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Melon Fruits: Genetic Diversity, Physiology, and Biotechnology Features

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Among Cucurbitaceae, *Cucumis melo* is one of the most important cultivated cucurbits. They are grown primarily for their fruit, which generally have a sweet aromatic flavor, with great diversity and size (50 g to 15 kg), flesh color (orange, green, white, and pink), rind color (green, yellow, white, orange, red, and gray), form (round, flat, and elongated), and dimension (4 to 200 cm). *C. melo* can be broken down into seven distinct types based on the previously discussed variations in the species. The melon fruits can be either climacteric or nonclimacteric, and as such, fruit can adhere to the stem or have an abscission layer where they will fall from the plant naturally at maturity. Traditional plant breeding of melons has been done for 100 years wherein plants were primarily developed as open-pollinated cultivars. More recently, in the past 30 years, melon improvement has been done by more traditional hybridization techniques. An improvement in germplasm is relatively slow and is limited by a restricted gene pool. Strong sexual incompatibility at the interspecific and intergeneric levels has restricted rapid development of new cultivars with high levels of disease resistance, insect resistance, flavor, and sweetness. In order to increase the rate and diversity of new traits in melon it would be advantageous to introduce new genes needed to enhance both melon productivity and melon fruit quality. This requires plant tissue and plant transformation techniques to introduce new or foreign genes into *C. melo* germplasm. In order to achieve a successful commercial application from biotechnology, a competent plant regeneration system of in vitro cultures for melon is required. More than 40 in vitro melon regeneration programs have been reported; however, regeneration of the various melon types has been highly variable and in some cases impossible. The reasons for this are still unknown, but this plays a heavy negative role on trying to use plant transformation technology to improve melon germplasm. In vitro manipulation of melon is difficult; genotypic responses to the culture method (i.e., organogenesis, somatic embryogenesis, etc.) as well as conditions for environmental and hormonal requirements for plant growth and regeneration continue to be poorly understood for developing simple in vitro procedures to culture and transform all *C. melo* genotypes. In many cases, this has to be done on an individual line basis. The present paper describes the various research findings related to successful approaches to plant regeneration and transgenic transformation of *C. melo*. It also describes potential improvement of melon to improve fruit quality characteristics and postharvest handling. Despite more than 140 transgenic melon field trials in the United States in 1996, there are still no commercial transgenic melon cultivars on the market. This may be a combination of technical or performance
Regardless, the future for improvement of melon germplasm is bright when considering the knowledge base for both techniques and gene pools potentially useable for melon improvement.

**Keywords**  cucurbit, transgenic plants, *Cucumis melo*, plant tissue culture, ripening

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IMPORTANCE OF MELON

Introduction

The Cucurbitaceae consist of mostly frost sensitive, tendril-bearing vine plants that are found in subtropical and tropical regions around the world (Robinson and Decker-Walters, 1999). Nevertheless, some species are well adapted to low temperatures and xerophytic conditions (Wien, 1997). Species in the Cucurbitaceae family are commonly known as cucurbits, and, according to their geographic origin, they also can be classified as new-world or old-world species. Two well-defined subfamilies, 8 tribes, about 120 genera and more than 800 species of plants are found in this family (Jeffrey, 1990). They are largely cultivated as vegetables, but several parts of the plants also are utilized for foodstuff. Fruits are the most commonly eaten part of the plant, but seeds, flowers, tendrils, very young shoots, and roots are also used for food. In addition, some cucurbits such as Cucurbita andreana are exploited as medicines, which have chemical compounds (cucurbitacins) with anticancer and anti-inflammatory activities (Jayaprakasam, Seeram, and Nair, 2003). In China, fruits and roots of C. melo are taken as an emetic, the leaves and seeds used to treat hematoma, and the stems to reduce hypertension. Cucurbits are utilized for other practical purposes as well, such as to store food (gourd) or to be used as a sponge (loofah) (Robinson and Decker-Walters, 1999).

The most important cultivated cucurbits, based on total production and harvestable area, around the globe, are watermelon (Citrullus lanatus Thunb.), cucumber (Cucumis sativus L.), cantaloupe and other melons (Cucumis melo L.), pumpkin (Cucurbita pepo L.), and squash (Cucurbita species) (Table 1 (Food and Agriculture Organization [FAO], 2005).

Among the major cucurbit vegetables, C. melo has one of the highest polymorphic fruit types and botanical varieties. This is a consequence of genetic diversity in this species (Mliki et al., 2001). Compared to other varieties, some melon fruits have excellent aroma, variety of flesh colors, deeper flavor, and more juice (Goldman, 2002).

Botany and Origin of Cucumis melo

Most melons are trailing indeterminate vines up to 15 m long; nevertheless, some modern cultivars with shortened internodes, bushy appearance, and concentrated-yield have been bred (McCollum et al., 1987; Paris et al., 1982, 1985, 1988). All melons are frost sensitive, but many differ in their ability to survive low- and high-temperature environments (Wien, 1997).

The main stem is almost round in shape. Stems may have some pubescence or not, but when present it is not so pronounced as in other cucurbits (Zitter, Hopkins, and Thomas, 1996). Leaves are simple, either three- or five-lobed, and borne singly at the nodes, and they may have a great variation in size, color, and shape (Kirkbride, 1993). Tendrils are borne in the leaf axis and are simple (unbranched).

