1992). Of this second group, there has been interest in domesticating buffalo gourd, *C. foetidissima* (De Veaux and Schultz 1985). Its storage roots contain starch, and the lipids extracted from its seeds could serve as edible oil (Gathman and Bemis 1990).

The current state of knowledge on the origin and evolution of the domesticated *Cucurbita* species has been summarized by Merrick (1995) and Sanjur et al. (2002). The five domesticated *Cucurbita* species differ significantly in terms of their early distribution in the Americas. Current research suggests that each species is likely to have been domesticated independently from the others in distinct regions: (1) *C. maxima* Duchesne (Figure 8.10) in southern South America, *C. ficifolia* perhaps in the northern or central South American highlands; (2) *C. moschata* Duchesne in the southern Central American or northern South American lowlands; (3) *C. argyrosperma* C. Huber in southern Mexico (Figure 8.6); and (4) *C. pepo* L. (Figure 8.11) in northern Mexico and most probably also in the south-central United States (Sanjur et al. 2002). There is strong evidence for two domestication events in *C. pepo*, but the precise locations of those events remains obscure.
Figure 8.9 (See color insert following page 304.) *Cucurbita* species seed variation (composite photo: L.C. Merrick) 1 = *C. argyrosperma*, 2 = *C. ficifolia*, 3 = *C. maxima*, 4 = *C. moschata*, 5 = *C. pepo*.

The wild taxon *C. maxima* subsp. *andreana* (Naudin) Filov has been considered the progenitor of *C. maxima* by many authors yet sometimes has been considered as a feral escape and not the wild ancestor (Nee 1990). Recently, Sanjur et al. (2002) presented evidence confirming the close relationship between these two taxa. The progenitor of *C. ficifolia* has not been identified (Nee 1990; Sanjur et al. 2002).

The ancestor of cultivated *C. moschata* also remains unknown, but will probably be found among wild cucurbits in northern Colombia (Nee 1990; Sanjur et al. 2002; Andres 2004b). *Cucurbita lundelliana* L.H. Bailey had been considered to be its probable ancestor by Whitaker and others (Merrick 1995). However, Merrick (1991) demonstrated that *C. argyrosperma* subsp. *sororia* (L.H. Bailey) L. Merrick & D.M. Bates has a closer genetic affinity to *C. moschata* than does any other known wild *Cucurbita* taxon (Merrick 1995). *Cucurbita argyrosperma* subsp. *sororia* is also the most likely progenitor of cultivated forms of *C. argyrosperma* (Merrick 1995; Sanjur et al. 2002) with data providing interesting geographic correlations between the center of origin of this domesticate and that of *Zea mays* L. (Doebley 1990; Sanjur et al. 2002). Finally,

Figure 8.10 (See color insert following page 304.) Examples of *Cucurbita maxima* morphotypes (classification according to Whitaker, T. W. and Davis, G. N., Cucurbits: Botany, Cultivation, and Utilization, New York: Interscience Publishers, 1962. With permission.) 1 = hubbard, 2 = field pie pumpkin, 3 = turban, 4 = banana. (Composite photo courtesy of E. Krístková.)
C. pepo was domesticated independently in two areas, most likely in the south-central U.S., from wild C. pepo subsp. texana (Scheele) Filov, and in Mexico, from the wild taxon, C. pepo subsp. fraterna (L.H. Bailey) Filov (Decker 1988; Wilson, Doebley, and Duvall 1992; Sanjur et al. 2002). A recent critical summary of the infraspecific classification of cultivated Cucurbita species, based primarily on the fruit morphology, was given by Jeffrey (2001) with in-depth discussions of classification within C. moschata, including cultivar origin and domestication presented by Andres (2004a, 2004b) and within C. pepo presented by Paris and his colleagues (Paris 1986, 1989, 2001a, 2001b; Paris and Nerson 2003; Paris et al. 2003).

8.3.6 Citrullus

The domestication of the watermelon, C. lanatus (Thunb.) Matsum & Nakai, is still somewhat unclear. All Citrullus species originated in tropics and subtropics of the Old World, primarily in Africa. Watermelon is an important crop in warmer parts of Russia and other parts Asia Minor, the Near East, China, and Japan. It was brought to the New World by Spanish explorers (Robinson and Decker-Walters 1997). By one interpretation (Bates and Robinson 1995), the edible cultivated watermelon was derived from C. colocynthis (L.) Schrad., a closely related species endemic to northern Africa, southwestern Asia and the eastern Mediterranean with a long archaeological history (Zohary and Hopf 2000). A more recent synthesis by Robinson and Decker-Walters (1997) indicated that wild populations of C. lanatus var. citroides (L.H. Bailey) Mansf., which are common in central Africa, probably gave rise to the domesticate, subsp. lanatus. This is supported by viewpoint that domesticated watermelons were derived from indigenous African populations of C. lanatus in or near the Kalahari Desert of Namibia and South Africa (Jeffrey 2001). It has been hypothesized that the initial site of intentional breeding and wide diversification of C. lanatus was in southwestern central Asia where it had spread from Africa as a wild or weedy plant (Filov 1959, in Sinskaja 1969).

Four species of Citrullus have been generally recognized, all sharing a chromosome number of \(2n=2x=22\) (Table 8.11). In addition to the two widely distributed species noted above, the genus includes two species native to the desert regions of Namibia: the perennial vine, C. ecirhosus Cogn., and the annual, C. rehmiit De Winter (De Winter 1990; Jarret and Newman 2000; Levi et al. 2000). Jarret and Newman (2000) presented evidence from a comparative study of ITS sequences...
that the annual species, *C. lanatus* and *C. rehmiii*, were both derived from perennial ancestors, that *C. colocynthis* was more remotely related to *C. lanatus* than was *C. ecirrhosus*, and that *C. lanatus* var. *citroides* is likely the immediate ancestor of cultivated watermelons. Their data supports the views of Robinson and Decker-Walters (1997) and the results of molecular studies based on SSRs by Jarret et al. (1997) who divided *C. lanatus* into two basic groups ( *C. lanatus* var. *citroides* and *C. lanatus* var. *lanatus*). However, a recent summary of the classification of cultivated *Citrullus* species (Jeffrey 2001; Table 8.11) partly differs from this concept.

An alternative classification that treats cultivated forms of *C. lanatus* was presented by Jeffrey (2001) who subdivided the species into three subspecies. These subspecies are subsp. *lanatus* that includes: (1) wild populations and one cultivar group, Group Citroides (including fodder melon, citron, and preserving melon); (2) subsp. *vulgaris* (Schrad. ex Eckl. and Zeyh.) with an inexacty defined Group Cordophanus with bitter and non-bitter bland forms and a Group Dessert, including sweet edible cultivars; and (3) subsp. *mucosospermus* Fursa that includes a single cultivar group, Group Mucosospermus, with large, soft-coated seeds rich in oil and protein.

Two additional species, closely related to *Citrullus* and once included therein, are *Praecitrullus fistulosus* (Stock) Pang., which is cultivated in India and Pakistan for its edible fruits and *Acanthosicyos naudinianus* (Sond.) C. Jeffrey, a wild species native to southern Africa (Robinson and Decker-Walters 1997; Levi et al. 2000).

### 8.3.7 Other Cultivated Genera

A useful summary of modern taxonomic understanding of secondary cucurbit crops, including *Benincasa, Lagenaria, Luffa,* and *Sechium,* was compiled by Bates, Merrick, and Robinson (1995). Noteworthy specific examples of biosystematic studies of these crops include an assessment of cultivar groups in *Benincasa hispida* (Walters and Decker-Walters 1989) and broader investigations of taxonomic relationships among members of tribe Benincaseae (Walters et al. 1991; Chung, Decker-Walters, and Staub 2003) and the evolution of the Cucurbitaceae (Decker-Walters, Chung, and Staub 2004a). In addition, Decker-Walters et al. (2001) combined information about geographic origins and fruit and seed types with RAPD data to investigate patterns of evolution and genetic diversity among landraces and cultivars of *L. siceraria,* which provided base-line data in support of a remarkable, recent report of a wild lineage of *L. siceraria* from Zimbabwe (Decker-Walters et al. 2004b).

Examining the evolution of another domesticate, Marr, Mei and Bhattarai (2004) investigated patterns of morphological and isozyme variation among wild and cultivated populations of *Momordica charantia,* and they observed comparable levels of morphological variation in wild and cultivated populations but a great reduction in isozyme polymorphisms among cultivated

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### Table 8.11 Organization of the Genus *Citrullus*

<table>
<thead>
<tr>
<th><em>Citrullus</em> spp.</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. colocynthis</em> (L.) Schrad.</td>
<td>Northern Africa, southwestern Asia, eastern Mediterranean</td>
</tr>
<tr>
<td><em>C. ecirrhosus</em> Cogn.</td>
<td>Namibia and South Africa</td>
</tr>
<tr>
<td><em>C. lanatus</em> (Thunb.) Matsum &amp; Nakai</td>
<td>Kalahari of Namibia, southern Africa, cultivated elsewhere</td>
</tr>
<tr>
<td>subsp. <em>lanatus</em></td>
<td>As wild plant in the Kalahari</td>
</tr>
<tr>
<td>subsp. <em>vulgaris</em> (Schrad. ex Eckl. &amp; Zeyh.)</td>
<td>Semicultivated forms in Sahara, Sudan, Egypt; warmer areas</td>
</tr>
<tr>
<td>subsp. <em>mucosospermus</em> Fursa</td>
<td>Western Africa (e.g., Senegal, Mali, Guinea, Ghana, Niger)</td>
</tr>
<tr>
<td><em>C. rehmi De Winter</em></td>
<td>Namibia</td>
</tr>
</tbody>
</table>

populations. Their results can be placed into the larger context of variation within the genus *Momordica* in southeast Asia as a result of de Wilde and Duyfjes's (2002) recent taxonomic treatment.

### 8.4 GENE POOLS AND GENETIC DIVERSITY

#### 8.4.1 General Characterization

Gene pools serve as a tool for conceptualizing the ability of plant populations to cross with conspecific populations and those of other species, usually of the same genus (Harlan and de Wet 1971). The primary gene pool is represented by interfertile populations, generally of a specific biological species and may include other species that are fully cross-compatible. The primary gene pool typically encompasses a crop and any fully interfertile wild progenitor(s). The secondary gene pool is represented by all other populations that can be crossed with the crop; the gene flow is possible but is connected with a reduction of fertility within hybrid generations. Species from the tertiary gene pool cannot be crossed with the crop species except through special biotechnological approaches such that the resulting hybrids often express abnormalities and often are lethal or completely sterile. Until the advent of transformation technologies for cucurbits (Clough and Hamm 1995), access to the genetic diversity present in tertiary gene pools by breeding programs was severely limited by sexual incompatibility (Gepts and Papa 2003).

In its most basic sense, however, a gene pool can also be thought of as consisting of a collection of gametes with the potential of intermating. The different types and frequencies of gametes that occur in a gene pool ultimately depend upon the pool's original parental genotypes and selection at the gametic level. If alleles having a positive overall fitness are present in the parental genotypes, they will frequently be predominant in the gene pool and, consequently, in genotypes within the resulting population.

Vavilov (1926, 1997), in his work on the origin of cultivated plants, used the concept of "centres of diversity" to predict where crop species were initially domesticated. This system works well for a high percentage of crops (Hancock 2004), including cucurbits (Smartt and Simmonds 1995). Secondary centers of diversity can be described from parts of the world where centers of diversity occur distinct from centers of origin. Such secondary centers can be created by the migration and continued domestication of genotypes beyond their original centers of origin.

Genetic diversity analyses provide estimates of allelic frequencies for the determination of genetic associations among populations within gene pools and to help determine the relationships of such gene pools to centers of origin. Such analyses are based upon the characterization of genetic diversity through the measurement of heritable phenotypic traits and molecular markers, which may not always be correlated (Reed and Frankham 2001). This situation can be attributed principally to weak linkages between molecular markers (mainly neutral) and genes coding for phenotypic traits (which often confer selective advantage), differences in gene action and heritability among marker types, and mutation rates and mutation input (Gepts and Papa 2003).

In cucurbit crop species, genetic diversity analysis has been rigorously applied to cucumber and melon to determine the nature and structure of their gene pools and to examine hypotheses that may provide insights into the nature of their domestication. The following section describes gene pools in cucumber and melon in relationship to their evolution, migration, and differentiation.

#### 8.4.2 Cucumis

##### 8.4.2.1 Gene Pools of *C. sativus* and *C. melo*

Considerable research has been conducted to help delimit primary, secondary, and tertiary gene pools within the genus *Cucumis*. However, results are insufficient to support a complete synthesis
of domestication of *Cucumis* species. The nature of these relationships is important for the application of appropriate technologies to transfer desirable genes from wild *Cucumis* species to cucumber and melon and between cucumber and melon (Bates and Robinson 1995). Many researchers have attempted to make interspecific crosses in *Cucumis*, and data resulting from initial efforts was summarized by Raamsdonk, den Nijs, and Jongerius (1989). Complex experiments on crossing ability within the genus have been conducted by den Nijs and Custers (1990) with the aim of transferring valuable characters from wild species from Africa cultivated *C. sativus* and *C. melo*. Their results confirmed the division of the genus into two distinct gene pools that can be treated as subgenera, *Cucumis* and *Melo*, and the further subdivision of subgenus *Melo* (den Nijs and Custers 1990). Although young embryos have been recovered from crosses between *C. sativus* and *C. melo*, these consistently abort at an early stage (Bates and Robinson 1995).

The species *C. heptadactylus*, *C. humifructus*, *C. melo*, and *C. sativus* have never been successfully crossed with any other species of *Cucumis* to directly produce a fertile F₁ progeny (Kirkbride 1993).

The primary gene pool of *C. sativus* consists solely of that species and its two interfertile varieties, the domesticate, var. *sativus*, and the wild or feral type, var. *hardwickii* (Royle) Gabaev. Its secondary gene pool includes *C. hystrix* Chakr. (Bates and Robinson 1995) because fertile interspecific hybrid progeny have been synthetically made through F₁ embryo rescue with subsequent chromosome manipulation (Chen and Adelberg 2000). *Cucumis hystrix* has been collected only in the Yunnan Province of China. *C. sativus* var. *hardwickii* (R.) Alef. grows in the foothills of the Himalayan Mountains sympatrically with var. *sativus* and is used by native peoples of northern India as a laxative (Deakin, Bohn, and Whitaker 1971). It possesses a multiple fruiting and branching habit not present in *C. sativus* var. *sativus* (commercial cucumber; Horst and Lower 1978), suggesting considerable potential for increasing the genetic diversity available for improvement of commercial cucumber (Staub and Kupper 1985). There is evidence for the relative recent introgression of *C. sativus* var. *hardwickii* genes into some *C. sativus* var. *sativus* landraces in northern India (Horejsi and Staub 1999). Another biological variation with respect to prefertilization barriers in crosses with *C. sativus* was also found in *C. zeyheri* (den Nijs and Custers 1990).

Gene pools of *C. melo* remain poorly defined. Analyses of chloroplast DNA (Perl-Treves et al. 1985; Perl-Treves and Galun 1985) clearly grouped *C. melo* and *C. sagittatus* together, distinct from the rest of the genus with just two mutations separating them. Fruit set is stimulated in *C. melo* and *C. sagittatus* by members of *Cucumis* ser. *Angurioidei*, but not by *C. humifructus* or *C. metuliferus* (Raamsdonk, den Nijs, and Jongerius 1989). Promising results were obtained by den Nijs and Custers (1990) after crossing *C. sativus* var. *hardwickii* accession IVT Gbn 1811A with *C. melo* because hybrid embryos developed much further than usual in this combination and appeared to accept pollen of both *C. metuliferus* and diploid *C. zeyheri* (den Nijs and Custers 1990). A hybrid between *C. melo* and *C. metuliferus* has been reported but not confirmed (Puchalski and Robinson 1990).

Isozyme studies provide two interpretations of the position of *C. melo* within subgenus *Melo*. In one interpretation, it constitutes a sister group with *C. humifructus* and *C. sagittatus* by sharing a common ancestor (Perl-Treves et al. 1985); in the second, it is a sister group with *C. humifructus* and *C. hirsutus* (Puchalski and Robinson 1990). In contrast, cpDNA results suggest different relationships among these taxa (Bates and Robinson 1995).

### 8.4.2.2 Genetic Diversity of *C. sativus* and *C. melo*

*Cucumis sativus*

Genetic relationships among cucumber accessions in relation to their geographic origins are consistent with generally accepted, historic dispersal patterns (Horejsi and Staub 1999). Distinct
C. sativus var. sativus gene pools exist in Europe (Dijkhuizen et al. 1996; Horejsi and Staub 1999), Africa (Mliki et al. 2003), North America (Dijkhuizen et al. 1996), and Asia (Staub, Serquen, and McCreight 1997b, 1999; Horejsi and Staub 1999).

Genetic markers (morphological and biochemical) have been employed for the characterization of genetic diversity present in cucumber (Knerr et al. 1989; Meglic, Serquen, and Staub 1996; Staub and Ivandic 2000). Assessment of genetic diversity in C. sativus var. sativus and var. hardwickii using isozymes, restriction fragment length polymorphisms (RFLPs), and random amplified polymorphic DNAs (RAPDs) indicated that diversity in C. sativus var. sativus is relatively low (3–8%) when compared with other allogamous Cucumis species (10–25%) (Dane 1976, 1983; Esquin- Alcazar 1977; Knerr et al. 1989; Dijkhuizen et al. 1996; Horejsi and Staub 1999). Levels of allelic polymorphism in C. sativus var. hardwickii (17–25%) are predictably higher than in C. sativus var. sativus (Dijkhuizen et al. 1996; Meglic, Serquen, and Staub 1996; Horejsi and Staub 1999) as is the case for many pairs of wild progenitors and domesticates. These data lend support to the hypothesis that cucumber originated in India (Leppik 1966). Chinese (China is a putative secondary center for cucumber diversity; Leppik 1966; Staub et al. 1999) and Indian germplasm accessions, when examined collectively, were distinct from each other and from all other C. sativus var. sativus and C. sativus var. hardwickii accessions examined by Staub et al. (1999). Chinese (Staub et al. 1999) and Indian (Staub, Serquen, and McCreight 1997b) accessions represent the two most diverse subsets of the primary gene pool present in nature and in the world’s major genebanks such as the U.S. NPGS (Staub et al. 2002).

It is thought that northern and southern Chinese cucumber cultivars have different origins (Leppik 1966; Staub et al. 1999), and it has been hypothesized that the genetic variation present in cucumber germplasm in southern China is endemic to that region and/or has been historically augmented by infrequent introductions of germplasm from northern Indian sources via ancient Himalayan trade routes (Staub et al. 1999). Southern Chinese germplasm has been isolated by the Himalayas and the region’s social structure. In contrast, it is thought that the genetic diversity of northern Chinese cucumber germplasm has been a direct beneficiary of the Silk Road where germplasm (genetic variation) has been continually shared across central Asia and the Near East based on observed protein and DNA polymorphisms (Staub et al. 1999; Horejsi and Staub 1999). Such findings (Staub et al. 1999) suggest that northern and southern Chinese cultivars may be more similar than previously thought. The fact that the Indian and Chinese accessions examined in several studies are genetically different (Meglic, Serquen, and Staub 1996; Staub, Serquen, and McCreight 1997b, 1983) supports the hypothesis that cucumber germplasm exchanged between these countries has been limited, allowing for unimpeded genetic changes through divergent selection resulting from different growing conditions and unique human needs.

Test arrays constitute artificially constructed subsamples of gene pools based on unique characteristics (e.g., specific disease-resistance alleles; Bretting and Widrlechner 1995) and, ideally, also on broad genetic diversity as defined by geography and marker-based genetic distance. The variation observed in cucumber germplasm has allowed for the construction of test arrays for reaction to angular leafspot (Pseudomonas syringae pv. lachrymans), anthracnose (Colletotrichum orbiculare), downy mildew (Pseudoperonospora cubensis), Rhizoctonia fruit rot (Rhizoctonia solani), target leafspot (Corynespora cassiicola), and water and heat stress and for the designation of a core collection consisting of 115 accessions from those test arrays and 32 more accessions to assist in encompassing the global genetic diversity in the NPGS collection (Staub et al. 2002). At the time of its selection, the core collection of 147 accessions (115 + 32) represented about 11% of the total collection’s size (1352).

Provisional classification of cultivar groups has been proffered for C. melo by Pitrat, Hanelt, and Hammer (2000) and Jeffrey (2001). Similar to melon, horticultural market classes exist for cucumber; however, a similar subdivision of C. sativus with regard to morphological and evolutionary aspects has not yet been elaborated, and a core collection for melon has not been proposed.
**Cucumis melo**

Melon is a morphologically diverse outcrossing species (Kirkbride 1993). Since the time of its domestication, melon cultivation has been expanding from points of origin along well-defined trade routes (e.g., the Silk Road and international shipping routes) (McCreadt, Nerson, and Grumet 1993; Robinson and Decker-Walters 1997).

Based on vegetative and fruit variation, Munger and Robinson (1991) defined melon morphotypes of *C. melo* as botanical varieties [treated herein as essentially synonymous with Groups as defined by Brickell et al. (2004) in the ICNCP] to include Agrestis, Flexuosus, Conomon, Cantalupensis, Inodorus, Chito, Dudaim, and Momordica. The economically important *C. melo* morphotypes are commonly partitioned into market classes according to their culinary attributes (Staub et al. 2000). For instance, Group Cantalupensis includes marker classes Earl's, House, Galia, Charentais, and Ogen; Group Inodorus includes Honeydew and Casaba; and Group Conomon includes Oriental with each having a different fruit morphology valued for their unique aromas and flavors and variable shelf life. While Groups Cantalupensis and Inodorus are of commercial importance in the United States and Europe as well as in Mediterranean and Asian countries, Group Conomon types have their origin and are widely grown in Asia (McCreadt, Nerson, and Grumet 1993; Robinson and Decker-Walters 1997).

Recently, Pitrat, Hanelt, and Hammer (2000) assessed earlier studies and conducted a morphological analysis of melon germplasm and provisionally classified 6 *C. melo* morphotypes into five groups in subspecies *agrestis* (Naudin) Pangalo (i.e., wild, weedy, and free-living types) and 11 groups in subspecies *melo* (to include diverse commercial types having specific consumer-based culinary attributes). A more rigorous taxonomic treatment of this provisional classification may allow for the formal designation of these morphotypes as Groups, consistent with the ICNCP (Brickell et al. 2004) as discussed by Spooner et al. (2003).

Attempts to clarify interspecific taxonomic relationships within *C. melo* and evaluate its patterns of genetic diversity, assess market-class relationships, and determine likely centers of origin for the various groupings have employed a diverse array of molecular markers, including isozymes (Esquinas-Alcazar 1981; Perl-Treves et al. 1985; Staub, Frederick, and Marty 1987; Staub et al. 2000; Akashi et al. 2002b; McCreadt et al. 2004), RFLPs (Neuhausen 1992; Silberstein et al. 1999), RAPDs (Staub et al. 1997a, 2000; Garcia et al. 1998; Silberstein et al. 1999; Stepansky, Kovalski, and Perl-Treves 1999a; Mliki et al. 2001; López-Sesé et al. 2002, López-Sesé, Staub, and Gomez-Guillamon 2003), SSRs (Katzir et al. 1996; Staub et al. 2000; Danin-Poleg et al. 2001; Decker-Walters et al. 2002a; López-Sesé et al. 2002; López-Sesé, Staub, and Gomez-Guillamon 2003; Monforte, García Mas, and Arus 2003), ISSRs (Danin-Poleg et al. 1998b; Perl-Treves et al. 1998; Stepansky, Kovalski, and Perl-Treves 1999a), and AFLPs (Garcia-Mas et al. 2000). Although both morphological and DNA (molecular-marker) variation has been used to define melon taxonomic groups and market classes, results derived from different lines of evidence do not always agree (López-Sesé, Staub, and Gomez-Guillamon 2003).

Melons have been the subject of numerous ethnobotanical studies (Jacquat and Berstossa 1990; Robinson and Decker-Walters 1997; Staub et al. 2000; Goldman 2002), and it is thought that the species is of African origin (Robinson and Decker-Walters 1997). However, Bates and Robinson (1995) noted the possibility of multiple origins in that at least some melons may have been domesticated in Asia. Typical of many vegetable crops, the paucity of archaeological remains of melon (because of its perishable tissues) does not allow for intense scrutiny of its domestication (Zohary and Hopf 2000). The only important exception to this limitation can be found in Egypt where, because of its unique climatic conditions and cultural and agricultural history, domestication theories can be postulated. A combination of archaeological evidence, interpretations of mural paintings, and genetic analyses supports the hypothesis that wild ancestors of cultivated melon originated in Africa and were domesticated in multiple areas of...
secondary diversity to include the Middle and Near East and India (Staub et al. 1992; Robinson and Decker-Walters 1997; Mliki et al. 2001). These diverse geographic areas house several related, but differentiated, gene pools.

Cultivation of *C. melo* in India began as early as 2000 BC where early forms of Groups Momordica, Agrestis, and Flexuosus were cultivated and selected for local culinary preferences and medicinal purposes (Robinson and Decker-Walters 1997). These non-sweet melon types are, based on phenotype and fruit morphology, clearly distinct from Group Cantalupensis and Inodorus types, which are cultivated primarily in Europe and the Middle East (Stepansky, Kovalski, and Perl-Treves 1999a). Thus, it is likely that Indian subsp. *melo* types were developed independently from those in Europe and the Middle East (López-Sesé, Staub, and Gomez-Guillamon 2003; Staub, Fanourakis, and López-Sesé 2004).

The Oriental Asian melons collectively form Group Conomon and comprise a distinct germplasm pool (Nakata et al. 2005). However, Pitrat, Hanelt, and Hammer (2000) described how Group Conomon morphotypes could be further partitioned into vars. *makuwa* Makino and *conomon* Thunberg. According to Kitamura (1950), var. *makuwa* melons were established in northern China whereas var. *conomon* melons were solely cultivated in southern China. Seed size and isozyme analyses by Akashi et al. (2002b) suggested that ancestral types of vars. *makuwa* and *conomon* melons were distinct lineages before their introduction to China. Although the introduction of these melons to China may have not been directly from India, they are, based on their small seed size, most closely related to the melons of eastern India. Thus, Akashi et al. (2002b) hypothesized that melons grown in China originated in central India, and then, through progressive domestication, were transported eastward to Laos and eastern China. Alternatively, melon types could have been introduced to western China via the Silk Road (from Baghdad to Iran to Kashmir and then to China; ca. 700–1000 AD; Kitamura 1951).

Relationships among the gene pools of Groups Flexuosus and Inodorous and African melons have not yet been clearly defined. But according to Stepansky, Kovalski, and Perl-Treves (1999a) and Silberstein et al. (1999), Group Conomon and African melons (no distinct market classes) possess closer genetic affinities than are shared between Group Conomon and Groups Flexuosus and Inodorus. In fact, the hypothesized close genetic relationship between African landrace melons and Group Conomon Oriental market class melons was confirmed by Mliki et al. (2001), suggesting a common origin. These landraces are, in many respects, morphologically distinct from Indian populations of Group Agrestis (McCreight and Staub 1993; Mliki et al. 2001), and their progenitor(s) may have reached Africa from southeast or western Asia relatively late (ca. 1600 AD) by sea-trade routes.

The history of European melons' domestication is also obscure, confounding an understanding of relationships among European market classes. The variation in fruit morphology of European melons is, however, broad and dramatic. Genetic-marker studies have documented differences among market classes, creating unique gene pools (García et al. 1998; Stepansky, Kovalski, and Perl-Treves 1999a; Staub et al. 2002). For example, the Group Cantalupensis market classes of central European origin clearly differ from Group Inodorus Spanish landraces as do the Spanish landraces from Asian Group Conomon and Cantalupensis melons (López-Sesé, Staub, and Gomez-Guillamon 2003; Nakata et al. 2005). These differences suggest divergent selection for culturally based culinary attributes (Staub et al. 2000; López-Sesé et al. 2002; López-Sesé, Staub, and Gomez-Guillamon 2003). Within Group Inodorus, Casaba types may have originated in the Middle East and were then transported to central and southern Europe where they were subsequently refined to form a distinct gene pool designated as modern Casaba melons (López-Sesé, Staub, and Gomez-Guillamon 2003; Staub, Fanourakis, and López-Sesé 2004).

Based on fruit morphology, the Group Flexuosus and Inodorus accessions from Greece are similar to west Asian and Mediterranean market classes, respectively (Staub, Fanourakis, and López-Sesé 2004). Their RAPD-based genetic similarity to reference accessions from Japan and Europe argues that this may be the case (Table 8.12). The Japanese reference accessions employed in
such studies typified variation in common Japanese market classes (i.e., House, Earl’s, and Oriental) (Nakata et al. 2005). Moreover, genetic affinities between the Greek accessions and Japanese accessions reported by Staub, Fanourakis, and López-Sesé (2004) might have been predicted because Japanese market classes were developed in part from European germplasm beginning in the middle to late 1800s (Nakata et al. 2005).

Fruits of many of the Greek accessions examined by Staub, Fanourakis, and López-Sesé (2004) resembled the Group Inodorus Casaba market class. In fact, the RAPD data they presented indicated that accessions from Crete also share genetic affinities with that market class. Upon closer inspection of relationships among Spanish melon landraces, López-Sesé, Staub, and Gomez-Guillamon (2003) detected strong genetic affinities between Spanish landraces originating from southwestern and central growing regions (Extremadura and Andalucia) and Group Flexuosus accessions from Greece. Moreover, some Greek Group Inodorus landraces (e.g., Agiou Vassiliou and Argous) are similar in fruit shape, size, and color to that of Piel de Sapo and other green Spanish melon cultivar types (López-Sesé, Staub, and Gomez-Guillamon 2003; Staub, Fanourakis, and López-Sesé 2004). Intentional, strict selection among Spanish melon cultivars for specific morphological characteristics and their culinary uniqueness (distinctive textures and specialized tastes) may have prevented the introgression of genes from additional germplasm sources of diverse origin despite the recent lack of geographic isolation (Esquinas-Alcázar 1977). Differences in melon fruit morphology and plant phenotype are controlled by relatively few genes (Pirat 1994); therefore, these genetic relationships may have been forged either by common ancestral origins or through gene introgression from one or multiple sources.

The proximity of Greece and Crete to ancient trade routes might partially explain the genetic relationships detected by Staub, Fanourakis, and López-Sesé (2004). Immigrants from Asia Minor first inhabited Crete during the Neolithic period (6th millennium BC). Crete and mainland Greece became important crossroads (by sea and land), linking the continents of Asia, Africa, and Europe with vital local and international commerce during the Minoan (2600–1100 BC), Achaean (1450 BC), and Dorian (1100 BC) cultural periods (Zohary and Hopf 2000; Staub, Fanourakis, and López-Sesé 2004).
Figure 8.14

Figure 8.15
Greeks consumed melon in the third century BC. Mediterranean trade routes were well established by the second century AD, and by the third century AD, Rome was importing melons from Armenia in Asia Minor. The King's Highway (ca. AD 400) was a well-travelled route of agricultural commerce, linking Asia with the Mediterranean and extending from Egypt across the Sinai Peninsula, through Jordan into Syria, through Damascus ending at the Euphrates River. Somewhat later, the early Islamic diffusion of the eighth to eleventh centuries AD also allowed for the westward transport of Indian and south Asian crops, including several fruit tree species, condiments, and vegetables (Zohary and Hopf 2000). This Arabic expansion purportedly facilitated the dissemination of unique melon germplasm westward through the Mediterranean region to Spain. Such trade routes afforded Spain and Crete doors of commercial opportunity during these periods and the avenues for gene exchange to produce distinct gene pools.

Most accessions from Crete studied by Staub, Fanourakis, and López-Sesé (2004) shared genetic similarities with the original Galia hybrid accession from Israel as well as Galia cultivars derived from it (Staub et al. 2000). The pedigree of Galia is complex, and its relatively recent development from Ogen (Italian) and Charentais (French) market class types and a line of Russian origin now represent a unique market class (released in 1974 by Zvi Karchi, Agricultural Research Organization). Ogen market class melons, in fact, have strong genetic marker-based affinities with Galia melon types (Staub et al. 2000) and with Spanish Group Inodorus Casaba landrace melons (López-Sesé, Staub, and Gomez-Guillamon 2003). The melon accessions of Greek origin examined by Staub, Fanourakis, and López-Sesé (2004) were cultivated on the mainland, Crete, and other islands of the Aegean Sea (Samos) and Ionian Sea (Zakynthos) before the release of the original Galia. Because genetic affinities were detected between Greek accessions and Charentais but not Ogen accessions, it is likely that western European germplasm is in the pedigree of the Greek accessions examined by Staub, Fanourakis, and López-Sesé (2004), or that Charentais shares common ancestors with the Greek landraces.

8.4.3 Cucurbita

8.4.3.1 Gene Pools of Cultivated Cucurbita Species

The five cultivated species of Cucurbita are believed to have been derived from mesophytic progenitors. The xerophytic, long-lived perennial species of Cucurbita (including the buffalo gourd) are considered by Merrick (1995) to be terminal evolutionary lineages distantly related to the mesophytic species, yet Sanjur et al.'s (2002) phylogeny indicated that at least one of the xerophytic species (C. foetidissima) is basal to four of the mesophytic species.

Generally, the domesticated Cucurbita species are reproductively isolated from one another. The primary gene pools of each species are represented by their landraces and commercial cultivars as well as by their infraspecific taxa (Table 8.13).

Experimental crosses among them can be made with difficulty, and interspecific progenies are usually either sterile or sparingly fertile (Merrick 1995). Spontaneous crosses between the cultivated Cucurbita species are uncommon, but hybrids interspecific natural can occasionally be detected in landraces mostly from Mexico (Decker-Walters et al. 1990; Merrick 1990, 1991). In addition, Křišťková (1991) reported a spontaneous hybrid of C. maxima by C. pepo under central European field conditions.

However, despite the high degree of genetic differentiation within Cucurbita, none of the genus's species is completely reproductively isolated from the others in terms of barriers to hybridization. Of the domesticates, C. moschata is considered to be the extant species with the most ancestral-like genome and displays wide cross-compatibility (Merrick 1995). The domesticated species C. argyrosperma, C. pepo, and C. maxima can be crossed with their wild or feral relatives (Lira-Saade 1995; Merrick 1995). In the Americas, pairs of closely related domesticated and wild
species can occur sympatrically, and genetic interchange between them takes place, providing a natural source of variation within populations (Decker 1988; Wilson 1990; Merrick 1991).

Hybridization experiments (and some field observations) involving *C. argyrosperma* and other wild and cultivated *Cucurbita* taxa have revealed (Lira-Saade 1995) that, among the cultivated species, *C. moschata* has the highest degree of compatibility with *C. argyrosperma*, placing it into its secondary gene pool (Table 8.13). The next level of cross-compatibility involves the wild and cultivated taxa of *C. pepo*, some cultivars of *C. maxima*, and the wild perennial species *C. foetidissima*, which collectively represent the tertiary gene pool. The wild species that have shown some degree of compatibility with *C. argyrosperma s.l.* possess genes of resistance to some viral diseases that have a high incidence in the cultivated species (Lira-Saade 1995).

There are strong hybridization barriers between *C. ficifolia* and other species of this genus, making the definition of its gene pools problematical. Some interspecific hybrids have been obtained from crosses with *C. pedatifolia*, *C. foetidissima*, and *C. lundelliana*, but they often lack the capacity to produce an F2 generation (Lira-Saade 1995).

The secondary gene pool of *C. maxima* is represented by *C. ecuadorensis*; and its tertiary gene pool includes *C. lundelliana*, *C. argyrosperma*, *C. ficifolia*, and *C. pepo* (Lira-Saade 1995).

The secondary gene pool of *C. moschata* is represented by *C. argyrosperma* as both species possess very close evolutionary relations. The tertiary gene pool is formed by *C. lundelliana* and some taxa of the "groups" Maxima and Pepo (Lira-Saade 1995).

The primary gene pool of *C. pepo* is formed by its various edible and ornamental cultivars (Figure 8.11) as well as populations of the wild taxa, var. *fraterna* and var. *texana*, until recently considered as distinct species or subspecies of *C. pepo*. There are a great many commercial cultivars with particular characteristics that, together with local landraces (grown mainly in Mexico), constitute an extraordinary genetic stock. However, in contrast to other *Cucurbita* species, this diversity does not represent an important source of resistance genes to pests and diseases because *C. pepo s.l.* is probably the one species with the greatest susceptibility to the most important viral diseases that attack cultivated *Cucurbita* species (Whitaker and Robinson 1986; Provvidenti 1990; Lira-Saade 1995). Populations that could be considered as part of its

### Table 8.13 Gene Pools of Cultivated Cucurbita Species

<table>
<thead>
<tr>
<th>Cucurbita spp.</th>
<th>Primary</th>
<th>Secondary</th>
<th>Tertiary</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. argyrosperma</em></td>
<td><em>C. argyrosperma</em> subsp. <em>sororia</em></td>
<td><em>C. moschata</em></td>
<td><em>C. pepo</em></td>
</tr>
<tr>
<td></td>
<td><em>C. argyrosperma</em> (sensu lato)</td>
<td></td>
<td><em>C. maxima</em></td>
</tr>
<tr>
<td><em>C. ficifolia</em></td>
<td><em>C. ficifolia</em></td>
<td><em>C. pedatifolia</em></td>
<td><em>C. lundelliana</em></td>
</tr>
<tr>
<td><em>C. maxima</em></td>
<td><em>C. maxima</em> subsp. <em>maxima</em></td>
<td><em>C. ecuadorensis</em></td>
<td><em>C. argyrosperma</em></td>
</tr>
<tr>
<td><em>C. moschata</em></td>
<td><em>C. moschata</em></td>
<td><em>C. argyrosperma</em></td>
<td><em>C. ficifolia</em></td>
</tr>
<tr>
<td><em>C. pepo</em></td>
<td><em>C. pepo</em> subsp. <em>pepo</em> (sensu lato)</td>
<td><em>C. argyrosperma</em> subsp. <em>okeechobensis</em></td>
<td><em>C. lundelliana</em></td>
</tr>
<tr>
<td></td>
<td><em>C. pepo</em> subsp. <em>fraterna</em></td>
<td><em>C. moschata</em></td>
<td><em>C. maxima</em></td>
</tr>
<tr>
<td></td>
<td><em>C. pepo</em> subsp. <em>texana</em></td>
<td><em>C. ecuadorensis</em></td>
<td><em>C. pepo</em></td>
</tr>
</tbody>
</table>

secondary gene pool are scarce as most attempts at hybridizing C. pepo with other wild or cultivated species have required special techniques such as embryo culture (see Section 8.6.3).

8.4.3.2 Genetic Diversity of Cultivated Cucurbita Species

Studies of isozyme variation in *Cucurbita* by Andres (1990), Decker (1988), Decker-Walters et al. (1990, 1993), and Merrick (1991) revealed moderate amounts of genetic differentiation within *C. pepo* and *C. moschata* while, in marked contrast, little genetic differentiation has occurred within *C. argyrosperma* and *C. ficifolia* (and perhaps in *C. maxima* as well, although the survey of the latter species has been less comprehensive than those of the others; Merrick 1995). Similar analyses to survey the variation present in DNA markers have been conducted, but on a somewhat more limited scale, with the most comprehensive work accomplished within *C. pepo* (Katzir et al. 2000; Decker-Walters et al. 2002b; Ferriol, Picó, and Nuez 2003a; Paris et al. 2003).

The diversity of *C. argyrosperma* is less than that found within the other three most widely cultivated domesticates, *C. pepo*, *C. moschata*, and *C. maxima*. Local varieties have evolved in a rather restricted geographic range including the southeastern United States, Mexico, and Central America (Lira-Saade 1995). The most important variation observed within cultivated morphotypes corresponds to fruit size, shape, and color pattern and to seed morphology (Lira-Saade 1995). Three major lineages which comprise the domesticated forms of *C. argyrosperma* have been recognized at the varietal level by Merrick (1990): vars. *argyrosperma*, *stenosperma*, and *callicarpa*. The different degrees of variation in the nutritionally important parts of the three cultivated varieties suggest a strong association with human interests. The relatively large seed size of var. *argyrosperma* indicates that it was mainly selected to obtain seeds for human consumption while the great diversity of shapes, colors, and sizes of the fruits and seeds of vars. *stenosperma* and *callicarpa* indicate that selection had two aims: to obtain flesh as well as seeds (Lira-Saade 1995).

The wide range of elevations at which *C. moschata* is cultivated within the New World suggests that this species has evolved diverse adaptations to various environmental conditions (Andres 2004b). The species is highly polymorphic (Andres 2004a) with considerable morphological diversity of its seeds and fruit (color, shape, thickness, and durability of the fruit's skin). The existence of varieties with life cycles of differing phenology as well as the breadth of its numerous cultivars developed in other parts of the world (see Gwanama, Labuschagne, and Botha 2000) and of local varieties with excellent horticultural characteristics strongly suggest that its collective genetic variation is very extensive (Filov 1959; Lira-Saade 1995).

Some interesting Latin American landraces have been noted in the traditional agroecosystems of the Yucatán Peninsula where two types with distinct life cycles are grown and in the Mexican states of Guanajuato and Chiapas where landraces with resistance to some viral diseases have been noted (Lira-Saade 1995). Among the landraces from Yucatán, the short-cycle type commonly grown in Mayan vegetable gardens is of great interest because it was a likely progenitor of the most commercially important variety in the region. And, notably, selected landraces from Guanajuato and Chiapas were incorporated into genetic improvement programs (Lira-Saade 1995).

With regard to the range of variation of *C. moschata* populations developed outside its center of origin, the best example may be that of a landrace native to Nigeria, which represents the only source of resistance to certain viral diseases (Provvidenti 1993). Another part of the gene pool of *C. moschata* is represented by the numerous commercial cultivars that have been mainly developed in the United States and, to a lesser extent, in Brazil. Some of these commercial cultivars also have different levels of resistance and/or susceptibility to certain diseases, indicating a wide genetic variation of this species (Lira-Saade 1995). The possibilities of hybridization that *C. moschata* has shown with other cultivated species (e.g., *C. maxima*) suggest that there are good prospects for the improvement of these other species as well (Lira-Saade 1995).
Cucurbita maxima has been shown to encompass a high degree of diversity for both morphological and molecular characters (Ferriol, Picó, and Nuez 2004b). Its variation includes local varieties and numerous commercial cultivars with different plant habits, fruit (Figure 8.10) and seed shapes and colors, and levels of host-plant resistance to viral diseases (Lira-Saade 1995). Also, the variability in the duration of its life cycle and, in some cases, its adaptation to marginal ecological conditions are remarkable (Filov 1959; Lira-Saade 1995). On the basis of morphological variation primarily in Spanish landraces, Ferriol, Picó, and Nuez (2004b) defined eight different groups, yet these were not congruent with concomitant molecular analyses of AFLP and SRAP variation. In that same study, Ferriol, Picó, and Nuez (2004b) noted that the high degree of genetic variability present in their small sample of New World populations (as compared to their larger sample of Spanish accessions) suggests that much work remains to be done to understand the full range of variation found in this species.

The diversity of C. pepo is comparable to or higher than that found in C. maxima (Lira-Saade 1995). Two major evolutionary lineages that collectively comprise the cultivated forms of C. pepo were recognized at the subspecific level by Decker (1988): subsp. pepo and subsp. ovifera. More recently, Decker-Walters et al. (2002b) used RAPD polymorphisms to characterize three distinct, New World populations of wild and weedy C. pepo. Local varieties and commercial cultivars have been categorized into at least ten cultivar groups (Figure 8.11) related to their fruit shape and quality and reflecting their evolutionary histories (Paris 1989; Paris et al. 2003).

Morphological and genetic variation is quite limited in C. ficifolia (Andres 1990) with a limited range of landraces and no commercial cultivars. Its scant morphological variation is consistent with its limited degree of isozyme variation. Yet, there may be cryptic variation for environmental adaptation because it is cultivated over a wide geographic range at various elevations in both agroecosystems with high competition, such as in maize fields in high rainfall areas, and in those with less competition and more intensive cultivation (Lira-Saade 1995).

8.4.4 Citrullus

Species of the genus Citrullus share a common chromosome number, and all its taxa are cross-compatible with each other to varying degrees. Citrullus lanatus and C. ecirrhosus are evidently more closely related to each other than either is to C. colocynthis (Navot and Zamir 1987; Jarret and Newman 2000). Yet even C. lanatus and C. colocynthis can be crossed experimentally and also cross spontaneously in nature to produce partially or fully fertile hybrids (Bates and Robinson 1995), indicative either of a single, primary gene pool or of a secondary gene pool encompassing two, poorly differentiated primary pools.

Considerable research has been conducted to describe the intra- and interspecific variation in Citrullus within this broad gene pool. However, a comprehensive synthesis of patterns of variation remains to be completed. The nature of phylogenetic and geographic relationships may guide the application of appropriate technologies to improve the efficiency of transferring desirable genes from wild Citrullus species to cultivated watermelon (Bates and Robinson 1995).

Watermelon fruits vary in size and shape, rind and seed color, and color and density of their flesh. Many of these variant forms were recorded by the European Middle Ages (Sturtevant 1919) and led to the description of a moderately complex array of species and botanical varieties, which for the most part, could be placed in cultivar groups (Bates and Robinson 1995). Complicating the situation is the enormous diversity of forms, developed and cultivated in southeastern countries of the former Soviet Union, that were described by Filov (1954). A recent overview of cultivar groups in C. lanatus was given by Jeffrey (2001), which addressed Fursa and Filov’s (1982) recognition of thirty-one cultivar types based on fruit form and color and their parallel description of ten ecological-geographical groups.
Levi et al. (2001a) analyzed a group of watermelon cultivars, representing a wide range of horticultural traits (fruit size and shape, flesh texture and color, and firmness), but RAPD-based genetic variation among them was relatively low. That study did demonstrate, however, that molecular markers can be useful in evaluating groups based on their genetic similarity values. Such a classification can enable the selection of representatives from each group of closely related accessions for evaluation of such traits as disease or pest resistance.

Levi et al. (2001b) noted that low genetic diversity among watermelon cultivars bred in the U.S., which are widely grown throughout the world, increases the need to expand the genetic base of elite cultivars. Their study also indicated a higher genetic diversity within the wild subspecies *C. lanatus* var. *citroides* than in *C. lanatus* var. *lanatus* although accessions of *C. lanatus* var. *lanatus* are preferred in watermelon breeding programs because of their horticultural qualities, their close proximity to elite lines, and their reported resistance to anthracnose (Boyhan et al. 1994) and Watermelon mosaic virus (WMV) (Gillaspie and Wright 1993). The wild species *C. colocynthis*, which has the widest natural geographic distribution, also has the highest genetic diversity among *Citrullus* species. Wide genetic diversity among *C. colocynthis* plant introductions (Pls; Levi et al. 2000) indicated that this species may possess various genes that could confer pest resistance to cultivated watermelon. Beyond the traditional use of watermelon flesh, the gene pool of wild *C. colocynthis* can be explored as a source of genes for developing watermelon as an edible oilseed (Schafferman et al. 1998).

### 8.5 GENETIC MAPPING AND GENOMICS

#### 8.5.1 Introduction

The primary utility of genetic maps in plant improvement is their deployment in marker-assisted selection (MAS) and breeding. The predictive value of genetic markers used in MAS depends upon their inherent repeatability, map position, and linkage with economically important traits (quantitative or qualitative) (Staub, Serquen, and Gupta 1996). While genetic mapping experiments have been well documented in *Cucumis* species, there are comparatively few examples for other cucurbit genera and species. The creation of genetic maps for watermelon, *C. lanatus* var. *lanatus*, has been hampered by lack of genetic diversity (Navot, Sarfatti, and Zamir 1990; Jarret et al. 1997; Levi et al. 2001a), few mapable, co-dominant markers (Hawkins et al. 2001; Levi et al. 2001a), and distorted marker segregation patterns (Levi et al. 2001c). This has necessitated the use of wide intervarietal crosses (*C. lanatus* var. *lanatus* × *C. lanatus* var. *citroides/cocolynthis*) for the construction of relatively unsaturated maps (Levi et al. 2002, 2004a; Hashizume, Shimamoto, and Hirai 2003; Zhang et al. 2004). Genetic diversity analysis (Gwanama, Labuschagne, and Botha 2000; Ferriol et al. 2004a; Ferriol, Picó, and Nuez 2004b) and map construction (Brown and Myers 2002) is also in its infancy in *Cucurbita*. Given the paucity of information on other cucurbit genera, the following discussion on genetic mapping will focus solely on *Cucumis* species.

#### 8.5.2 *Cucumis sativus*

##### 8.5.2.1 Mapping

Cucumber linkage maps have been constructed using phenotypic markers (Fanourakis and Simon 1987; Pierce and Wehner 1990; Vakalounakis 1992), isozymes (Knerr and Staub 1992), isozymes and phenotypic markers (Meglic and Staub 1996a, 1996b), and combinations of molecular (RFLP, RAPD, isozyme) and phenotypic markers (Kennard et al. 1994; Serquen, Bucher, and Staub 1997a). The maps of Fanourakis and Simon (1987), Knerr and Staub (1992a),
Vakalounakis (1992), Kennard et al. (1994), Meglic and Staub (1996a,b), Serquen, Bacher, and Staub (1997a), and Park et al. (2000) spanned 168, 166, 95, 766 (narrow-based) and 480 (wide-based), 584, 600, and 816 cM, respectively (750–1000 cM total genome length). These reports have been augmented more recently with markers associated with basic cell functions (Xie, Wehner, and Conkling 2002; Xie et al. 2003) and cytoplasmic factors controlling economically important traits (Chung and Staub 2003).

Park et al. (2000) employed 347 RAPD, RFLP, and AFLP marker loci and those conditioning virus resistances to construct a map with 12 linkage groups (LOD ≤ 3.5) and a mean marker interval of 4.2 cM. Resistances to Papaya ringspot virus (PRV) and ZYMV were closely linked (2.2 cM) to each other and were also tightly linked (~5.2 cM) to three AFLP markers. A map constructed by Serquen, Bacher, and Staub (1997a) defined nine linkage groups and spanned ca. 600 cM with an average distance between RAPD markers of 8.4 cM. Information from the Serquen, Bacher, and Staub (1997a) map was recently merged with other maps (Fanourakis and Simon 1987; Knerr and Staub 1992; Kennard et al. 1994; Meglic and Staub 1996a, 1996b; Horejsi, Staub, and Thomas 2000) to synthesize a consensus map containing 255 markers, including morphological traits, disease resistance loci, isozymes, RFLPs, RAPDs, and AFLPs spanning ten linkage groups (Bradeen et al. 2001). The mean marker interval in this consensus map was 2.1 cM, spanning a total length of 538 cM. More recently, Fazio, Staub, and Stevens (2003) constructed a map containing 14 SSR, 24 sequence characterized amplified region (SCAR), 27 AFLP, 62 RAPD, one single nucleotide polymorphism (SNP), and three morphological markers (131 total markers) spanning seven linkage groups (the theoretical number based on the haploid chromosome number) by using recombinant inbred lines (RILs). This map spanned 706 cM with a mean marker interval of 5.6 cM. Because the map of Fazio, Staub, and Stevens (2003) contains several anchor markers common to the maps of Serquen, Bacher, and Staub (1997a) and Bradeen et al. (2001), it is possible that these maps and that of Park et al. (2000) could be merged for syntenic comparison with melon (C. melo) using anchor markers (Danin-Poleg et al. 2000a).

### 8.5.2.2 QTL Analysis

The wide cross [GY-14 (U.S. elite processing) × PI 432860 (China)] used by Kennard et al. (1994) was employed to study QTL associated with fruit quality by using different mating designs (Kennard and Havey 1995). The two-year, single-location study identified five, three, three, and two QTL for fruit length, diameter, seed-cavity size, and color, respectively. More recently, Serquen, Bacher, and Staub (1997a) defined nine linkage groups and spanned ca. 600 cM with an average distance between RAPD markers of 8.4 cM. Information from the Serquen, Bacher, and Staub (1997a) map was recently merged with other maps (Fanourakis and Simon 1987; Knerr and Staub 1992; Kennard et al. 1994; Meglic and Staub 1996a, 1996b; Horejsi, Staub, and Thomas 2000) to synthesize a consensus map containing 255 markers, including morphological traits, disease resistance loci, isozymes, RFLPs, RAPDs, and AFLPs spanning ten linkage groups (Bradeen et al. 2001). The mean marker interval in this consensus map was 2.1 cM, spanning a total length of 538 cM. More recently, Fazio, Staub, and Stevens (2003) constructed a map containing 14 SSR, 24 sequence characterized amplified region (SCAR), 27 AFLP, 62 RAPD, one single nucleotide polymorphism (SNP), and three morphological markers (131 total markers) spanning seven linkage groups (the theoretical number based on the haploid chromosome number) by using recombinant inbred lines (RILs). This map spanned 706 cM with a mean marker interval of 5.6 cM. Because the map of Fazio, Staub, and Stevens (2003) contains several anchor markers common to the maps of Serquen, Bacher, and Staub (1997a) and Bradeen et al. (2001), it is possible that these maps and that of Park et al. (2000) could be merged for syntenic comparison with melon (C. melo) using anchor markers (Danin-Poleg et al. 2000a).

The RIL-based QTL analysis by Fazio, Staub, and Stevens (2003) re-examined those traits studied by Serquen, Bacher, and Staub (1997a, 1997b). Fazio, Staub, and Stevens (2003; four-location study) confirmed the QTL detected by Serquen, Bacher, and Staub. (1997a, 1997b; two-location study). In general, Fazio, Staub, and Stevens (2003) mapped these QTL to smaller intervals than those identified by Serquen, Bacher, and Staub (1997a). The highest $R^2$ value indicated that the major genes controlling $F$ (female flowering) and determinate ($de$; dwarf plant type) (Figure 8.12)
were linked to SSR loci CSWCT28 and CSWCT14 at distances of 5.0 cM and 0.8 cM, respectively. Moreover, Fazio, Staub, and Stevens (2003) revealed four, location-independent factors that cumulatively explained 42% of the observed phenotypic variation for MLB. QTL conditioning lateral branching (mlbl.1), fruit length/diameter ratio (ldrl.2), and sex expression (sexl.2) were associated with de, confirming the results of Serquen, Bacher, and Staub (1997a). Six QTL were identified for sex expression, five for MLB, two for earliness, and five for fruit length. Sex expression was influenced by three genomic regions corresponding to \( F \) and de, both on Linkage Group 1 and a third locus (sex6.1) on Linkage Group 6 (Figure 8.13). The potential value of these marker-trait associations with other yield components for plant improvement has been documented (Serquen, Bacher, and Staub 1997a, 1997b; Fazio, Staub, and Stevens 2003; Fazio and Staub 2003). Their efficacy is portended by the relatively high LOD scores (2.6-13.0) and associated \( R^2 \) values (1.5%-32.4%) resulting from comparatively few genetic factors (perhaps three to ten). These and other QTL-based data (Dijkhuizen and Staub 2003) confirm the reliability and consistency of these QTL-marker associations over multiple environments, populations, and various experimental designs.

### 8.5.2.3 Application of Marker-Assisted Selection

A test of the efficiency of MAS in cucumber was conducted in concert with efforts to modify MLB (Figure 8.2), a metric trait controlled by at least five effective factors (Serquen, Bacher, and Staub 1997b; Fazio, Staub and Stevens 2003). Yield increase in processing cucumber is positively correlated with increased number of fruit-bearing branches (Cramer and Wehner 2000). Fazio and Staub (2003) compared three breeding schemes for MLB in a backcross population: Phenotypic selection under open-field conditions (PHE), random intermating without selection (RAN), and MAS, employing five markers for the selection of greenhouse-grown plants. Their study
Figure 8.13 Relative positions of QTL on linkage Groups 1 and 6 of cucumber (Cucumis sativus) which contain RAPD, SCAR, AFLP, and morphological markers (italicized) (Modified from Fazio, G., Staub, J. E., and Stevens, M. L., Theor. Appl. Genet, 107, 864-874, 2003a.); Linkage groups are designated by numbers (1 and 6) above Roman numerals and letters corresponding to linkage groups in maps by Bradeen et al. (2001) and Serquen, Bacher, and Staub (1997a) (From Serquen F. C., Bacher, J., and Staub J. E., Mol. Breed., 3, 257-268, 1997a.), respectively; RAPDs are identified by the preceding letters OP and BC according to Serquen, Bacher, and Staub (1997a), SSR by the preceding letters CS, CM and NR, AFLP by E_M_, and SCARs by the designation SCAR according to Fazio (2001); The vertical bars to the left of each linkage group represent the QTL regions detected with their respective LOD score; Markers associated with and/or bracketing economically important regions are given in bold.

employed two SSRs, two RAPDs, and one SNP marker to independently confirm previously determined linkages and their utility in MAS. No significant differences (p < 0.001) were detected between the mean values of MLB from PHE and MAS. However, values for both PHE and MAS populations were significantly higher than for the RAN control. Because MAS BC populations were produced in one year and phenotypic selection required three years to complete, markers linked to MLB increased overall breeding efficiency.

8.5.3 Cucumis melo

The first melon gene list was published in 1979 and has been updated every four years since 1986 (Pitrat 2002). Initially, this list included genes for simply inherited traits such as disease resistance and morphological characters (Pitrat 1990). The most recent melon gene list, however, included 162 genes (forty-seven mapped unto linkage groups as phenotypic markers), with seven QTL for disease resistance, forty-three QTL for fruit-quality traits, and forty-six cloned genes with complete sequences (Pitrat 2002).
8.5.3.1 Mapping of Disease and Pest Resistance

Molecular markers (e.g., AFLPs, CAPs, ISSRs, RAPDs, RFLPs, SCARs, SNPs, and SSRs) have been used to identify and map disease and pest-resistance genes in melon. Marker–trait associations have been identified for host reaction to fusarium wilt (fom1 and fom2; Wechter et al. 1995; Wechter, Thomas, and Dean 1998; Wang, Thomas, and Dean 1998, 2000; Zheng et al. 1999; Karsies, Dean, and Thomas 2000; Zheng and Wolff 2000; García-Mas et al. 2001; Brotman et al. 2004), ZYMV (Zym2 and Zym3; Danin-Poleg et al. 2000b, 2002), PRV (Prv; García-Mas et al. 2001; Brotman et al. 2002, 2004), powdery mildew (Pm; Fukino et al. 2002), Melon necrotic spot virus (MNSV) (Nsv; García-Mas et al. 2004; Morales et al. 2004a, 2004b), and for resistance to the transmission of viruses by aphids (Vat; Brotman et al. 2002).

8.5.3.2 Linkage Maps

The genome size of melon has been estimated to be $4.5-5.0 \times 10^8$ bp, about three times that of Arabidopsis (Arumuganathan and Earle 1991). In 1984, Pitrat initiated a systematic characterization of linkage relationships among phenotypic markers and developed the first linkage map for melon (Pitrat 1984, 1991). Initial reports were followed by a map consisting of 28 loci partitioned into eight linkage groups (Pitrat 1994). Some of these linkage groups contained genes for economically important traits, including disease resistance (Fom-2, Nsv, Pm, Pvr, and Zym), floral characters (a and ms), and plant architecture (si and lmi).

The development of molecular markers has led to the construction of numerous linkage maps in melon. However, most of the initial maps were relatively unsaturated and identified the position of relatively few traits of economic importance. Baudracco-Arnas and Pitrat (1996) constructed the first DNA-based map based on 218 F$_2$ progeny from the cross Vedrantais × Songwhan Charmi (PI 161375). The 103-point map consisted of 34 RFLPs, 63 RAPDs, one isozyme, four disease-resistance markers (Fom-1, Fom-2, Nsv, and Vat), and one morphological marker (p, conditioning carpel number). The total length of this map was 1,390 cM, where marker loci were fairly evenly dispersed among 14 linkage groups at an average distance of 17.7 cM. Wang, Thomas, and Dean (1997) constructed a 204-point map based on 66 BC$_1$ lines derived from a cross between line MR-1 and Anas Yokneam. That map consisted of 14 major and six minor linkage groups composed of 197 AFLPs, six RAPDs, and one SSR marker, spanning 1942 cM, with an average distance between markers of 11 cM (Wang, Thomas, and Dean 1997, 1998). Similarly, Liou et al. (1998) used 64 F$_2$ progeny (Makuna #SLK-V-052 × "Sky Rocket") and 125 RAPD markers to construct a map consisting of twenty-nine linkage groups spanning 1348 cM, with markers spaced at an average distance of 10.8 cM. Staub, Meglic, and McCreight (1998) also used F$_2$ and BC$_1$ progenies originating from strategic crosses among 400 USDA Pis to construct two linkage groups consisting of 11 isozyme loci spaced at an average distance of 9 cM.

These initial efforts were followed by the publication of more robust maps with higher degrees of saturation by French, Spanish, and Israeli research groups. Pépin et al. (1998) reported the use of 122 RILs derived from the Ved161 population used by Baudracco-Arnas and Pitrat (1996) for the creation of a map consisting of 354 markers (294 AFLP, 46 ISSR, and 14 morphological markers). That map consisted of twelve major and five minor linkage groups covering 1,366 cM with an average marker interval of 3.9 cM (Pépin et al. 1998). Dogimont et al. (2000) subsequently constructed an improved 527-point map based on the backbone map of Pépin et al. (1998). That map consisted of 12 linkage groups spanning 1583 cM where the average marker interval was 3 cM. Pépin et al. (2000) continued this mapping effort by using 120 RILs from Ved161 and 60 RILs from Ved414 (Vedrantais × PL 414723) to construct a composite map spanning 1590 cM. This consensus map consisted of 777 markers, including 608 AFLP, 25 RFLP, 128 ISSR, 16 RAPD, and 14 morphological markers evenly dispersed in 12 linkage groups. More recently, Pépin et al. (2002b) developed a 668-point map by
using 163 RILs from Ved161 and 63 RILs from Ved414 and AFLP, RAPD, RFLP, SSR, and morphological markers, which were grouped into 12 linkage groups spanning 1654 cM (Pépin et al. 2002a, 2002b, 2002c).

Spanish researchers published several reports that culminated initially in the creation of 411-point map (Oliver et al. 1998, 2000, 2001). This map was constructed using 93 F₂ progeny from a cross between Songwhan Charmi and Pinyonet Piel de Sapo consisting of 234 RFLP, 94 AFLP, 47 RAPD, 29 SSR, five ISSR, two isozyme, and one phenotypic marker (p). These markers were randomly distributed across 12 linkage groups that spanned 1197 cM where the average marker interval was 3.1 cM (Oliver et al. 2001). Gonzalo et al. (2005) more recently published a more saturated map by adding 26 SSRs to the extensive array used by Oliver et al. (2001). The 287-point map consisted of 12 linkage groups and spanned 1240 cM with an average marker interval of 4.3 cM. In addition, Gonzalo et al. (2005) mapped 173 polymorphic markers to 12 linkage groups using 77 dihaploid (DH) lines derived from the F₂ mapping population of Oliver et al. (2001). This mapping effort included 33 previously described SSRs (Katzir et al. 1996; Danin-Poleg et al. 2000a, 2001; Fazio, Staub, and Chung 2002), 41 newly developed SSRs, 79 RFLPs, 16 EST-SSR, three SNPs (Morales et al. 2004b), and the Nsv locus (Morales et al. 2004a). The map consisted of 12 linkage groups spanning 1223 cM with a mean marker interval of 7 cM. These two maps were merged (Gonzalo et al. 2005), resulting in a consensus map consisting of 327 loci (226 RFLPs, 97 SSRs, 3 SNPs, and the Nsv locus) distributed over 12 linkage groups spanning 1021 cM, with an average distance between loci of 3.1 cM.

Important contributions for the development of molecular markers and genetic maps in melon have also been made by Israeli scientists. Initially, Katzir et al. (1995, 1996) and Danin-Poleg et al. (1996, 2001) developed SSR markers, which lead to the construction of three SSR-based maps designed to assess the utility of this marker class for map construction and merging (Danin-Poleg et al. 1998a, 2000a; 2002). The first map (Danin-Poleg et al. 1998a, 2000a) was constructed from 60 F₂-derived F₃ bulks randomly chosen from the population used by Baudracco-Arnas and Pitrat (1996). Their initial 123-point map (138 RFLPs, 65 RAPDs, one isozyme, Fom-1, Fom-2, Nsv, and Vat, and 14 SSRs) consisted of 13 linkage groups spanning 1716 cM. A second 82-point map (Danin-Poleg et al. 1998a, 2000a) spanning 11.30 cM and consisting of 12 linkage groups was constructed using 93 F₂ progeny from a Songwhan Charmi × Pinyonet Piel de Sapo mating (Oliver et al. 2001). A third map (Danin-Poleg et al. 2000a, 2002) in this initial series was developed using 122 F₂ progeny from the cross PI 414723 × Dulce consisting of 22 SSR, 46 RAPD, 2 ISSR, and four phenotypic markers (a, Fom-1, st, and pH) in 14 linkage groups, spanning 610 cM. A common set of SSR markers resident in these three maps allowed for marker-order confirmation and synteny assessment. More recently, maps have been developed based on 113 F₂ individuals derived from the cross between PI 414723 × Top-Mark (Brotman et al. 2000; Silberstein et al. 2003). Progeny segregation allowed for the construction of a 179-point consensus map using AFLPs, RAPDs, ISSRs, SSRs, RFLPs, and two phenotypic markers (vat and a), consisting of 24 linkage groups spanning 1421 cM (Silberstein et al. 2003).

8.5.3.3 QTL Analysis and Map Merging

At this time, there are only four known studies involving the molecular dissection of QTL controlling horticulturally important traits in melon. These include the characterization of QTL for Cucumber mosaic virus (CMV) resistance (Dogimont et al. 2000), ethylene production during fruit maturation (Pépin et al. 2002a), and ovary and fruit shape, fruit weight, sugar content, external color, and flesh color (Pépin et al. 2002c; Monforte et al. 2004).

The paucity of QTLs studies in melon is in part due to a lack of linkage maps constructed from populations designed for the extensive replication required for effective QTL analysis. Most melon maps have been developed from F₂ or BC₁ populations, which are not particularly well-suited for extensive replicated, multi-location evaluation (Baudracco-Arnas and Pitrat 1996;
CUCURBITS (CUCURBITACEAE: CUCUMIS SPP., CUCURBITA SPP., CITRULLUS SPP.)

Liou et al. 1998; Wang, Thomas, and Dean 1998; Oliver et al. 2001; Danin-Poleg et al. 2002; Silberstein et al. 2003). Although immortalized populations (e.g., DH and RIL populations) are ideal for analyzing complex trait QTL, saturated melon maps constructed from such populations are relatively new (Périn et al. 2002a,b,c; Gonzalo et al. 2005).

The development of more densely saturated maps and the use of DH or RIL populations will likely lead to the placement of economically important traits conditioned by QTL. However, many initial melon maps included many dominant markers (Baudracco-Arnas and Pitrat 1996; Liou et al. 1998; Wang, Thomas, and Dean 1998; Danin-Poleg et al. 2002; Silberstein et al. 2003) with information that is usually not readily transferable to other populations (Périn et al. 2002a). Codominant markers (e.g., SSRs) are often syntenic and can be used in map comparison and merging experiments (Danin-Poleg et al. 2001; Gonzalo et al. 2005). Such markers will likely be important for interspecific synteny analysis (melon and cucumber) and the identification, characterization, and mapping of QTL for complex traits (Katzir et al. 1996; Danin-Poleg et al. 2000a, 2001, 2002; Oliver et al. 2001; Périn et al. 2002a, 2002b, 2002c; Monforte, Garcia Mas, and Arns 2003; Silberstein et al. 2003; Fukino et al. 2004; Gonzalo et al. 2005).

8.6 GERmplASM ENHANCEMENT

8.6.1 Introduction

The process of genetic improvement often involves the incorporation of genetic material [gene(s)] from one or more exotic accessions (Walters and Wehner 1994a; Wehner and St. Amand 1995) into a germplasm pool (Wehner, Elsey, and Kennedy 1985, Wehner et al. 1989; Wehner, Shetty, and Clark 2000a; Wehner, Shetty, and Wilson 2000b; Wehner and Shetty 1997, 2000; Shetty and Wehner 2002) with subsequent cycles of enhancement of the broadened pool followed by testing of derived populations or lines for hybrid or per se performance (Rubino and Wehner 1986; Cramer and Wehner 1999a). This general strategy has resulted in important public germplasm releases in cucumber (Peterson et al. 1982, 1986b; Peterson, Staub, and Palmer 1986; Wehner et al. 1996b; Walters and Wehner 1997, Wehner 1998a, 1998b, 2001), melon (Jagger and Scott 1937; McCreight, Kishaba, and Bohn 1984; Thomas 1986), and squash (Coyne and Hill 1976; Mutschler and Pearson 1987).

The selection scheme used in cucurbits depends on the objectives of the enhancement/breeding project, the inheritance patterns and heritability of the characters to be selected, the available germplasm, and the species selection history. Cucurbits are typically genetically diverse and allogamous, but tolerant to some degree of inbreeding, making them amenable to a myriad of selection procedures. Selection procedures often evolve over time because breeders react to new information about the genetics of their target species and its key traits and changes in economic conditions (e.g., market target), management practices (e.g., hand vs. machine harvesting operations), and environmental factors that affect abiotic and biotic stresses. For traits that are conditioned by few genes (such as many disease resistances), segregating progeny can be tested against simple Mendelian ratios. In contrast, response of metric traits conditioned by multiple allelic arrays (such as yield and quality components) to selection is measured by changes in population means and variances that can be used to define heritabilities (Wehner 1984; Strefeler and Wehner 1986; Serquen, Bacher, and Staab 1997b; Cramer and Wehner 1998a; St. Amand and Wehner 2001a, 2001b) and develop optimal recurrent selection procedures (Wehner and Cramer 1996a, 1996b; Cramer and Wehner 1998b, 1999b).

8.6.2 Gene Pools: Evaluation and Utilization

Gathering appropriate germplasm is a critical step in any enhancement program. Plants can be visualized on a continuum, based on degree genetic improvement and local adaptation, from exotic,
unadapted types, which may possess specific traits of interest to elite, locally-adapted materials that are uniform, possessing many commercially acceptable traits. Exotic cucurbit germplasm typically includes genetic diversity absent in elite types. Because of this and the fact that exotic germplasm usually also exhibits undesirable characteristics (e.g., lack of disease resistance, bitterness, relatively small fruit), refinement of exotic populations often requires a substantial, long-term effort. Therefore, the types and proportions of adapted and exotic germplasm that are selected to form a base population that maximizes the genetic diversity for desirable traits is dictated by program objectives (e.g., long vs. short-term), time (reproduction cycles/year) and cost considerations (e.g., molecular-aided vs. conventional), and potential benefits. Strategic intercrossing of the germplasm forming the base population is needed when the breeder requires that the population eventually utilized is random-mating and at linkage equilibrium (e.g., F2, three-way, mass intercrossing). Often, where the objective of the enhancement program is the development of an elite population for further improvement, a large array of diverse parental stocks based on their performance (disease resistance, yield heterosis) is initially chosen for intermating, followed by selection over seasons and years for a range of specific characteristics (e.g., sex expression, flowering date, disease resistance, and fruit number and quality; e.g., Wehner et al. 1989; Wehner and Cramer 1996b).

The breeding of cucurbits has primarily focused on improving the production and quality of fruits by increasing resistance to a wide range of pathogens and diseases and by modifying plant architecture and sex expression. Until the end of twentieth century, interspecific hybridization, even when aided by embryo culture and other biotechnologies, had not been yet used to breed cucumber and melon cultivars (Bates and Robinson 1995; Robinson and Decker-Walters 1997). Principal attention was focused on the search for valuable characters within cultivated species and on basic genetic studies (i.e., inheritance of useful features and identification of genes). Gene lists for cucurbitaceous vegetables summarize many of the results of above mentioned approaches (Pitrat 2002).

The identification of genetic variation within cucurbit germplasm collections to overcome the challenges of abiotic and biotic stresses and reduce the cost and/or increase the value of production has motivated considerable research. This body of research encompasses both the commonly cultivated taxa and, to a lesser extent, their wild and weedy relatives as evaluated for many different traits. These include horticultural and agronomic characteristics, biochemical and quality traits, and reaction to biotic and abiotic stresses. Given such an extensive body of literature, the following section will focus on published literature reviews (where such exist) and on recent findings.

### 8.6.2.1 Horticultural and Agronomic Characteristics

An excellent example of a long-term project where standardized morphological evaluations of cucurbit germplasm were used to identify superior horticultural forms for breeding and/or direct introduction has been conducted by the Institute for Introduction and Plant Genetic Resources in Bulgaria, primarily for *C. sativus* (Neykov 1994, 1998; Neykov and Alexandrova 1997) but also for *Citrullus, C. melo,* and *Cucurbita* (Stefanova, Neykov, and Todorova 1994; Krasteva 2002). Brazilian researchers have also directed considerable attention to the evaluation of local cucurbit (*Citrullus* and *Cucurbita*) germplasm with the goal of identifying valuable accessions for breeding (Choer 1999; Romão et al. 1999; Ramos et al. 2000; Queiroz et al. 2004). *Cucurbita* germplasm has received similar attention by Chinese researchers (Chen 1993; Zhou et al. 1995; Ren 1998). Other reports on horticultural evaluations directed toward the identification of superior genotypes among local landraces include studies of *Cucurbita* landraces from Cuba (Ríos Labrada, Fernández Almirall, and Casanova Galarraga 1998) and Jordan (Kasrawi 1995) and of *C. melo* landraces from India (Seshadri and More 2002), Italy, and Albania (Ricciardi et al. 2003).

While horticultural production of cucurbits in Europe and North America focuses on *Citrullus, C. melo, C. sativus,* and *Cucurbita,* worldwide production is much more diverse, including many
genera and species of local importance (Ng 1993). The body of literature on horticultural evaluation includes a number of reports on these less widely cultivated genera and species. For example, two uncommon species of *Cucumis* have been evaluated for specialty fruit production. Marsh (1993) evaluated 26 accessions of *C. metuliferus* under field conditions in Missouri, U.S., and Dhaliwal (1997) evaluated 48 accessions of *C. anguria* under field conditions in Punjab, India. Both of these projects identified superior lines for potential commercial production. Evaluations of *Sechium edule* germplasm have been reviewed by Newstrom (1990), of *Lagenaria siceraria* germplasm have been reported by Ram et al. (1996) and Upadhyay, Ram, and Singh (1997–1998), and of *Momordica charantia* germplasm by Xu and Huang (1995), Ram et al. (1996), and Marr, Mei, and Bhattarai (2004).

As noted above, there are many papers that report the results of general horticultural and morphological evaluations. However, there is also a body of research that has investigated particular plant traits that contribute to fruit yield and marketability; on the basis of such work, in-depth evaluations of specific traits have also been conducted. This has been true especially for *C. sativus* where germplasm has been evaluated for variation in root growth (Grumet et al. 1992; Walters and Wehner 1994a), postharvest storage (Wehner, Shetty, and Wilson 2000b), combining ability for yield as measured via testcrosses with a common inbred tester (Wehner, Shetty, and Clark 2000a), specific yield components such as fruit number and weight (Shetty and Wehner 2002), and precocious staminate flowering (Walters and Wehner 1994b). In addition, Sugiyama (1998) evaluated *Citrullus lanatus* germplasm for variation in the number of pistillate flowers and its relationship to fruit yield.

### 8.6.2.2 Biochemical and Quality Traits

Variation in soluble solids, individual sugars, and the enzymes that control sugar production has received special attention especially in *C. melo* (Stepansky et al. 1999b; Burger et al. 2004), and to a lesser extent, in *C. sativus* (Robinson 1987). Burger et al. (2004) also surveyed variation in ascorbic acid (vitamin C) production in *C. melo* germplasm.

The evaluation of seed composition, especially of fatty acids, has been conducted in the domesticated oilseed forms of *Cucurbita pepo* (reviewed by Teppner 2004) and in both *Cucurbita foetidissima* (Scheerens et al. 1978; De Veaux and Schultz 1985) and *Citrullus colocynthis* (Schafferman et al. 1998) with the goal of developing them as new, arid-land perennial oilseed crops. Research to develop *Citrullus colocynthis* as a new crop in India has been summarized by Mal, Rana, and Joshi (1996). Thompson, Dierig, and White (1992) presented a succinct review of research that had been conducted in an unsuccessful attempt to domesticate *Cucurbita digitata* and *Cucurbita foetidissima* in the United States between the 1940s and 1990.

Information on the challenges and opportunities related to the domestication of *C. foetidissima* has been reported by Gathman and Bemis (1990). The substantial production of cucurbitacins, a class of unusual tetracyclic triterpenoids by *C. foetidissima*, has potential application in the production of insect attractants (baits). The complex roles of cucurbitacins as vertebrate toxins, insecticides, and attractants has been reviewed by Metcalf and Rhodes (1990). This review included papers that summarized the evaluation of cucurbit germplasm for variation in the production of these distinctive compounds.

### 8.6.2.3 Reaction to Biotic and Abiotic Stresses

The discovery of valuable genes that allow plants to resist pathogens and pests and to tolerate abiotic stresses and their subsequent incorporation into commercial crops are key justifications for the conservation of *ex situ* germplasm collections. As described below, cucurbit germplasm collections have received considerable attention for evaluation, both as sources of pathogen and pest resistance and for their responses to a wide range of abiotic stresses.
Viral Pathogens

Provvidenti (1982, 1986, 1989, 1993) published three review papers that summarize the body of knowledge on screening cucurbit germplasm for its reaction to viral pathogens. In addition, a single, more specialized review focused exclusively on the genus Cucurbita (Provvidenti 1990). Munger (1993) also reviewed the genetic control of viral resistance in relation to cucurbit breeding. Many germplasm viral pathogen evaluations have been published since these early reviews.

Three destructive viruses have received much of the attention for screening: CMV, ZYMV, and Watermelon mosaic virus-2 (WMV-2) (Lebeda and Křístková 1996; Křístková and Lebeda 2000a; Paris and Cohen 2000). Evaluations discovered new sources of resistance in both C. maxima and C. pepo. New sources of resistance to ZYMV infection have been reported for both Citrullus (Boyhan et al. 1992; Guner and Wehner 2004) and C. melo (Herrington and Prytz 1990), Cucumis. hystrix, which is sexually compatible with C. sativus, evidently also harbors some degree of resistance to CMV and ZYMV and comparatively strong resistance to PRV (Chen et al. 2004).

Horváth (1993a) screened a set of 67 accessions of 12 Cucumis species for the resistance to seven viruses [Cucumber green mottle mosaic virus (CGMMV), Cucumber leaf spot virus (CLSV), CMV, MNSV, Melon yellow fleck virus (MYFV), WMV-2, ZYMV]. An immune reaction was found within cultivated C. melo and C. sativus genotypes, but potentially valuable sources of resistance were also identified within C. africanus (CGMMV, CLSV, CMV, WMV-2, ZYMV), C. anguria (CLSV, CMV, WMV-2, ZYMV), C. ficifolius (CGMMV, MNSV, WMV-2, ZYMV), C. figurei (MNSV), C. mesuillii (CGMMV, WMV-2), C. myriocarpus (CLSV, CMV, WMV-2), C. melo var. agrestis (CMV), and C. zeyheri (WMV-2). Horváth (1993b) conducted a similar screening of four Cucurbita species.

Other viral pathogens that have received special attention for germplasm evaluation include the Cucurbit yellowing stunting disorder virus in Citrullus and Cucumis (Hassan et al. 1990, 1991; López-Sesé and Gómez-Guillamón 2000) and Cucurbit aphid-borne yellows luteovirus (Dogimont et al. 1996), Cucumber vein yellowing virus (CVYV) (Montoro et al. 2004), and Kyuri green mottle mosaic virus (KGMMV) (Daryono, Somowiyarjo, and Natsuaki 2004) in C. melo.

Bacterial Pathogens

The major bacterial diseases of cucurbits have been described by Zitter, Hopkins, and Thomas (1996). Important germplasm evaluation reports include those by Barry, Burnside, and Myers (1976) for reaction to bacterial wilt (Erwinia tracheiphila) in Cucumis by Sowell and Schaad (1979), Hopkins and Thompson (2002) for reaction to bacterial fruit blotch (Acidovorax avenue subsp. citrulli) in Citrullus and by Kudela and Lebeda (1997) and Olczak-Woltman et al. (2004) for reaction to angular leaf spot (Pseudomonas syringae pv. lachrymans) in wild Cucumis species and C. sativus, respectively. Earlier publications describing reactions of C. sativus germplasm to angular leaf spot were reviewed by Kudela and Lebeda (1997) and Olczak-Woltman et al. (2004) with the exception of an extensive screening project conducted on C. sativus germplasm by Staub et al. (1989).

Fungal Pathogens

Any comprehensive reviews of research to evaluate cucurbit germplasm for reaction to the diverse array of known fungal pathogens (Zitter, Hopkins, and Thomas 1996) has not been found. However, considerable work has been conducted on screening for reaction to downy and powdery mildews and, to a lesser extent, on many other fungal pathogens. Much of this work is complicated by variation in pathogen isolates and screening protocols.

Downy Mildew: Downy mildew (Pseudoperonospora cubensis) damages many cucurbit species with pathotypes differing in host range and virulence. Lebeda and Widrlechner (2003) reviewed
germplasm evaluation reports for all cucurbits as part of the establishment of a comprehensive set of differential lines that discriminate pathotypes. In addition to evaluations reviewed by Lebeda and Widrlechner (2003), extensive screening of more than 2000 accessions of C. melo has been conducted by Thomas and Jourdain (1992), Thomas (1999), More (2002) and More, Dhakare, and Sawant (2002) evaluated 368 accesses of C. melo; Staub and Palmer (1987) evaluated seedling response in nine wild species of Cucumis; and Lebeda (1992a, 1992b) and Lebeda and Prášil (1994) evaluated large sets of wild Cucumis species and C. sativus germplasm. However, to date, efficient and highly effective sources of resistance in Cucumis species are unavailable; only significant differences in field resistance have been recorded (Lebeda 1999). Recently, Lebeda and Widrlechner (2004) published the results of downy mildew screening on wild and weedy accessions of Cucurbita. Cultivated C. pepo, represented by eight groups of morphotypes, expressed significant differences in resistance/susceptibility to P. cubensis and both powdery mildews (G. cucurbitacearum and P. xanthii). Generally, there was an inverse relationship detected in resistance to the two groups of mildews. While zucchini, cocozelle, and vegetable marrow were highly resistant to P. cubensis, they had relatively high powdery mildew sporulation. Cultivars with the fruit type acorn, straightneck, and ornamental gourd were quite susceptible to P. cubensis; however, they were considered resistant to powdery mildew in laboratory and field evaluations (Lebeda and Křístková 2000).

**Powdery Mildew.** Another widespread foliar disease of cucurbits is powdery mildew, that is caused by *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*) and *Golovinomyces cucurbitacearum* (syn. *Erysiphe cichoracearum*). Jahn, Munger, and McCreight (2002) published a comprehensive review of sources and genetic control of resistance to powdery mildew in cucurbits, which included some references to past germplasm evaluations. Two more recent extensive evaluations for reaction of *C. sativus* germplasm to inoculation by *P. xanthii* have been reported by Morishita et al. (2003) and by Block and Reisma (2005). Among *C. sativus* PI accessions, Lebeda and Křístková (1997) found significant differences in field resistance to both powdery mildews.

For *C. melo*, Pitrat, Dogimont, and Bardin (1998) reviewed the literature on screening germplasm for powdery mildew resistance. Since that time, Fanourakis, Tsekoura, and Nanou (2000) reported results of an evaluation of Greek landraces of *C. melo* for reaction to *P. xanthii*. The only report of a comprehensive screening of wild *Cucumis* germplasm to *P. xanthii* and *G. cucurbitacearum* was contributed by Lebeda (1984) who identified the best resistance in *C. anguria*, *dinteri*, *ficifolius*, and *sagittatus*. Recently, Thomas, Levi, and Caniglia (2005) presented the results of screening 266 *C. lanatus* accessions for reaction to race 2 *P. xanthii*, identifying 23 accessions with intermediate levels of resistance. Evaluations of *Cucurbita* germplasm for resistance to powdery mildew have been well summarized by Jahn, Munger, and McCreight (2002). One additional report, examining differences in damage to leaf surfaces and stems and petioles of *C. pepo*, germplasm was published by Křístková and Lebeda (2000b).

**Other Foliar Pathogens.** Cucurbit germplasm has been evaluated for reaction to other important foliar pathogens, including scab (*Cladosporium cucumerinum*) in wild *Cucumis* species (Staub and Palmer 1987), *C. sativus* (Lebeda 1985), and *C. species* (Strider and Konsler 1965), anthracnose (*Colletotrichum orbiculare*) in *C. sativus* (Staub et al. 1989; Wehner and St. Amand 1995) and *C. lanatus* (Wang and Liu 1989), and target leaf spot (*Corynespora cassiicola*) in *C. sativus* (Staub et al. 1989). Gummy stem blight (*Didymella bryoniae*) damages both foliar and vascular tissues. The results of three extensive evaluations for reaction to gummy stem blight, emphasizing *C. melo* germplasm, have been published (Zhang et al. 1997; Sakata et al. 2000; Wako et al. 2002) that also include reviews of past work. And more recently, Chen, Moriarty, and Jahn (2004b) reported finding resistance to *D. bryoniae* in *C. hystrix*. Higher levels of resistance to *D. bryoniae* were also found in some wild *Cucumis* species (*C. ficifolius*, *C. melo var. agrestis*, and *C. myriocarpus*) (Lhotsky, Lebeda, and Zvara 1991). Also inbred pickling cucumber with resistance to *D. bryoniae* was released (Wehner, St. Amand, and Lower 1996).

**Fusarium Wilt.** Fusarium wilt (*Fusarium oxysporum*) can cause significant economic losses in many cucurbits. Results of germplasm screening have been summarized for *Citrullus* landraces
from Botswana (Wang and Zhang 1988) and for a broader sampling of more than 100 Citrullus accessions, including C. lanatus var. lanatus, C. lanatus var. citroides, and C. colocynthis (Huh, Om, and Lee 2002), for C. sativus germplasm challenged by isolates representing three different races (Armstrong, Armstrong, and Netzer 1978), for a diverse sampling of C. melo cultivars tested against a single race (Zink, Gubler, and Grogan 1983), for selfed progeny of landraces and cultivars against two races (Pitrat et al. 1996), and for Iberian landraces of C. melo tested against various local races (Alvarez and Gonzalez-Torres 1996). Vine decline in C. melo can also be caused by Acremonium cucurbitacearum and Monosporascus cannonballus. Crosby (2001) has evaluated C. melo var. agrestis for resistance to M. cannonballus in Texas, and de C.S. Dias et al. (2001) conducted a field assay of a diverse sampling of C. melo accessions and related species in southeastern Spain where highly aggressive isolates of both fungi had been isolated.

Belly Rot, Phytophthora Fruit Rot. Screening has also been conducted to evaluate resistance to two fungal pathogens that can cause fruit damage: belly rot (R. solani) and Phytophthora fruit rot (Phytophthora capsici). Sloane, Wehner, and Jenkins (1983, 1984) described the most resistant of 1063 C. sativus accessions they evaluated for reaction to R. solani in field and laboratory tests. Lopes, Brune, and Henz (1999) presented results from an inoculation of 150 Cucurbita accessions with P. capsici.

Nematodes, Insects, and Mites. Nematodes are serious pests of cucurbits in both field and greenhouse production with the root-knot nematodes (Meloidogyne spp.) among the most destructive. Thies (1996) presented an overview of nematode damage on cucurbits and reviewed key evaluations of germplasm conducted to identify sources of resistance to Meloidogyne. Two evaluations, unnoted by Thies (1996), have been published. Zhang, Qian, and Liu (1989) presented the results of an extensive screening of Citrullus germplasm to three species of Meloidogyne, and Dalmasso, Dumas de Vaulx, and Pitrat (1981) published a brief summary of research on wild species of Cucumis and Cucurbita. In addition, Walters, Wehner, and Barker (1997) characterized a recessive gene for resistance to Meloidogyne javanica from C. sativus var. hardwickii, and resistance has also been reported from C. hystrix (Chen and Lewis 2000; Chen et al. 2001).

Elsey (1989) and Robinson (1992) assembled comprehensive reviews of research conducted to evaluate cucurbit germplasm for reaction to insect and mite pests. However, in the intervening years, results from more recent evaluations for reaction to destructive insects and mites have been published. For example, extensive screening of C. melo germplasm to identify sources of host-plant resistance to whiteflies (Bemisia spp.) has been conducted with promising results (McCreight 1995; Simmons and McCreight 1996). In addition, resistance to whiteflies has been identified among Citrullus colocynthis germplasm accessions (Simmons and Levi 2002). C. melo germplasm has also been evaluated for tolerance to the melon aphid, Aphis gossypii (Bohn, Kishaba and McCreight 1996), and to melonworm, Diaphania hyalinata (Guillaume and Boissot 2001), where field tests identified moderate levels of tolerance in some C. melo lines and higher levels of resistance in C. metuliferus and C. pustulatus. Western flower thrips, Frankliniella occidentalis, is a serious pest of cucurbits under greenhouse conditions. Balkema-Boomstra and Mollema (1996) identified sources of resistance to this pest within a geographically diverse sample of C. sativus germplasm.

Early reports by Leppik (1968), Tulisalo (1972), and Knipping et al. (1975) suggested that wild species of Cucumis might harbor valuable sources of resistance to spider mites (Tetranychus spp.). To that end, Lebeda (1996) evaluated a broad sampling of wild Cucumis accessions and identified useful levels of resistance to Tetranychus urticae in C. africanus and C. zeyheri. Very broad variation for resistance to greenhouse whitefly (Trialeurodes vaporariorum) has also been found in wild Cucumis germplasm (Láska and Lebeda 1989). Even after the completion of a diverse array of insect and mite evaluations by the 1990s, however, Webb (1998) found only a few cucurbit breeding lines and one cultivar with insect resistance among releases from that time period.
Abiotic Stresses. Extensive evaluations of *C. sativus* germplasm have been conducted to identify sources of tolerance to the herbicides, clomazone (Staub et al. 1991) and atrazine (Werner and Putnam 1977), and to high temperatures (Staub and Krasowska 1990) and moisture deficits (Wann 1992). Wehner (1982) evaluated the ability of *C. sativus* accessions to germinate under low-temperature conditions and determined the heritability of this characteristic based on parent-progeny regressions (Wehner 1984). And for *C. melo* accessions, Hutton and Loy (1985) compared rates of seedling development at 15 and 30°C. Ríos Labrada, Fernández Almirall, and Casanova Galarraga (1998) evaluated Cuban landraces of *Cucurbita moschata* for tolerance to high temperatures and drought, and they developed a stress-tolerance index as part of a larger project to identify germplasm to breed new cultivars for low-input, tropical agriculture.

Sulfur dusting is used to control powdery mildew in *C. melo*, but it can induce severe foliar damage. Perchepied et al. (2004) recently evaluated more than 200 *C. melo* accessions for reaction to sulfur and reported interesting geographic patterns of variation in the distribution of resistant germplasm. They subjected one source of resistance to QTL analysis through an examination of recombinant inbred lines and detected one major and two minor QTL.

8.6.3 Cross-Compatible Breeding Strategies

8.6.3.1 Interspecific Hybridization: Conventional Approaches

Transfer of economically important characteristics such as host-plant resistance to diseases and pests or stress tolerance from wild cucurbit species to their cultivated counterparts is one of the most important challenges for breeders. Substantial differences can be found among the three major genera of cultivated Cucurbitaceae (*Cucumis, Cucurbita,* and *Citrullus*) with regard to their cross-compatibilities with wild relatives. They exhibit a wide range of crossing barriers at both the presyngamic (failure of pollen tube growth) and postsyngamic (breakdown of embryo development) developmental phases. Many techniques, including embryo and callus culture, bridge crosses, bud pollination, repeated pollination, regulating ploidy levels, and the use of growth regulators, have been employed in attempts to improve the success of interspecific hybridization (Kalloo 1988).

To date, conventional crosses between *C. sativus* and other *Cucumis* species, with the exception of *C. hystrix*, have not been successful; however, somatic hybridization via protoplast fusion, especially asymmetrical fusion, has been suggested as a solution to this problem (Tatlıoğlu 1993).

Melon, *C. melo*, is not easily crossable with a majority of wild *Cucumis* species in spite of sharing the same base chromosome number and ploidy level with many of those species. In some cases, *in vitro* culture of hybrid embryos (discussed in Section 8.6.5.3) has been successfully employed for their recovery (Sauton 1987).

Similarly, among *Cucurbita* species, conventional interspecific pollination followed by *in vitro* culture of hybrid embryos can result in the production of fertile hybrids (Šiško, Ivančič, and Bohaneć 2003). Information related to hybridization among *Cucurbita* species and techniques to overcome crossing barriers and hybrid sterility has been summarized by Lira-Saade (1995).

As the cultivated watermelon, *C. lanatus* var. *lanatus*, is cross-compatible with its congeneric wild relatives, biotechnological methods (discussed in Section 8.6.5) have been applied to primarily increase the fertility of obtained hybrids. Although unique crossing approaches are used for the creation of triploid seedless cultivars (Kihara 1951), such approaches have not been needed for overcoming crossing barriers. Specific examples documenting the application of various conventional techniques to improve the success of wide hybridization follow.

The power of the bridge cross emanates from its ability to exploit the natural potential of crossing ability among species. If two species are not crossable directly, the breeder searches for a third species that is compatible with both. Within the genus *Cucurbita, C. moschata* is
crossable with many wild and cultivated species, and it has been exploited as a bridge species (Provvidenti, Robinson, and Munger 1978). Three-way crosses have been used to transfer resistance genes for powdery mildew and CMV from \textit{C. martinezii} to \textit{C. pepo}. Initially, \textit{C. martinezii} was crossed with \textit{C. moschata}, then the resulting hybrid was crossed to \textit{C. pepo} (Whitaker and Robinson 1986).

Bridging species can also be used to circumvent sterility in interspecific crosses. Whitaker (1959) found that \textit{C. lundelliana} could be crossed with each of the cultivated species of \textit{Cucurbita}. Rhodes (1959) developed an interbreeding population from crosses involving \textit{C. lundelliana} and the cultivated species: \textit{C. pepo}, \textit{C. argyrosperma}, \textit{C. moschata}, and \textit{C. maxima}. Thus, \textit{C. lundelliana} served as a bridge to transfer genes between species that would otherwise be difficult to cross (Whitaker and Robinson 1986).

Cross-compatibility also depends upon the particular accessions selected to represent a given species in interspecific hybridization. Cultivars of the same species may vary greatly in crossability. For example, crosses with \textit{C. moschata} Butternut were more easily made with Scallop than with other \textit{C. pepo} cultivars (Whitaker and Robinson 1986). In the genus \textit{Cucumis}, there is ample variation within two species (\textit{C. anguria} and \textit{C. zeyheri}) that carry CGMMV resistance. This variability has, in fact, been exploited to overcome hybridization barriers in wild African \textit{Cucumis} species (Visser and den Nijs 1983). These examples and many others (see Raamsdonk, den Nijs, and Jongerius 1989) suggest that biosystematic and gene-pool studies can guide the selection of genotypes to increase the probability of successful interspecific hybridization.

Common problems that occur in the \textit{F}_1 and early succeeding generations of distant crosses are sterility and poor seed development. Often the embryo does not abort, but the nutritive tissue of the seed fails to develop normally. Embryo culture may be required in such cases (see Section 8.6.5.3). Poorly developed seeds from interspecific hybrids or their progeny often germinate better when their seed coats are removed (Whitaker and Robinson 1986). \textit{F}_1 hybrid seedlings so obtained from a cross between \textit{C. pepo} Black Jack and \textit{C. martinezii} were resistant to \textit{G. cichoracearum} and CMV as was the male parent (\textit{C. martinezii}) (Metwally, Haroun, and El-Fadly 1996). This method has also been recommended for use when making compatible crosses in \textit{Cucurbita} species (Hong and Hyo-Guen 1994). Interspecific hybrids of \textit{C. moschata}×\textit{C. maxima} are known as Kabocha and have been offered by Sakata Seed Company (Japan) under different cultivar names (e.g., Alguri, Kikusui, Tetsakabuto). Fruits are reported to combine favorable characters from both species, and there is considerable heterosis for degree of female sex expression and for yield (Whitaker and Robinson 1986).

Researchers have also explored the ability of mentor pollen to help overcome prezygamic crossing barriers. In such cases, the stigma is pollinated with a mixture of pollen grains from a compatible species with those from a typically incompatible species possessing important traits. The presence of compatible pollen can facilitate pollen-tube growth. This approach improved hybrid seed set in the cross \textit{C. metuliferus}×\textit{C. africanus} (Kho, den Nijs, and Franken 1980). In \textit{Cucumis}, success has been achieved when mentor pollen combined with chemical treatment was followed by embryo culture (den Nijs and Oost 1980; den Nijs, Custers, and Kooistra 1980).

The use of irradiated pollen has been investigated for various purposes. One experimental approach was based on the assumption that irradiation leads to the selective elimination of chromosomes of \textit{C. melo} in the early stages after pollination. This approach was attempted by Custers and Bergervoet (1984) in crossing \textit{C. sativus} with \textit{C. melo}. However, they found that with increasing doses of irradiation, there was a decrease in the number and size of embryos and endosperm.

Irradiated pollen has also been used to produce haploid plants. Pollen can be irradiated at dosages that retain its capacity for fertilization but destroy its potential to deliver viable chromosomal material to offspring. This approach (pollen irradiated by gamma rays) has been applied to \textit{C. melo} for the induction of parthenogenetic ovule development. Haploid embryos excised at the globular or heart stage were cultured in vitro (Sauton 1988), producing haploid melon plants where there was no spontaneous doubling of chromosomes, but there are other reports of the recovery of
both haploid and dihaploid plants (Sauton and de Vaulx 1987; Savin et al. 1988; Dirks and van Buggenum 1991).

Hybridization barriers can be overcome by changing ploidy levels, either to restore fertility or to match ploidy levels between two species (Kalloo 1988). Different chromosome numbers in C. sativus and other Cucumis species are the main reason of their non-crossability (Dane 1991). Polyploidization could favorably influence the constitution of chromosome bivalents in the first stage of the zygotic cell division and to promote subsequent growth and development (Colijn-Hooymans et al. 1994; Lebeda, Kříštková, and Kubaláková 1996). Colchicine has been used to restore fertility in sterile C. maxima × C. moschata progeny (Pearson, Hopp, and Bohn 1951; Whitaker and Robinson 1986). Moreover, Bemis (1973) reported that the original interspecific crosses of C. moschata × C. palmetta were successful at the tetraploid level.

Another potential method to overcome either presyngamic or postsyngamic crossing barriers is the application of growth regulators (AVG, BAP, IBA, 4-CPA) to the peduncles of female flowers. This technique was first applied in crosses among Cucumis species (Deakin, Bohn, and Whitaker 1971), but it was without the expected positive results (Chatterjee and More 1991; Lebeda, Kříštková, and Kubaláková 1996).

8.6.3.2 Developing Unique Genetic Stocks

Bulk segregant analysis (Michelmore, Paran, and Kesseli 1991), in combination with near isogenic lines (NILs) and other unique genetic stocks, has been used in cucumber to identify markers linked to disease resistance (Horejši, Staub, and Thomas 2000; Park et al. 2000) and to study sex-expression genes (Trebitsh, Staub, and O'Neill 1997; Witkowicz, Urbańczyk-Wochniak, and Przybecki 2003). The cucumber RILs developed by Fazio, Staub, and Stevens (2003) are useful in mapping markers and QTL, but they are not, by themselves, immediately useful to plant breeding programs. Genes for yield components are dispersed throughout these RILs, and the lines themselves are not entirely homozygous (Fazio, Staub, and Stevens 2003). Therefore, it would be desirable to create genetic stocks containing QTL in useful homozygous genetic backgrounds for use as introgression lines. One method to introgress QTL into breeding lines, called "advanced backcross QTL analysis" (AB-QTL analysis), has been proposed by Fulton et al. (2000). This method involves backcross breeding in combination with MAS to create NILs that vary in useful donor alleles in the elite parent background. Once created, these NILs could be used for the efficient introgression of economically important QTL into elite germplasm ( Tanksley et al. 1996; Fulton et al. 1997; Bernacchi et al. 1998a, 1998b). For instance, NILs (BC2S3) strategically created in cucumber (e.g., Gy-7 × H19; Serquen, Bacher, and Staub 1997a; Fazio, Staub, and Stevens 2003) could carry single-gene donor introgressions for desirable QTL alleles (i.e., branching; fruit length) derived from the donor parent (i.e., H-19) but transferred to the genetic background of the elite recurrent parent (i.e., Gy-7). Such genetic stocks would allow for the evaluation of epistatic interactions to determine efficacy of gene action under different environments.

In addition to NILs, Ezura, Kikuta, and Oosawa (1994) have produced aneuploid plants by crossing tetraploid and triploid melon lines followed by in vitro culture. From these aneuploid plants, trisomic lines could be developed, which have long served as useful tools in genetic analysis (reviewed by Kasha 1973).

8.6.3.3 Broadening Genetic Diversity

Genetic diversity is essential for the continued development of commercial cultivars. The NPGS cucumber core collection (147 accessions; Staub et al. 2002) effectively samples genetic diversity at molecular loci to allow for the incorporation genetically diverse exotic accessions into the core...
However, genetic analysis of the NPGS cucumber collection (~1350 accessions of worldwide origin) and elite commercial germplasm (>150 accessions developed between 1846 and 1995) documented reduced diversity among commercial germplasm (~3% polymorphism in elite commercial germplasm and ~12% across the entire primary gene pool) (Meglic and Staub 1996b). Thus, rigorous genomic characterization of the elite lines suffers from a paucity of genetic variation (Dijkhuizen et al. 1996; Staub et al. 2002), highlighting the need for the genetic enrichment of elite cucumber germplasm to ensure continued potential for plant improvement.

The inbred backcross line (IBL) breeding method and analytical procedures, as originally described by Wehrhahn and Allard (1965), could be applied in combination with MAS for broadening the genetic base of commercial cucumber germplasm. The IBL procedure applies no selection (as opposed to AB-QTL analysis) in the production of BC$_2$S$_3$ lines in distinct genetic backgrounds (e.g., U.S., European, and Asian cucumber) with extensive replicated evaluation. A modification of this procedure involves the initial cross of a donor (exotic germplasm) to a recurrent (elite germplasm) parent in a target genetic background followed by a backcross to the recurrent parent to produce the BC$_1$ (this procedural modification of IBL proposed initially herein). In this modified IBL procedure, germplasm at various stages of introgression (i.e., BC$_1$ and BC$_2$) are genotyped by using a standard marker array (Lopez-Sese, Staub, and Gomez-Guillamon 2003; Milki et al. 2003) that has proven useful across various gene pools to maximize genetic diversity. For example, the BC$_1$ progeny could be genotyped by molecular markers and about 200 of the most genetically diverse BC$_1$ would then be crossed to the recurrent parent to produce 200 BC$_2$ progeny (either marker-genotyped or chosen at random), which can then be self-pollinated thrice after marker genotyping to obtain BC$_2$S$_3$ lines prior to replicated evaluation in appropriate target environments (e.g., European greenhouse cucumber under controlled environments and U.S. processing under open-field conditions) for qualitative and quantitative traits of interest. The best lines could then be further refined (e.g., inbreeding or backcrossing) or used directly in breeding programs (e.g., evaluated for combining ability).

### 8.6.4 Cross-Incompatible Breeding Strategies

The identification and incorporation of resistance to economically important pests such as root-knot nematode (Thies 1996) and various foliar pathogens (den Nijs and Custers 1990) has been required to sustain cucumber production. However, resistance genes for several crop-limiting pests and pathogens have not been found within the primary gene pool of *C. sativus*.

The incorporation of wild *Cucumis* species to broaden the genetic base of cucumber has been a goal of plant geneticists and breeders for over one hundred years (Robinson and Decker-Walters 1997). Meeting this goal has been daunting because nearly all wild *Cucumis* species are cross-incompatible with *C. sativus* and *C. melo* (Staub et al. 1992). However, these species are potentially important for plant improvement programs as sources of resistance to economically important pathogens such as powdery mildew, downy mildew, anthracnose, and fusarium wilt (Leppik 1966; Lower and Edwards 1986; Kirkbride 1993). In the last 60 years, conventional and biotechnological approaches for overcoming species crossing barriers in *Cucumis* have often been applied without success (Whitaker 1930; Batra 1953; Smith and Venkat Ram 1954; Deakin, Bohn, and Whitaker 1971; Fassuliotis and Nelson 1988). Until recently, repeated attempts at interspecific hybridization of wild *Cucumis* species (e.g., *C. metuliferus, C. melo*) with cucumber were either unsuccessful or irreproducible (Fassuliotis and Nelson 1988). Thus, it had been concluded that mating of *C. sativus* with any other *Cucumis* species possessing a chromosome series of 2n=24 or 48 would be unsuccessful (Kristkova and Lebeda 1995).

However, in 1995, a successful interspecific hybridization was accomplished between *C. hystrix* Chakr. (2n=2x=24), native to Yunnan Province, China (Chen et al. 1995), and...
Cucumis sativus var. sativus (Chen et al. 1997b). Cucumis hystrix appears to possess economically important traits such as root-knot nematode resistance (Chen and Lewis 2000; Chen et al. 2001), gummy stem blight (D. bryoniae) resistance, downy mildew (P. cubensis) resistance, unique nutritional qualities, and tolerance to growth under low irradiance and temperature (Chen et al. 2003b; Chen, Moriarty, and Jahn 2004b).

The cross was attempted based on Cucumis phylogenetic relationships as revealed by isozyme (Chen et al. 1995, 1997a) and RAPD/SSR (Zhuang et al. 2004) analyses. Because the initial F₁ hybrids (2n = 19, with 12 and 7 chromosomes contributed by C. hystrix (H) and C. s. var. sativus (C), respectively) were completely heterologous, chromosome-doubling experiments were initiated to restore fertility (Chen and Staub 1997). This doubling of chromosome number in F₁ interspecific hybrid progeny was accomplished by monitoring in vitro cultures for somaclonal variation (Chen et al. 1998, 2003b; Chen and Adelberg 2000). Putative tetraploid somaclonal variants were initially identified by their unique morphological characters (e.g., serrated leaf edge) and then by chromosome counts (Chen et al. 2003a, 2003b). Reciprocal differences have been observed between F₁ progeny from C. sativus var. sativus × C. hystrix matings, indicating maternal and paternal inheritance for certain morphological and molecular (RAPD) characters (Chen et al. 2004c).

The resulting amphidiploid (HHCC, 2n = 4x = 38) was designated as a new synthetic nothospecies (C. Xhytivus Chen) and has been reproduced in subsequent experiments, ultimately resulting in an array of amphidiploids that have been self-pollinated to produce viable seeds (Chen and Kirkbride 2000). More recently, hybridization experiments have produced allotriploids (2n = 3x = 26; genome designated as HCC) from amphidiploid × diploid (C. sativus var. sativus) matings as well as diploid derivatives (2n = 2x = 14), which are fully fertile with C. sativus var. sativus (Chen et al. 2004a; Qian et al. 2005). The amphidiploid and its allotriploid and diploid derivatives provide a species bridge in Cucumis, and a source for broadening the genetic base of C. s. var. sativus and perhaps eventually also of C. melo (Chen et al. 2003a; Zhuang et al. 2004; Qian et al. 2005).

An attempt to transfer resistance to P. cubensis from C. melo (line MR-1) to C. sativus combined the conventional pollination of intact C. sativus flowers with C. melo pollen with in vitro culture of excised young seeds and embryos (Lebeda, Křístková, and Kubaláková 1996), resulting in the regeneration of seven embryos. Five embryos developed small roots and/or shoot meristems. However, only callus formation was observed during the culture of these embryos that is likely due to an increase in ploidy level (Lebeda, Křístková, and Kubaláková 1996). Isozyme analyses of callus derived from superior embryos verified the presence of hybrid zones. Two embryos developed into flowering plants after which their hybrid origin was confirmed through isozyme analyses. Although the morphology of these plants resembled their maternal (C. sativus) parent (Lebeda et al., 1999), increased resistance to P. cubensis was observed (Lebeda et al., 1999). Lebeda et al. (1999) concluded that the regenerated plants were not symmetrical interspecific hybrids, but they were more likely asymmetrical hybrids (Lebeda et al. 1999). More detailed studies of the mechanism and methodology of interspecific crossing between C. melo and C. sativus are ongoing (Ondřej, Navrátilová, and Lebeda 2000, 2001, 2002; Skálová, Lebeda, and Navrátilová 2004).

8.6.5 Biotechnological Approaches

8.6.5.1 Haploid Technology

Cucumber (Cucumis sativus)

The first cucumber haploids were obtained by stimulating parthenogenesis through pollination with irradiated pollen (Troung-Andre 1988; Niemirowich-Szczytt and Dumas de Vaulx 1989;
Three weeks after pollination, 0.49–1.70 embryos per fruit were isolated, but only 3.3–7.7% of the haploid embryos developed into haploid plants. Some plants showed abnormal development such as lack of a primary meristem, short internodes, rosette growth habit, or thick curled leaves. To help optimize the efficiency of haploid production, Faris, Nikolova, and Niemirowicz-Szczytt (1999) examined the effects of radiation dosage. Of the three conditions they tested, a dose of 0.1 kGy from a gamma ray (60Co) source stimulated development of the largest number of haploid embryos.

Haploid cucumber plants are infertile and typically do not undergo spontaneous diploidization. Thus, haploids from four different genotypes were treated with colchicine to induce doubled haploids (DH) (Nikolova and Niemirowicz-Szczytt 1996). The following procedure was used in the production of haploid plants: (1) an apical shoot-meristem treatment, (2) the soaking of shoot explants, and (3) the culture of shoot explants on a medium containing colchicine. Optimal results (20.9% DH) were obtained with repeated treatment of apical shoot meristems with colchicine. Many chimeras (28.5%) as well as haploids and tetraploids resulted from this treatment protocol. The DH plants were fertile and gave uniform progeny. Chimeras had decreased fertility when compared to untreated control plants and showed disturbances in meiotic divisions. DH lines have also been occasionally produced by regenerating spontaneous diploid shoots from tissue cultures (Faris et al. 1997; Sztanger et al. 2004).

Haploid cucumber plants have also been produced via in vitro gynogenesis with limited success (Gémes-Juhász, Vencze, and Balogh 1996; Gémes-Juhász et al. 2002). By optimizing culture conditions, including a special heat treatment, gynogenic plants of cucumber were successfully produced in vitro from unpollinated ovules, including both haploids and spontaneous DH plants directly through embryogenesis. The highest frequency of gynogenesis obtained was 18.4% with rates of plant regeneration of up to 7.1%. Analysis with flow cytometry indicated that 87.7% of the regenerants were haploid.

In vitro conservation and storage of haploid cucumber lines has been attempted by Niemirowicz-Szczytt et al. (2000) who reported that lines at optimal developmental stages could be maintained through timely subculturing for two years.

**Melon (Cucumis melo)**

Rapid production of homozygous lines is extremely valuable for commercial hybrid melon breeding. However, several generations of inbreeding are typically required to produce such lines. The significance of haploid and DH lines in accelerating inbred-line development has been recognized by melon breeders for many years. Spontaneous haploid production can occur via parthenogenensis, but at a very low frequency (Bhojwani and Razdan 1983).

The recovery of gynogenetic haploid melon plants obtained from an interspecific cross between *C. melo* and *C. ficifolius* was first reported by Dumas de Vaulx (1979). However, efficiency was quite low. In situ haploid gynogenesis has been induced by using irradiated pollen (Sauton and Dumas de Vaulx 1987; Cuny et al. 1992) treated with either gamma radiation or soft x-rays (Sauton 1989). The frequency of haploid production was usually less than a few percent of the melon ovules cultured in vitro, although frequency was influenced by season and genotypes (Sauton 1988; Ficcadenti et al. 1995) with the best results obtained in summer. Most of plants recovered from gynogenesis were haploid, in contrast to DH plants in other species (see Antoine Michard and Beckert 1997; Gu, Zhou, and Hagberg 2003). Melon haploids are sterile, and chromosome doubling must be induced to obtain fertile homozygous lines. Colchicine is commonly used for this purpose (Yashiro et al. 2002; Lotfi et al. 2003; Yetisir and Sari 2003).

Yashiro et al. (2002) treated lateral shoots of 31 haploid melon plants grown in a greenhouse with a 0.1% colchicine solution. Eventually, 68% of the treated haploid plants were doubled by this method. Lotfi et al. (2003) treated haploid melon plants with colchicine in vitro. From 167 micropropagated haploid shoots, 10 diploid and 100 mixoploid plants were produced.
Yetisir and Sari (2003) compared both in vitro and in situ methods for the diploidization of haploid melon plants. Immersion of in vitro plantlets or of single-node explants in a colchicine solution was compared to immersion of shoot tips of greenhouse-grown plants. In addition, single drops of colchicine were applied to lateral buds of greenhouse-grown plants. The diploidization rate achieved by the immersion of shoot tips was about 89%, a rate three times greater than that attained by the treatment of in vitro plantlets or single-node explants.

Haploid technology has been put into practice by melon breeders; however, two challenges limit haploidization’s progress. Haploid production’s frequency is still low with the number of expected haploids per plant ranges from one to five. Recently, Kuzuya et al. (2003) investigated whether resistance to powdery mildew (P. xanthii) could be manifest in haploids derived two disease-resistant lines, PMR 45 and WMR 29. Encouragingly, the haploids responded to powdery mildew identically to their diploid progenitors.

**Squash (Cucurbita pepo)**

The production of haploids from unfertilized ovules of summer squash (C. pepo) has been reported by Dumas de Vaulx and Chambonnet (1986), Metwally et al. (1998a). Ovaries from squash plants were collected one day before anthesis and exposed to low temperature (4 °C) for zero, two, four, or eight days. Ovules were then cultured on MS medium, supplemented with four concentrations of 2,4-D. After incubation at 25 ± 1°C under 16-h photoperiod for four weeks, the ovules were transferred to an MS medium lacking 2,4-D for four weeks. The highest rate of plant recovery was from ovules that did not receive a cold treatment when cultured on MS medium supplemented with 1 or 5 mg/L 2,4-D. Of the plants recovered, one third of those examined were haploid and the others were DH.

Haploid squash plants have also been derived from anther culture by Metwally et al. (1998b) who excised anthers at the mid or late uninucleate microspore stage from sterilized buds and plated them on twenty different induction media. The most plantlets resulted from an induction medium supplemented with 150 g/L sucrose and 5 mg/L 2,4-D. Root tips from twenty plantlets were cytologically examined, revealing that ten were diploid plants and ten plants were haploid.

In addition, haploids have been obtained by stimulating parthenogenesis through pollination with irradiated pollen (Kurtar, Sari, and Abak 2002). Haploid embryos and plants were obtained with production strongly influenced by gamma ray dosage, embryo development, and maternal genotypes with gamma ray doses of 25 and 50 Gy giving the greatest response. Different shapes and stages of embryos were derived from seeds extracted from fruits harvested four to five weeks after pollination. All of the pointed globular arrow-tip and stick-shaped embryos developed into haploid plants. However, only 53.8% of torpedo and 23.1% of heart-shaped embryos resulted in haploid plants. In contrast, cotyledonary embryos or those with amorphous shapes produced only diploid plantlets.

No publications describing successful haploid production in other species of Cucurbita are currently known.

**Watermelon (Citrullus lanatus)**

Parthenogenetic haploid embryos of Crimson Sweet, Halep Karasi, Sugar Baby, and Panonia F1 watermelon were obtained after pollination with gamma-irradiated (200 or 300 Gy) pollen (Sari et al. 1994). Some globular and heart-shaped embryos were observed in fruit harvested two to five weeks after pollination. The number of embryos per 100 seeds was highest for Halep Karasi. After in vitro culture, Sari et al. (1994) obtained seventeen haploid plants, and DH lines were generated after chromosome doubling with colchicine.

Both direct (chromosome counting) and indirect (flow cytometry, stomatal size, chloroplast number of the guard cells, and morphological observations) methods were used by Sari, Abak, and Pitrat (1999) to determine the most efficient method(s) for ploidy level identification. Their results
revealed that all the techniques tested could be successfully used. Although counting chromosomes is cumbersome, producing plants for morphological observations requires considerable time, and flow cytometry is expensive. On the other hand, measurement of stomata and chloroplast counting methods are relatively simple and less labor intensive than cytological or morphological inspection and are recommended as practical alternatives (Sari, Abak, and Pitrat 1999).

8.6.5.2 Polyploid Technology

Cucumber (Cucumis sativus)

Tetraploid cucumbers were discovered among plants regenerated via somatic embryogenesis by Custers, Zijlstra, and Jansen (1990) who noted that tetraploid comprised 11% of the recovered regenerants. More recently, Plader et al. (1998), Ladyzynski, Burza, and Malepszy (2002) have reported even higher frequencies of tetraploid generation after in vitro culture with some cases reaching over 50%.

Triploid plants have been regenerated from embryos isolated from crosses between diploid and tetraploid plants (Mackiewicz et al., 1998; Malepszy et al., 1998). In vitro culture of late heart-stage tripl oid embryos isolated from reciprocal crosses of tetraploid and diploid forms of three cucumber lines produced variable results, depending on genotype and the direction of the cross. Five main obstacles that decrease the probability of obtaining triploid plants have been reported (Malepszy et al. 1998): (1) inability of embryos to germinate; (2) non-rooting plantlets; (3) albinism; (4) mixoploid chimeras; and (5) death after transplanting to soil. The lowest failure rate occurred when the tetraploid was used as the female parent. Depending on the line, 11.7–45.8% of embryos obtained from maternal tetraploids developed into mature plants. Surprisingly, triploid plants did not show visible abnormalities. Flow-cytometry analyses of DNA content revealed that normal plants were triploid while poorly growing and abnormal plants were hexaploid or mixoploid (Malepszy et al. 1998). Triploid plants of various origins were characterized (Mackiewicz et al. 1998) where the parthenocarpy and seedlessness, larger floral corollas and fruits, increased production of female flowers, new classes of pollen size, and morphology, and decreased pollen fertility were reported as unique traits. Notably among these triploid plants, parthenocarpic fruit set ranged between 13 and 41% without pollination and a 9–11-fold increase in fruit set as a result of pollination by diploid plants.

Melon (Cucumis melo)

Melon has a basic chromosome number of \(x=12\), and cultivated melons are mostly diploid \((2n=24)\), but triploids \((2n=36)\) have also been reported (Dane 1991) with the triploids resulting from hybridization between diploid and tetraploid \((2n=48)\) lines. Tetraploid melons have been discovered spontaneously growing in field and greenhouse plantings (Suzuki 1958; Nugent and Ray 1992; Nugent 1994) and have also been induced by colchicine treatment of germinating seedlings (Batra 1952) although their incidence is generally quite low. In addition, tetraploids can occur as a result of adventitious-shoot organogenesis (Bouabdallah and Brauchard 1986) and among plants regenerated via other in vitro culture systems, including somatic embryogenesis and shoot-primordial aggregates (Ezura et al. 1992b). The frequency of tetraploidy generated by somatic embryogenesis and adventitious shoot organogenesis can be remarkably high, up to 30% of regenerated plants (Ezura et al. 1992a, 1992b; Adelberg et al. 1994), which is favored by selective regeneration conditions (Ezura and Oosawa 1994a, 1994b). Tetraploid plants have been characterized by low fertility, large male and female flowers, thickened leaves, short internodes, round seeds, and flat, lighter fruits, and no significant improvement over diploid fruits have been displayed (Ezura et al. 1992a).
Although triploid melon plants are produced by crossing tetraploid and diploid lines, their frequency is relatively low (Suzuki 1959, 1960). However, when seeds produced by crossing diploid and tetraploid plants are sown in vitro, triploid plants can be obtained at comparatively high frequencies (Ezura, Amagai, and Oosawa 1993). Pollen fertility in triploid melons is low. Moreover, while triploid lines cannot set fruit by simple self-pollination, they are reported to set fruit by self-pollination following growth-regulator treatment (Ezura, Amagai, and Oosawa 1993) or when pollinated by adjacent diploid pollen sources (Adelberg et al. 1995). The fruits of triploids also have shown no significant improvement over those of diploids.

**Watermelon (Citrullus lanatus)**

Seedless watermelon fruits have been produced either by induction of triploid lines, as reviewed by Compton, Barnett, and Gaba (2004), or by using pollen irradiated with soft X-rays on diploid parents (Sugiyama and Morishita 2000a, 2000b; Sugiyama, Morishita, and Nishino 2002a). Triploid seeds are produced by crossing diploid and tetraploid plants (Kihara 1951). Traditionally, tetraploid plants have been generated by treating diploid seedlings with colchicine (Kihara 1951). However, that method is difficult; thus, only a few tetraploid breeding lines are currently used to produce commercial triploid hybrids.

As with other cucurbits, tetraploid watermelons can be identified among plants regenerated in vitro from diploids (Compton, Barnett, and Elmstrom 1994; Zhang, Rhodes, and Adelberg 1994a), and a method to identify tetraploids in vitro has been developed (Compton, Gray, and Elmstrom 1996; Compton, Barnett, and Gray 1999) based on differences in the number of chloroplasts per guard-cell pair in leaves (Compton, Gray, and Elmstrom 1996). The mean number of chloroplasts for tetraploid regenerants was 19.1, whereas diploids averaged 11.2. An enhanced method was developed by painting the lower epidermis of intact in vitro-derived leaves with fluorescein diacetate and observing fluorescence of guard cell chloroplasts under a microscope with UV light in comparison to fluorescence from known diploid cultivars and tetraploid breeding lines (Compton, Barnett, and Gray 1999).

Plant regeneration protocols in watermelon have been improved and more reliable protocols to produce tetraploids developed to expand the genetic diversity of tetraploid breeding lines (Compton 1999, 2000; Chaturvedi and Bhatnagar 2001). By germinating embryos in darkness, Compton (1999) significantly improved the number of explants that then produced harvestable shoots during a six-week incubation period on a shoot-regeneration medium under a 16-h photoperiod. The organogenic competence of different explant sizes and sources from young watermelon seedlings was determined by calculating the percentage of various cotyledon explants that produced adventitious shoots (Compton 2000). Although about 52% of explants prepared from the proximal region of cotyledons formed shoots, only about 6% of distal explants did so. Shoot formation was limited to the proximal end of basal explants but was not restricted to any specific region on distal ones. This study indicated that watermelon lines which respond poorly to in vitro procedures may have fewer cells competent for shoot regeneration, requiring special care during explant preparation.

### 8.6.5.3 Embryo Rescue

**Cucumis**

To overcome post-zygotic barriers to interspecific hybridization, immature zygotic embryos can be removed from developing seeds and cultured in vitro (Figure 8.14). Recent progress, problems, and future trends in the development of embryo-rescue and ovule culture methods for *Cucumis* and their
Figure 8.14  (See color insert following page 304.) Embryo culture in the genus Cucumis (adapted from Skálová, D., Lebeda, A., and Navrátilová, B., in Progress in Cucurbit Genetics and Breeding Research, Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, A., Lebeda and H.S., Paris, eds., Olomouc, Czech Republic: Palacký University in Olomouc, pp. 415–430, 2004.); A=mother flowers (Cucumis sativus) in the glasshouse; B=isolated embryos in the test-tubes in the cultivation room; C=germinated embryo (Cucumis melo, cv. Solartur) 2 weeks after self-pollination; D=embryo (Cucumis melo) after 3 weeks; E=in vitro pollination in the cross-pollination between Cucumis sativus (female flowers) and Cucumis melo (male flowers); F=germinated pollen tubes (Cucumis melo, colored FDA).

utilization in interspecific hybridization were summarized above and discussed in detail by Chen and Adelberg (2000) and Skálová, Lebeda, and Navrátilová (2004). Key developments in the utilization of embryo rescue for interspecific hybridization in the genus Cucumis are summarized in Table 8.14.

**Cucurbita**

Several attempts have been made to produce interspecific Cucurbita hybrids with the assistance of embryo rescue, following techniques reviewed by Rakoczy-Trojanowska and Malepszy (1989a) and Rakoczy-Trojanowska, Płader, and Malepszy (1992). By using this strategy, one could expect novel traits to be introgressed or the generation of hybrid genotypes with entirely new characteristics. Limited success in obtaining fully developed seeds that germinated and produced viable plants has been reported for a few interspecific combinations, including C. andreana × C. ficifolia (Whitaker 1954), C. lundelliana × C. moschata (Whitaker 1959), and C. maxima × C. ecuadorensis (Paran, Shifriss, and Raccah 1989). Embryo rescue was employed in transferring resistance to powdery mildew, CMV, and WMV from C. ecuadorensis to C. pepo (de Vaulx and Pirat 1980). However, the successful rescue of interspecific Cucurbita hybrid embryos has also been reported for many other combinations, including C. pepo × C. moschata (Wall 1954; de Oliveira et al. 2003;
Table 8.14  Key Examples of the Use of Embryo Rescue or Embryos Production in Conjunction with Interspecific Hybridization in *Cucumis*

<table>
<thead>
<tr>
<th>Species</th>
<th>Results</th>
<th>Reference</th>
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<tbody>
<tr>
<td><em>C. sativus</em> × <em>C. melo</em></td>
<td>Embryos (5 callus formation, 2 flowering plants)</td>
<td>Lebeda, Kristková, and Kubaláková (1996), Lebeda et al. (1997, 1999); Ondrej, Navrátilová, and Lebeda (2001)</td>
</tr>
<tr>
<td><em>C. sativus</em> × <em>C. hystrix</em></td>
<td>Plants 2n=19 (sterile), <em>Cucumis × hystrix</em> 2n=38 (amphidiploid, fertile)</td>
<td>Chen and Staub (1996), Chen and Staub (2000); Chen et al. (2003a,b)</td>
</tr>
<tr>
<td><em>C. melo</em> × <em>C. africanus</em></td>
<td>Fruit set with embryos</td>
<td>Custers, den Nijs, and Riepma (1981)</td>
</tr>
<tr>
<td><em>C. melo</em> × <em>C. melo</em></td>
<td>Embryos, plants</td>
<td>Wehner, Cade, and Locy (1990)</td>
</tr>
<tr>
<td><em>C. melo</em> × <em>C. zeyheri</em></td>
<td>Fertile, some embryos</td>
<td>Custers and den Nijs (1986)</td>
</tr>
<tr>
<td><em>C. melo</em> × <em>C. metuliferus</em></td>
<td>Pollen tubes penetrated into the upper part of the style</td>
<td>Wehner, Cade, and Locy (1990)</td>
</tr>
<tr>
<td><em>C. melo</em> × <em>C. ficifolius</em></td>
<td>Fruit set (irradiation), high irradiation dose increases fruit set</td>
<td>Beharav and Cohen (1994)</td>
</tr>
<tr>
<td><em>C. hystrix</em> × <em>C. sativus</em></td>
<td>Fertile plants (4n)</td>
<td>Kho, Franken, and den Nijs (1981)</td>
</tr>
<tr>
<td><em>C. sagittatus</em> × <em>C. melo</em></td>
<td>Embryos</td>
<td>Chen et al. (1998)</td>
</tr>
</tbody>
</table>


8.6.5.4 Protoplast Culture and Somatic-Cell Fusion

In melon, protoplasts were first prepared and cultured from leaves (Moreno et al. 1986), cotyledons (Roig et al. 1986b; Fellner and Lebeda 1998), and hypocotyls (Fellner and Lebeda 1998). Since that time, there have been successful reports of plant regeneration from cultured protoplasts (Li et al. 1990; Debeaujon and Branchard 1992; Tabei, Nishio, and Kanno 1992), but reported regeneration frequencies remained low. Clearly, efficient regeneration protocols from protoplasts are needed for protoplast culture and for somatic-cell fusion to be of practical importance (Figure 8.15 and Figure 8.16). Recent progress and future trends in protoplast culture for *Cucumis* and *Cucurbita* species were reviewed and discussed in detail by Gajdová, Lebeda, and Navrátilová (2004).

In cucumber, Burza and Malepszy (1995) described a protocol for the isolation and culture of protoplasts from embryogenic calli and embryogenic suspension cultures (ESC). From these isolated protoplasts, they observed a high degree of direct embryogenesis with up to ca. 5,000 embryo structures/g tissue. Some embryos developed into plants after six to eight weeks in culture. ESC-derived plants, when transferred into the glasshouse, flowered normally and set seed. Later,
Figure 8.15  (See color insert following page 304.) Protoplast culture of *Cucumis melo* (line MR-1) (adapted from Gajdová, J., Lebeda, A., and Navarátilová, B., In *Progress in Cucurbit Genetics and Breeding Research, Proceedings of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding*, A. Lebeda and H.S. Paris, Eds., Czech Republic: Palacký University, Olomouc, pp. 441-454, 2004.) A=mesophyll protoplasts immediately after isolation; B=viability of callus protoplasts after isolation (fluorescence staining with FDA); C=dividing hybrid cell—product of protoplasts fusion between *Cucumis melo* and *Cucumis metuliferus*, 7 days after fusion; D=viability of mesophyll protoplasts (13 days after isolation, FDA staining).

various protocols and enzyme mixtures were described for protoplast isolation from cotyledons and hypocotyls of cucumber (Fellner and Lebeda 1998).

With the culture systems described by Burza and Malepszy (1995), various attempts have been made to fuse protoplasts and create somatic hybrids. Roig et al. (1986a) attempted to fuse protoplasts of *C. melo* and *C. myriocarpus* and obtained putative hybrid plants, but hybridity was not confirmed. Yamaguchi and Shiga (1993) fused protoplasts of melon (*C. melo*) and pumpkin (interspecific hybrid *C. maxima* × *C. moschata*), and they also obtained putative somatic hybrid plants. At an early developmental stage, the regenerated plants included a chromosomal segment from *Cucurbita*, but it was evidently eliminated at a later stage of growth. Jarl, Bokelmann, and DeHaas (1995) attempted asymmetric protoplast fusion between *C. melo* and *C. sativus*, but recovered only callus. Molecular analysis of the callus showed the incorporation of genetic materials from cucumber to melon.

Cotyledonary protoplasts from an albino *C. melo* mutant were electrofused with protoplasts of *Cucumis anguria* var. *longipes* (Dabauza et al. 1998). Selection of putative somatic hybrids was based on competence of the albino cells to grow and regenerate shoots when combined with the photosynthetic wild-type cells. The ITS region of *C. anguria* ribosomal DNA was sequenced to design species-specific primers, which were used to distinguish between parental lines and fusion products by PCR amplification. All the organogenic lines characterized by this method proved to be somatic hybrids. Three of 16 selected lines produced shoots with
albino and green sectors. Eleven lines remained green, but shoots developed abnormally and did not produce roots in vitro. Two hybrid lines regenerated normal shoots, but with a limited ability to produce roots in vitro or in vivo.

Cotyledonary protoplasts from an albino C. melo mutant were also electrofused with protoplasts of C. myriocarpus (Bordas et al. 1998). Putative somatic hybrids were selected on the basis of albinism complementation and the differential behavior of protoplast-derived cells of each of the parental species in a defined sequence of culture media. Somatic hybrids were characterized at a molecular level, and the green calli recovered after fusion were shown to be interspecific hybrids. RFLP patterns that identified both the C. melo and C. myriocarpus genomes could be obtained by digestion of PCR-derived DNA fragments containing the 18S–25S ribosomal DNA...
(rDNA) spacer region with the endonuclease Sau3A. The selected calli recovered after fusion contained part of the *C. myriocarpus* genome. Because these calli also showed the RFLP melon pattern, they were clearly a combination of both parental genomes.

### 8.6.5.5 Somaclonal Variation

The selection of useful phenotypes, known as somaclonal variants, created by revealing cryptic, somatic genetic variation through *in vitro* culture or through the *de novo* generation of variation as part of the culture process became a prominent topic for research beginning in the late 1970s, in part because of successes reviewed by Larkin and Scowcroft (1981). The following paragraphs summarize efforts to document and, in one case, to exploit somaclonal variation in cucurbits.

#### Cucumis sativus

Plader et al. (1998) examined somaclonal variation in a cucumber line subjected to five different *in vitro* culture systems: (1) micropropagation (MP); (2) direct leaf-callus regeneration (DLR); (3) leaf-callus regeneration (LCR); (4) recurrent leaf-callus regeneration (RLCR); and (5) direct protoplast regeneration (DPR). They measured the frequency of new phenotypes that appeared in R₁ lines and the stability of the rDNA region by using five probes. MP was not subject to change while DLR and LCR caused only infrequent changes. The highest frequencies of change arose through DPR (90% of lines) and RLCR (43%). Tetraploids were produced only through LCR (4.7%) and RLCR (28%).

The effects of seven *in vitro* culture protocols on somaclonal variation were also compared in an inbred cucumber line by Ladyzynski, Burza, and Malepszy (2002). They regenerated plants by using the following systems: 12- and 18-month-old liquid culture of meristematic clumps (LMC 12 and 18); 10-month-old, embryogenic, cytokinin-dependent suspension (CDS); 18-month-old, embryogenic, cytokinin-dependent suspension in medium with a modified NH₄⁺/NO₃⁻ ratio (CDS 1.7); 12-month-old, embryogenic, auxin-dependent suspension (ADS); 36-month-old, embryogenic, auxin-dependent suspension in medium with a modified NH₄⁺/NO₃⁻ (ADS 1.7); and recurrent leaf-callus regeneration (RLC). Differences were noted among the various systems for ploidy levels in R₀ plants and by the segregation of new morphological traits in the R₁ generation and the germination ability of R₁ seeds. While segregating R₁ families with new phenotypes were most numerous from CDS (62.5%) and LMC18 (57.9%), less so from CDS1.7 (35.7%), the smallest number was found from LMC12 (11.1%) and RLC (3.4%). Among the new phenotypes, Ladyzynski, Burza, and Malepszy (2002) noted some truly novel traits, including ginkgo-like leaf (gll), yellow-green chlorophyll mutants (y-gc), and flowers with serrate corolla margins (smc).

In addition, tetraploid and mixoploid plants were regenerated from ADS1.7 and ADS (100%), but only tetraploids were regenerated from CDS and RLC. There were no changes of ploidy after LMC12, LMC18, or CDS1.7.

#### Cucumis melo

High frequencies of somaclonal variation among melon plants regenerated after *in vitro* culture have been reported (Moreno and Roig 1990; Ezura et al. 1992a, 1992b). Selection among somaclonal variants to identify desirable traits at the germination or seedling stage can be performed effectively as demonstrated by Ezura et al. (1995). They were able to select somaclonal variants with low-temperature germinability from melon plants regenerated via somatic embryogenesis.
Selected variants also set larger fruits than did the original plants used for somatic embryogenesis. By using those mutants as a source of parental lines, a commercial F₁ hybrid cultivar was created.

8.6.5.6 Genetic Transformation

Cucumber (Cucumis sativus)

Transgenic cucumber plants were first produced by Agrobacterium rhizogenes-mediated transformation via somatic embryogenesis (Truson, Simpson, and Shahin 1986). Subsequent reports of the production of transgenic cucumbers mediated by A. tumefaciens via somatic embryogenesis include those by Chee (1990) and Tabei et al. (1994). Transgenic cucumber plants were also obtained from hypocotyl explants inoculated with A. tumefaciens, via organogenesis (Nishibayashi, Kaneko, and Hayakawa 1996b), in experiments where acetosyringone enhanced the transformation efficiency. Recently, Rajagopalan and Perl-Treves (2005) reported an increased efficiency of transformation resulting from special explant wounding methods and selection protocols.

Biolistic transformation of cucumber has also been reported (Chee and Slighton 1992; Schulze et al. 1995). Neomycin phosphotransferase (nptII) and the reporter, glucoronidase GUS genes were used in bombarding a highly embryogenic cell-suspension culture by using a particle gun, and transgenic plants were regenerated via embryogenesis.

Improvement of virus resistance by the introduction of the CMV coat protein (CP) gene was the first example of transgenic modification of a useful trait in cucumber (Chee and Slighton 1991). Nishibayashi, Hayakawa, and Nakajima (1996a) also introduced a CMV-O CP gene into cucumber plants by using a Ti-Agrobacterium-mediated transformation. Progeny of a cross between cv. Sharp 1 and transgenic plants possessing the CP gene displayed strong resistance to cotyledonary inoculation by a CMV-Y strain; although, both the control (Sharp 1) and segregating CP(−) plants displayed foliar disease symptoms 5–6 days after CMV-Y inoculation. However, the transgenic cucumber plants displayed no direct resistance to ZYMV, yet transgenic plants did show a reduced degree of disease symptom development following a double inoculation of CMV and ZYMV.

Various chitinase-encoding genes have also been introduced into cucumber to enhance resistance to fungal pathogens (Punja and Raharjo 1996; Raharjo et al. 1996; Tabei et al. 1998). For example, Tabei et al. (1998) introduced rice chitinase cDNA (RCC2) driven by the CaMV 35S promoter into cucumber. Sixty elongated shoots were examined for the presence of the integrated RCC2 gene. Of these, 20 were tested for resistance against gray mold (Botrytis cinerea) by conidial infection; 15 exhibited a higher level of resistance than did the non-transgenic plants. The three transgenic cucumber strains exhibiting the highest resistance against B. cinerea completely inhibited disease transmission. Different patterns of gene expression were observed among the highly resistant strains. One strain inhibited appressoria formation and hyphal penetration. Another permitted hyphal penetration but restricted further hyphal invasion. These transgenic cucumber strains have been further characterized by Ishimoto et al. (2002), Koga-Ban et al. (2004).

Szwicka et al. (2002) produced transgenic cucumber plants with stable integrated constructs consisting of the cauliflower mosaic virus 35S promoter and thaumatin II cDNA, which codes for a unique protein with a sweet flavor. Inter- and intra-transformant variability in the expression of the thaumatin II gene was observed. Observed variation was independent of integrated copy number. Variation in thaumatin II protein accumulation levels in ripe fruits and a lack of correlation between protein and mRNA levels were both noted, suggesting that protein production may be controlled both during transcription and translation. Transgenic fruits accumulating thaumatin II exhibited a sweet phenotype, and a positive correlation between thaumatin accumulation levels and the intensity of perceived sweetness was reported.
A novel use for transgenic cucumber fruits was proposed by Lee et al. (2003a), who introduced the CuZnSOD cDNA (mSOD1) gene from cassava to produce superoxide dismutase (SOD) for human cosmetic applications (putative anti-aging agent). They employed Agrobacterium-mediated transformation with an ascorbate oxidase promoter that gives high levels of gene expression in fruits. Southern blot analysis confirmed that the mSOD1 gene was properly integrated into the nuclear genomes of three cucumber plants. It was highly expressed in transgenic cucumber fruits; whereas, it was expressed at a low level in transgenic leaves. SOD-specific activity (units/mg protein) in transgenic fruits was approximately three times higher than were those of non-transgenic plants, suggesting that cucumber fruits with elevated levels of SOD could serve as a functional cosmetic material.

Endogenous ethylene levels have been correlated with different floral sex phenotypes in cucumber (Perl-Treves 1999). Rajagopalan et al. (2004) recently transformed cucumber plants for overexpression of an ethylene receptor, encoded by the cucumber CS-ERS1 gene, and produced two families segregating for plants with more female flowers produced earlier in plant development. These plants also exhibited accelerated yellowing of detached leaves. This approach could ultimately lead to earlier, high-yielding cultivars.

Field tests of these transgenic cucumber have been conducted in the United States (29 records) (Anonymous 2005). Most of these reports involve plants transformed with CP genes from CMV, ZYMV, and WMV-2, but they also include genes that enhance salt tolerance.

**Melon (Cucumis melo)**

Various Agrobacterium-mediated and particle-bombardment transformation procedures have reported in melon (reviewed by Guis et al. 1998), and Agrobacterium-mediated methods have been practically used for producing transgenic melon plants. The first successful Agrobacterium-mediated transformation event produced conferred kanamycin resistance in melon by introducing the NPT-II gene (Fang and Grumet 1990). The introduction and expression of the reporter gene, GUS, has also been noted (Dong et al. 1991; Valles and Lasa 1994).

However, from those early studies, two problems in melon transformation became clear. First, many of the transgenic melon plants were found to be tetraploid. In response, Guis et al. (2000) developed a simple and efficient regeneration system, facilitating the production of diploid transformants at a high rate.

A second problem was the low overall frequency of transformation events because of the presence of escapes (Guis et al. 1998). In initial studies, transgenic plants were generated via adventitious-shoot organogenesis. To reduce the frequency of escapes, an alternative regeneration system was needed. Several groups reported the production of somatic embryos from melon cell-suspension cultures (Oridate and Oosawa 1986). Although somatic embryogenesis can sometimes lead to problems such as abnormal embryos and hyperhydricity, the liquid-culture system is considered useful for efficient selection of transformed tissues as whole explants absorb antibiotics more easily when suspended in liquid media than when cultured on solidified media (Ezura, unpubl. results). Recently, Akasaka-Kennedy, Tomita, and Ezura (2004) published an efficient transformation and plant regeneration system via somatic embryogenesis for two melon types of *C. melo* subsp. *melo* Group Cantalupensis (i.e., vars. *cantalupensis* and *reticulata*). By following their protocol, transgenic plants were successfully produced at a rate greater than 2.3%, sufficient for practical use.

Other approaches to improve the frequency of transformation events have been proposed. Galperin (2003) screened melon genotypes for ease of transformation and regeneration and noted variation between 0.4 and 1.5 transgenic shoots per explant. Ezura et al. (2000) noted that during Agrobacterium inoculation, explants produced ethylene. By adding an ethylene biosynthesis inhibitor, AVG, to the co-cultivation medium, they reduced ethylene production from the explants.
resulting in increased transformation efficiency. And Atares et al. (2004) recently described a protocol that employs polyethylene glycol to increase direct (non-Agrobacterium mediated) transformation efficiency of melon protoplasts.

The first useful trait in melon modified by genetic transformation was virus resistance conferred by the expression of viral CP genes. A CP gene from CMV was introduced into a commercial melon variety (Yoshioka et al. 1992; Gonsalves et al. 1994), and the transgenic plants showed improved resistance to CMV (Yoshioka et al. 1993; Gonsalves et al. 1994). This CP-mediated strategy has been applied to improve the resistance to other cucurbit viruses in melon. Transgenic melon plants with a CP gene from ZYMV showed resistance to ZYMV and other potyviruses (Fang and Grumet 1993). Transgenic melon plants over-expressing both CPs of ZYMV and WMV-2 were produced and subjected to extensive field trials (Clough and Hamm 1995). A significant reduction in disease incidence in the transgenic lines was observed. In addition to CP-mediated virus resistance, ribozyme-mediated resistance has been used to produce virus-resistant melons. Transgenic plants with a polyrribosome construct showed resistance to a natural CMV infection (Plages 1997) and to WMV (Huttner et al. 2001). Gaba, Zelcher, and Gal-On (2004) has summarized the results from many field trials of these transgenic lines with virus resistance.

Recently, three genes, Vat, Nsv, and Fom-2, which are responsible for major disease resistance in melon, have been isolated by map-based cloning. Vat confers a double resistance, resistance to plant colonization by the important pest, melon/cotton aphid, Aphis gossypii, and to virus transmission by A. gossypii (Paquet et al. 2004). Functional validation was obtained by the stable transformation of susceptible melons with Vat. Nsv confers resistance to MNSV (Garcia-Mas et al. 2004), a single-stranded RNA virus that infects cucurbits grown under glass. Transformation for disease resistance has not been restricted to viruses and their vectors. The third gene, Fom-2, confers resistance to races 0 and 1 of soil-borne fungal pathogen, Fusarium oxysporum f. sp. melonis (Joobeur et al. 2004), which causes significant losses in cultivated melon.

Another major fungal pathogen, Pseudoperonospora cubensis, the causal agent of downy mildew in cucurbits (i.e., cucumber, melon, watermelon, and squash), can be controlled through transgenic manipulation. The wild melon line, PI 124111F, is highly resistant to P. cubensis, through its enhanced expression of the resistance genes, Atl and At2, which encode glyoxylate aminotransferases. These enzymes are important in photorespiration. Transgenic melon plants overexpressing either At1 or At2 displayed enhanced glyoxylate aminotransferase activity and remarkable resistance against P. cubensis (Taler et al. 2004).

The use of genetic transformation to improve tolerance to abiotic stresses has also received attention. The Saccharomyces cerevisiae HAL1 gene was introduced to two melon cultivars (Bordas et al. 1997). It confers salt tolerance to yeast by increasing intracellular K+ and decreasing Na+ levels by an unknown mechanism (Rios et al. 1997). Primary transformants carrying the 35S:HAL1 construct exhibited improved rooting in a NaCl-containing medium with respect to the control, but no clear differences were observed in aboveground plant parts.

Modification of postharvest fruit characteristics has been another target of genetic engineering in melon. Postharvest physiology has been extensively studied in melon (Martinez-Madrid et al. 1999; Yang and Oetiker 1998), and key genes have been isolated and characterized. For example, the aminocyclopropane-1-carboxylic acid (ACC) oxidase gene (Cm-ACO1) encodes the ACC-oxidase enzyme, which catalyzes the conversion of ACC to ethylene (Balague et al. 1993), a critical phytohormone responsible for fruit ripening. The antisense version of Cm-ACO1 was introduced to Charentais type melon (Ayub and Guis 1996). By suppressing the expression of Cm-ACO1, ethylene production was greatly reduced in transgenic fruit, and ripening was delayed as expected. Transgenic fruits could be stored for at least two weeks without over-ripening while the wild-type fruits became decomposed under the same conditions.

As previously noted for cucumber, melon lines also display a range of sex phenotypes that are subject to ethylene control (Rudich 1990). Direct evidence of the importance of endogenous ethylene production in floral sex determination was presented by Papadopoulou et al. (2002).
They transformed the melon cultivar, Hale's Best Jumbo, with the ACS gene encoding ACC synthase, responsible for the first step in ethylene biosynthesis, and with an Arabidopsis etr1-1 gene that blocks ethylene perception. The ACS transformants showed increased ethylene production and increased femaleness, and the Etrl-1 melons exhibited phenotypes associated with ethylene insensitivity and failed to produce pistillate flowers. In practice, the use of such genes may facilitate the development of melon lines with earlier fruit production.

Field tests of transgenic melons have been conducted (143 records; Anonymous 2005). The reports have predominantly been of transgenic plants exhibiting virus resistance through the expression of CP genes, but they have also included transformants exhibiting altered fruit ripening via the expression of S-adenosylmethione hydrolase from E. coli and those exhibiting male sterility from the expression of phosphinothricin acetyl transferase or glucanase.

Squash (Cucurbita maxima and C. pepo)

The production of transformants in Cucurbita species has been limited by the development of appropriate tissue-culture regeneration protocols. Juretic and Jelaska (1991) reported the induction of embryogenic callus derived from summer squash (C. pepo) hypocotyl segments, and Chee (1991) reported that summer squash cultivars were regenerated via somatic embryos produced from calli derived from shoot apices. Cucurbita pepo cultivars have also been regenerated via somatic embryogenesis from cotyledonal explants (Gonsalves, Xue, and Gonsalves 1995). An efficient regeneration protocol using cotyledonal explants of winter squash (C. maxima) has also been developed (Lee, Chung, and Ezura 2002, 2003b). Squash regeneration via organogenesis has also recently been reported (Anathakrishnan et al. 2003).

Methods to produce transformants of Cucurbita containing viral CP genes via Agrobacterium-mediated transfer have been described by Pang et al. (2000) based on systems initially developed by Tricoli et al. (1995). By using various protocols, both those published in the scientific literature and others found only in patent documentation, transgenic Cucurbita lines exhibiting CP-mediated resistance to CMV, ZYMV, and WMV-2 have all been produced. Field tests of these transgenic squash have been conducted (68 records; Anonymous 2005). There are two deregulated transgenic squash lines, ZW-20 and CWZ-3, in the United States permitted for commercial sale. The transgenic line ZW-20 exhibits resistance to WMV-2 and ZYMV while CWZ-3 is resistant to CMV, WMV-2, and ZYMV.

Watermelon (Citrullus lanatus)

Genetic transformation in watermelon was first reported by Choi, Soh, and Kim (1994) who used an Agrobacterium-mediated protocol to introduce a 35S:GUS fusion construct to yield transformants created by direct shoot organogenesis from cotyledon explants. Reed et al. (2001) described an in vitro method to select transformants via the production of phosphomannose isomerase (PMI). PMI catalyzes the reversible interconversion of mannose 6-phosphate and fructose-6-phosphate. Plant cells lacking this enzyme are incapable of surviving on synthetic media containing only mannose as a carbon source. Selection by PMI resulted in an average transformation frequency of 2% (Reed et al. 2001).

A more efficient and reproducible Agrobacterium-mediated protocol suitable for transferring interesting genes into different watermelon cultivars was reported by Ellul et al. (2003). Their protocol resulted in transformation efficiencies ranging from 2.8 to 5.3% depending on cultivar. Using this protocol, the HALI gene (as described in the above melon section) related to salt tolerance was introduced into watermelon cultivars. Transgenic plants expressing the HALI gene performed better than non-transformed plants did under salt-stress conditions.

Novel techniques for introducing foreign genes into intact plant organs have also been reported for watermelon. Chen et al. (1998) injected a phosphorylated GUS plasmid containing
the CaMV35S promoter and DNA from a *Cucurbita* cultivar resistant to *Fusarium oxysporum* f.sp. *niveum* into the ovaries of the F1 hybrid watermelon, Pink Orchid, after hand pollination. Transformation events were confirmed through staining, Southern blot analysis, and RAPD analysis. A transformation frequency of 0.5% (i.e., 10 of 200 transformants) yielded a low number of *Fusarium* plants. More recently, Hema, Prasad, and Vani (2004) introduced a chimeric *GUS* gene following electroporation of zygotic embryos and nodal buds. The stable integration of this chimeric gene in progeny was confirmed by dot and Southern blot analyses.

Genetic transformation of a wild relative of watermelon, *Citrullus colocynthis*, has also been reported (Dabauza, Bordas, and Salvador 1997). *C. colocynthis* exhibits resistance to various diseases, including to *Yellow-stunting disorder*, *ZYMV*, *CMV*, and *WMV-2*. A *GUS* gene was introduced into *C. colocynthis* by *Agrobacterium*-mediated protocol, and the transformation efficiency was 14.2%, which was considerably higher than that reported for cultivated types.

Using the above protocols, transgenic watermelon lines have been mainly generated by commercial companies and have been field tested in the United States and Europe (thirteen records; Anonymous 2005). Most of the transgenic watermelon lines field tested were for virus resistance conferred by the expression of CP genes from *CMV*, *ZYMV*, and *WMV-2*. However, no transgenic watermelon has been yet released for commercial use.

### 8.7 CONCLUSIONS

The Cucurbitaceae is a remarkable plant family; the fruits of wild and cultivated cucurbits display enormous variation in size, shape, and color patterns (Rubatzky and Yamaguchi 1997). A few cucurbit genera (e.g., *Cucumis, Cucurbita*, and *Citrullus*) are widely cultivated and have extraordinary human importance for many reasons reviewed (economic, aesthetic, cultural, medicinal, and botanical). Cucurbits are among the oldest domesticated plants, emerging as some of the first vegetable crops 8,000–12,000 years ago, both in the Old and New Worlds (Hancock 2004). However, the current state of knowledge about the origins, domestication processes, post-domestication diversification, and geographic expansion of important cucurbits is still relatively limited. Gaps in current understanding present many research opportunities to collect additional historical, geographical, biological, and genetical evidence related to the domestication and diversification of these crops.

The family Cucurbitaceae is taxonomically well-defined and treated as the only member of the monotypic order, Cucurbitales. Its putative relatives are members of the Begoniaceae and Datisca-ceae in the Begoniales (Jeffrey 1990). About 90% of cucurbit species occur in three tropical regions: Africa (south of the Sahara, including Madagascar), Central and South America, and southeast Asia and Malaysia. There are about 118 extant genera and 825 species (Jeffrey 1990, 2001). From the perspective of life form, the family is represented by annual and perennial herbs, semi-shrubs, aculeateous shrubs, and a few rare succulent trees. Trailing or climbing vine growth with nodal branching is typical of many species. Recent taxonomic treatments of the family build upon traditional morphological and anatomical studies by incorporating evidence from biochemistry, cytology, cytogenetics, molecular genetics, crossing barriers, and coevolution with insects and pathogens in delimiting taxa (Bates, Robinson, and Jeffrey 1990; Kirkbride 1993). Since 1990, comprehensive biosystematic and evolutionary monographs and/or detailed studies have been published for the genera *Cucumis* (Kirkbride 1993), *Cucurbita* (Merrick 1995; Sanjur et al. 2002) and *Citrullus* (Jarret and Newman 2000; Levi et al. 2000), and an overview of the classification of cultivated cucurbits was provided by Jeffrey (2001). However, careful comprehensive studies to elaborate taxonomic and phylogenetic relationships among most wild and cultivated cucurbits are still required, and information about biogeography and ecobiology of wild relatives is still rather limited.
Germplasm collections of cucurbits are among the oldest and the most extensive of horticultural plant germplasm collections. Recent information about the holdings of the world’s largest collections of *Citrullus*, *Cucumis*, and *Cucurbita* germplasm was summarized as part of the “The State of the World’s Plant Genetic Resources for Food and Agriculture” (FAO 1998). The world’s largest cucurbits collections are represent by the U.S. National Plant Germplasm System and the Vavilov Institute in Russia. Among European countries, research and long-term conservation of cucurbits germplasm is coordinated by the Working Group on Cucurbits (Diez, Pico, and Nuez 2002), which operates under the aegis of The European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR). In the areas of acquisition and exploration, since the 1980s, considerable effort has been directed toward expanding the diversity of *ex situ* germplasm collections by collecting wild, weedy, and landrace populations of cucurbits from their centers of diversity. For selected species such as crops in the genus *Cucurbita*, an excellent summary of exploration efforts has been published (Andres 2000); however, no analogous overview has been published for other important cucurbit genera. This summary of published exploration reports (Table 8.6) could serve as a starting point in the development of such overviews. An important topic related to the collection of cucurbits from traditional agroecosystems is the investigation of *in situ* gene flow and delimitation of populations. The most significant progress on this topic has been made with New World *Cucurbita* spp. (cf. Wilson 1990).

Substantial progress has been achieved in seed regeneration and maintenance protocols of cucurbits from both the perspective of controlled pollination through the manipulation of insects and from that of seed physiology. In addition to seed storage, tissue-culture methods for cucurbits are relatively well developed (see Molina and Nuez 1996), and pollen-storage methods, primarily for *Citrullus*, are being developed (Sugiyama, Morishita and Nishino 2002b).

Germplasm characterization can be considered a crucial component of effective germplasm management. Without accurate characterization, it is impossible to establish or verify taxonomic identity. This is not only important for meeting the germplasm needs of researcher, but it is also for efficient management of collections (e.g., fingerprinting for quality assurance, identification of duplicates, and gaps in collections) (Bretting and Widrlechner 1995). During the last two decades, diverse techniques to assess highly heritable phenotypic features (morphological, anatomical, biochemical, pest and pathogen reactions, polymorphic proteins) have been employed to characterize cucurbit germplasm, often in concert with analyses of polymorphic DNA markers. Nevertheless, in many germplasm collections, basic characterization data are incomplete, limiting the utility of the collections.

Given the importance of germplasm characterization, it is surprising that only a single internationally approved descriptor list for cucurbits has been published, one for *C. melo* (IPGRI 2003). The development of international descriptor lists for other important cucurbits has been identified as a crucial task (Diez, Picó, and Nuez 2002). Recently, however, detailed descriptor lists (*Cucumis* and *Cucurbita* spp.) have been developed for national gene bank collections (Křístková et al. 2003, 2005).

During the last two decades, considerable research has been directed toward the exploitation of wild relatives in cucurbit breeding (Robinson and Decker-Walters 1997). Closely related to this topic, various authors have applied the concept of gene pools (sensu Harlan and de Wet 1971) to cucurbits. In cucurbit crop species, genetic diversity analysis has been rigorously applied primarily to *C. sativus* and *C. melo* to determine the nature and structure of their gene pools and to examine hypotheses that may provide insights into the nature of their domestication (Bates and Robinson 1995). These gene pools are now relatively well described in relation to their evolution, migration, and differentiation (e.g., Raamsdonk, den Nijs, and Jongerius 1989; Horejsi and Staub 1999). The concept of gene pools is also rather well elaborated for *Cucurbita* spp. (e.g., Lira-Saade 1995). Species of the genus *Citrullus* share a common chromosome number, and all its taxa are cross-compatible (Bates and Robinson 1995), indicative either of a single primary gene pool or of a secondary gene pool encompassing two poorly differentiated primary pools.
The development of polymorphic protein and DNA markers and their use in germplasm characterization created fertile ground for application of genetic mapping and genomic technologies. Genetic mapping has progressed quickly for *C. sativus* and *C. melo*, but lags for other cucurbit genera. Genetic diversity analysis and map construction (Brown and Myers 2002) is in its infancy in *Cucurbita*, and the creation of genetic maps for watermelon, *C. lanatus* var. *lanatus*, has been hampered by insufficient genetic diversity (Levi et al. 2001a, 2001c).

Cucumber linkage maps have been constructed by using combinations of molecular and phenotypic markers (Kennard et al. 1994; Serquen, Bacher, and Staub 1997a) and more recently with markers associated with basic cell functions (Xie, Wehner, and Conkling 2002; Xie et al. 2003) and cytoplasmic factors controlling economically important traits (Chung and Staub 2003). During the last decade, important contributions toward the development of molecular markers and genetic maps in melon (*C. melo*) have also been made (Baudracco-Arnas and Pitrat 1996; Katzir et al. 1996; Pépin et al. 2002b; Gonzalo et al. 2005). The development of linkage maps for cucumber have spurred research on quantitative trait loci (QTL) associated with fruit quality (Kennard and Havey 1995) and important yield components (e.g., earliness, sex expression, multiple lateral branching, fruit number and weight, and fruit size) (Fazio, Staub, and Stevens 2003), leading to the application of MAS (Fazio and Staub 2003). In the related area of the development of unique genetic stocks, bulk segregant analysis (BSA) (Michelmore, Paran, and Kesseli 1991), in combination with near isogenic lines (NILs) and other similar techniques, has been used in cucumber to identify markers linked to disease resistance (Horejsi, Staub, and Thomas 2000) and to study sex-expression genes (Witkowicz, Urbanczyk-Wochniak, and Przybecki 2003). NILs could be also used for the efficient introgression of economically important QTL into elite germplasm (e.g., Bernacchi et al. 1998a, 1998b).

The breeding of cucurbits has primarily focused on improving the production and quality of fruits by increasing resistance to a wide range of pathogens and diseases and by modifying plant architecture and sex expression (Robinson and Decker-Walters 1997). Toward those ends, many different selection schemes have been used depending on the objectives of the enhancement/breeding project, the inheritance patterns and heritability of the characters to be selected, the available germplasm, and the species’ selection history.

Broadening the genetic diversity is considered to be essential for the continued development of commercial cucurbit cultivars. However, genetic analysis of the U.S. National Plant Germplasm System’s cucumber collection showed reduced diversity among commercial germplasm in relation to the entire primary gene pool (~3% polymorphism in elite commercial germplasm vs. ~12% across the entire collection; Meglic and Staub 1996b), highlighting the need for the genetic enrichment of elite cucumber germplasm to ensure continued potential for plant improvement (Staub et al. 2002).

To this end, considerable progress has been made in the areas of germplasm enhancement and introduction of novel traits into various cultivated cucurbits. This typically involves the incorporation of genes from exotic accessions into a more elite germplasm pool (Shetty and Wehner 2002). This strategy has resulted in important public germplasm releases in cucumber (Walters and Wehner 1997), melon (Thomas 1986), and squash (Mutschler and Pearson 1987). A crucial step in the enhancement process is an evaluation of available germplasm diversity for key phenotypic characters. Exotic and wild cucurbit germplasm often includes genetic diversity absent in elite types.

Germplasm evaluation projects have often focused on horticultural and agronomic characteristics. Many horticultural evaluations have been directed toward the identification of superior genotypes among local landraces of *C. sativus*, *C. melo*, *Cucurbita* spp., and *C. lanatus* with some of the most detailed evaluations of specific traits (e.g., root growth, postharvest storage, combining ability for yield, specific yield components) conducted on *C. sativus* (Wehner, Shetty, and Clark 2000a; Wehner, Shetty, and Wilson 2000b; Shetty and Wehner 2002). The characterization of biochemical and quality traits can also contribute significantly to crop marketability. Variation in soluble solids, ascorbic acid, individual sugars, and the enzymes that control sugar production...
have received special attention, especially in *C. melo* (e.g., Burger et al. 2004), and to a lesser extent, in *C. sativus* (Robinson 1987). Substantial progress has been made in the evaluation of seed composition (especially fatty acids) in *Cucurbita pepo* (Teppner 2004), *C. foetidissima* (Scheerens et al. 1978), and *C. colocynthis* (Schafferman et al. 1998). There is also interest in the roles of cucurbitacins as vertebrate toxins, insecticides, and attractants, which has led to evaluations for cucurbitacin composition and concentration (Metcalf and Rhodes 1990).

Cucurbit germplasm collections have received considerable attention for evaluation for pathogen, pest, and abiotic stress resistance. Clearly, the discovery and incorporation of resistance genes into commercial crops are key justifications for the conservation of *ex situ* germplasm collections. Comprehensive reviews have been published to summarize extensive research on the evaluation of cucurbit germplasm for its reaction to viral pathogens, including information on the genetics of resistance (Munger 1993; Provvidenti 1993). Since the publication of those reviews, many additional germplasm evaluations to different viral pathogens have been conducted and new sources of resistance identified (e.g., Horváth 1993a; Lebeda and Křístková, 1996; Křístková and Lebeda 2000b; López-Sesé and Gómez-Guillamón 2000). In contrast, reports on the screening of cucurbit germplasm for resistance to bacterial pathogens are relatively scarce.

A diverse array of known fungal pathogens has been reported to infect cucurbits (Zitter, Hopkins, and Thomas 1996); however, of this extensive list, only a smaller number are considered as economically important. Considerable progress has been achieved on research and screening of germplasm for reaction to key pathogens such as the downy and powdery mildews. Recent progress in pathotype discrimination, cucurbit germplasm evaluation, and resistance breeding against downy mildew (*Pseudoperonospora cubensis*) was reviewed by Lebeda and Widrlechner (2003). Cucurbit powdery mildew, caused by *Podosphaera xanthii* and *Golovinomyces cichoracearum*, is a widespread foliar disease. Jahn, Munger, and McCreight (2002) published a comprehensive review of sources and genetic control of resistance to powdery mildew in cucurbits, which included some references to past germplasm evaluations. More recently, additional data were published on the reactions of *C. sativus* germplasm to inoculation by *P. xanthii* (Morishita et al. 2003; Bioc and Reitsma 2005). Results of extensive cucurbit germplasm evaluations has been published for reactions to other important foliar pathogens as well [e.g., scab (*Cladosporium cucumerinum*), anthracnose (*Colletotrichum orbiculare*), target leaf spot (*Corynespora cassiicola*), gummy stem blight (*Didymella bryoniae*)]. Of the fungal root-rot diseases, the most damaging include fusarium wilt (*Fusarium oxysporum*) and vine decline (caused by *Acremonium cucbitaeacearum* and *Monosporas canosnballus*) in *C. melo*. Research to evaluate melon germplasm to vine decline is in progress (Crosby 2001; Dias et al. 2001).

Substantial progress has been made in the study of cucurbits resistance against nematodes, insects, and mites. Thies (1996) reviewed key evaluations of cucurbit germplasm conducted to identify sources of resistance to root-knot nematodes (*Meloidogyne* spp.). Robinson (1992) comprehensively reviewed research conducted to evaluate cucurbit germplasm for reaction to insect and mite pests. However, many additional results from more recent evaluations have been published and are reviewed herein (e.g., Bohn, Kishaba, and McCreadt 1996; Simmons and McCreadt 1996; Simmons and Levi 2002).

Abiotic stresses are often as important as are pests and pathogens in limiting the production of cucurbit crops. Extensive germplasm evaluations have been conducted to identify sources of tolerance to herbicides (Staub et al. 1991) and other chemical treatments such as sulphur dusting (Perchepie et al. 2004), high temperatures and moisture deficits (Staub and Krasowska 1990; Wann 1992; Ríos Labrada, Fernández Almirall, and Casanova Galarraga 1998), and the ability to germinate under low-temperature conditions (Wehner 1984).

Transfer of economically important traits from wild cucurbit species to their cultivated counterparts continues to be one of the most important challenges facing breeders. This is most crucial in cases where resistance genes for several crop-limiting pathogens and pests have not been found within the primary gene pools such as resistance to *P. cubensis* in
C. sativus. Substantial differences in ease of interspecific hybridization have been recognized among the three major genera of cultivated Cucurbitaceae (Cucumis, Cucurbita, and Citrullus; Robinson and Decker-Walters 1997). Many techniques, including embryo and callus culture, somatic hybridization via protoplast fusion, bridge crosses, bud pollination, repeated pollination, regulation of ploidy levels, and growth regulators use, have been developed in attempts to improve the success of interspecific hybridization (e.g., Kalloo 1988; den Nijs and Custers 1990; Tatlioglu 1993; Lebeda, Křístková, and Kubaláková 1996; Lebeda et al. 1999; Chen and Adelberg 2000; Gajdová, Lebeda, and Navrátilová 2004; Skálová, Lebeda, and Navrátilová 2004). More specifically, progress in overcoming post-zygotic barriers has been achieved in Cucumis by using embryo rescue (Lebeda, Křístková, and Kubaláková 1996; Lebeda et al. 1999; Chen and Adelberg 2000; Skálová, Lebeda, and Navrátilová 2004), and both haploid and polyploid technologies (e.g., Ezura and Oosawa 1994a, 1994b) have been developed and used in C. sativus, C. melo, C. pepo, and C. lanatus. The successful rescue of interspecific Cucurbita hybrid embryos has also been reported for many combinations (Šiško, Ivančič, and Bohanec 2003). Recent progress and future trends in protoplast culture and somatic-cell fusion in Cucumis and some Cucurbita species have been reviewed and discussed in detail by Gajdová, Lebeda, and Navrátilová (2004). Despite the large body of technical protocols to help optimize wide crosses, careful biosystematic and gene-pool studies will continue to guide the selection of genotypes that increase the probability of successful interspecific hybridization (see Raamsdonk, den Nijs, and Jongerius 1989).

Genetic transformation is one of the most quickly developing and expanding technologies being harnessed in the improvement of cucurbits. To date, transgenic plants of C. sativus (Truson, Simpson, and Shahin 1986; Chee 1990), C. melo (Guis et al. 1998), Cucurbita maxima and C. pepo (Pang et al. 2000), and C. lanatus (Choi, Soh, and Kim 1994) were released. Various methodologies, including Agrobacterium rhizogenes and A. tumefaciens-mediated transformation via somatic embryogenesis and organogenesis and biolistic transformation, have been used for production of these plants. A wide range of genes, including ones coding for chitinase, conferring resistance to viral and fungal pathogens, modifying fruit ripening, and influencing many other physiological processes, have now been introduced and expressed in vivo.

The array of biotechnological tools that are being brought to bear on the improvement of cucurbits in extensive and rapidly evolving. While the next generation of such tools cannot be accurately predicted, current advancements in functional genomics and metabolomics will play key roles in their development. What can be predicted with confidence is that the successful application of today’s technologies and those yet to come (no matter how promising or broadly applicable) will continue to rely upon researchers’ sound understanding of the organism and its inherent diversity. This comprehensive review of recent cucurbit research may help provide that understanding, connecting today’s researchers with their colleagues’ work and allowing those that follow to build upon it.

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REFERENCES


de Vaulx, R. D. and Pitrat, M., Realization of the interspecific hybridization (F1 and BC1) between Cucurbita pepo and C. ecuadorensis, Cucurbits, 3, 42, 1980.


Dijkhuizen, A. and Staub, J. E., Effects of environment and genetic background on QTL affecting yield and

Dogimont, C., Leconte, L., Doebley, J., Molecular evidence and the evolution of maize,

Dirks, R. and van Buggenum, M., Regeneration of plants from mesophyll protoplasts of haploid and diploid

Doebley, J. F., Isozymic evidence and the evolution of crop plants, In

Diez, Dhaliwal, M. S., Evaluation of gherkin germplasm,


Dijkhuizen, A., Kennard, W.

CUCURBITS (CUCURBITACEAE; CUCUMIS SPP., CUCURBITA SPP., CITRULLUS SPP.)

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Edwards, M. D., Lower, R. L., and Staub, J. E., Influence of seed harvesting and handling procedures on


Ellis, M. D., Jackson, G. S., Skrdla, W. H., and Spencer, H. C., Use of honey bees for controlled interpollination


El-Mahdy, I., Metwally, E. I. and EL-Fadly, G. A., In vitro differentiation of the immature interspecific

embryo derived from the crosses between Cucurbita pepo and Cucurbita ficifolia, In Proc. 4th Nat.


Elsey, K. D., Insect resistance in the cucurbits; status and potential In Proceedings of Cucurbitaceae 89:

Evaluation and Enhancement of Cucurbit Germplasm, Thomas, C. E., Ed., USDA/ARS, Charleston, SC,


Ezura, H., Amagai, H., Kikuta, I., Kobota, M., and Oosawa, K., Selection of somaclonal variants with low-


Filov, A. I., Rukovodstvo po aprobacii selskochozajstvennych kul’tur, Tom 6, Bach’chevye kultury, Gosudarstvennoje Izdatelstvo Selskochozijajstvennoj Literatury, Moskva, Leningrad, 1954.


Fursa, T. B., and Filov, A. I., Tykvennye (arbus, tykva), In Kulturnaja Flora SSSR, 21, Kolos, Moskva, 1982.


Jagger, L. C. and Scott, G. W., Development of powdery mildew resistant cantaloup no. 45, *USDA Circ.*, 441, 6, 1937.
CUCURBITS (CUCURBITACEAE; CUCUMIS SPP., CUCURBITA SPP., CITRULLUS SPP.)


Křištíková, E., Lze křižit dýni s cuketou (It is possible to cross pumpkin with squash), Zahrada, 16, 139, 1991.


Kurtar, E. S., Sari, N., and Abak, K., Obtention of haploid embryos and plants through irradiated pollen technique in squash (Cucurbita pepo L.), Euphytica, 127, 335–344, 2002.


Ladýžynski, M., Burza, W., and Malepszy, S., Relationship between somaclonal variation and type of culture in cucumber, Euphytica, 125, 349–356, 2002.


Nishibayashi, S., Kaneko, H., and Hayakawa, T., Transformation of cucumber

Nikolova, V. and Niemirowicz-Szczytt, K., Diploidization of cucumber

Niemirowicz-Szczytt, K. and Dumas de Vaulx, R.,

Nishibayashi, S., Hayakawa, T., and Nakajima, T., CMV protection in transgenic cucumber plants with

Niemirowicz-Szczytt, K., and Dumas de Vaulx, R.,

Nienhuis, J. and Lower, R. L., The effects of fermentation and storage time on germination of cucumber seeds


Nikolova, V. and Niemirowicz-Szczytt, K., Diploidization of cucumber (Cucumis sativus L.) haploids by


Nishibayashi, S., Hayakawa, T., and Nakajima, T., CMV protection in transgenic cucumber plants with


Nishibayashi, S., Kaneko, H., and Hayakawa, T., Transformation of cucumber (Cucumis sativus L.) plants


Norton, J. D. and Granberry, D. M., Characteristics of progeny from an interspecific cross Cucumis melo with


Nuez, F., Anastasio, G., Cortés, C., Cuartero, J., Gómez-Guillamón, M. L., and Costa, J., Germplasm resources


Nuez, F., Ayuso, M. C., Molina, R. V., Costa, J., and Cuartero, J., Germplasm resources of Cucumis sativus L.


Nuez, F., Díez, M. J., Palomares, G., Ferrando, C., Cuartero, J., and Costa, J. Germplasm resources of


Nuez, F., Fernández de Córdova, P., and Díez, M. J., Collecting vegetable germplasm in the Iberian Peninsula,


Nuez, F., Fernández de Córdova, P., Valcárcel, M., Ferriol, J. V., Picó, B., and Díez, M. J., Cucurbita spp. and

Lagenaria siceraria collection at the Center for Conservation and Breeding of Agricultural Biodiversity


Nugent, P. E., Tetraploid “Planters Jumbo” melon lines C883-m6-4x and 67-m6-1004x, HortSci., 29, 48–49,

1994.


Ogita, R., Ishikawa, M., Niwata, E., and Oosawa, K., Cryopreservation of shoot primordium cultures of melon


Olczak-Woltman, H., Bękowski, M., Schollenberger, M., and Niemirowicz-Szczytt, K., Cucumber screening

for resistance to angular leaf spot, Progress in Cucurbit Genetics and Breeding Research. Proceedings

of Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Lebeda, A.


Oliver, M., García-Mas, J., Arroyo, M., Morales, M., Dolcet-Sanjuan, R., de Vicente, M. C., Gomez, H. et al.

The Spanish melon genome project: construction of a saturated genetic map, Acta Hortic., 510, 375–378,

2000.

Oliver, M., García-Mas, J., Cardús, M., Pueyo, N., López-Sesé, A. I., Arroyo, M., Gomez-Paniagua, H., Arus, P.,

and de Vicente, M. C., Construction of a reference linkage map for melon, Genome, 44, 836–845, 2001.

Oliver, M., García-Mas, J., López-Sesé, A. I., Gomez, H., and de Vicente, M. C., Towards a sturdy map of

melon (Cucumis melo L.). In Cucurbitaceae 98: Evaluation and Enhancement of Cucurbit Germplasm,


Oltouch, M. O. and Welbaum, G. E., Effect of postharvest washing and post-storage priming on viability and

vigour of six-year-old muskmelon (Cucumis melo L.) seeds from eight stages of development, Seed Sci.


Ondřej, V., Navrátilová, B., and Lebeda, A., Embryo cultures of wild and cultivated species of the genus


Ondřej, V., Navrátilová, B., and Lebeda, A., Determination of the crossing barriers in hybridization of


Suzuki, E., Studies on muskmelon

Suzuki, E., Studies on muskmelon


Wehner, T. C., McCream, J. D., Rhodes, B., and Zhang, X., Mutually beneficial cucumber expedition to the People’s Republic of China leads to continued collaboration with the United States, *Diversity*, 12(1), 13, 1996.