Sex expression in C. melo is controlled by genetic factors, as well as by environment (Wien, 1997; Robinson and Decker-Walters, 1999). According to Wien (1997), at least four environmental factors, such as light energy, photoperiod, water supply, and temperature, have a strong influence on sex expression. Normally, physiological conditions that favor the increase of carbohydrates within the plant, such as low temperature, low nitrogen availability, short photoperiod, and high moisture accessibility, promote female sex expression (Robinson and Decker-Walters, 1999). These environmental factors affect plant hormonal balance, which in turn determine sex expression. In general, gibberelins promote male flower development, whereas auxins and ethylene induce female flower production (Karchi, 1970). Melon plants bear perfect or imperfect flowers in several combinations: perfect (hermaphroditic) flowers are capable of self-pollination, and imperfect flowers are either pistillate (female) or staminate (male) (Roy and Saran, 1990). Most melon cultivars are andromonoecious (hermaphroditic and stamine flowers present at the same plant), although monoecious (pistillate and stamate flowers) forms are found as well (Roy and Saran, 1990).

Melon fruits are generally classified as an indehiscent “pepo,” which is a modified berry, with three ovary sections or locules (Font-Quer, 1979). According to Robinson and Decker-Walters (1999), a pepo is a fleshy fruit with a leathery, nonseptate rind derived from an inferior ovary. The edible flesh is derived from the placenta or mesocarp tissue (Seymour and McGlasson, 1993). Among the different parts of a melon plant, fruits have the highest diversity in size, form, external ornamentation, and internal and external color (Kirkbride, 1993). For instance, Kirkbride (1993) and Goldman (2002) reported that fruits as short as 4 cm long (C. melo L. var. agrestis) and as long as 200 cm (C. melo L. var. flexuosus), attaining weights of between 50 g and more than 15 kg (a 300-fold variation size) are known (Naudin, 1859). Variation is also expressed in flesh color (orange, orange light or pink, green, white, or even mixture of these colors), rind color (green, yellow, white, orange, red, grey, or blend of these colors), rind texture (smooth, warty, striped, netted, rough, or combination of these textures), form (round, flattened, or elongated), and size (from 4 up to 200 cm) (Kirkbride, 1993; Goldman, 2002).

Some melon fruits (depending on cultivar) when ripe have an abscission layer at the attachment zone between the fruit and the stem, whereas others remain attached to the stem even after they are ripe (Kirkbride, 1993).

### Table 1

Production of various cucurbits in different world regions in 2004 (1,000 metric tons) (FAO, 2005)

<table>
<thead>
<tr>
<th>Region</th>
<th>Watermelon</th>
<th>Cucumber</th>
<th>Melon</th>
<th>Pumpkin &amp; squash</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asia</td>
<td>81,157</td>
<td>33,038</td>
<td>19,537</td>
<td>12,122</td>
</tr>
<tr>
<td>Europe</td>
<td>3,955</td>
<td>3,923</td>
<td>3,160</td>
<td>2,177</td>
</tr>
<tr>
<td>N&amp;C America</td>
<td>3,135</td>
<td>2,059</td>
<td>2,440</td>
<td>1,942</td>
</tr>
<tr>
<td>Africa</td>
<td>3,800</td>
<td>1,074</td>
<td>1,601</td>
<td>1,782</td>
</tr>
<tr>
<td>South America</td>
<td>1,315</td>
<td>77</td>
<td>565</td>
<td>722</td>
</tr>
<tr>
<td>World</td>
<td>93,481</td>
<td>40,190</td>
<td>27,371</td>
<td>19,016</td>
</tr>
</tbody>
</table>
Africa and Asia have been suggested as possible sites of origin (Robinson and Decker-Walters, 1999). Nevertheless, Kerje and Grum (2000) reported, based on genetic studies, crossing attempts with other Cucumis species, and world distribution of melon varieties, that the origin of melon appears to be Africa. The domestication process of melon started in Egypt over 3,000 years ago (Pangalo, 1929). From this area, melon dispersed throughout the Middle East and Asia, where a secondary domestication and diversification development may have occurred (Kerje and Grum, 2000).

Classification and Cultivars

According to Jeffrey (1990), melon classification may be listed as follows:

Class: Dicotyledoneae
Subclass: Dilleniidae
Superorder: Violanae
Order: Cucurbitales
Family: Cucurbitaceae
 Tribe: Melothrieae
Subtribe: Cucumerinae
Genus: Cucumis
Subgenus: Melo
Species: Cucumis melo

The high polymorphism of fruits in cultivated melons has led botanists to propose different infraspecific classifications. An excellent, updated and complete study on Cucumis genus was undertaken by Dr. Joseph H. Kirkbride, Jr. (1993) from the United States Department of Agriculture (USDA). His book titled “Biosystematic Monograph of the Genus Cucumis (Cucurbitaceae)” is a cornerstone in melon classification.

In 1859, the French botanist Charles Naudin using mostly living plants grown in the gardens of the Natural History Museum of Paris, proposed the first useful system of infraspecific categorization for C. melo L. Naudin (1859) subdivided this species into ten groups, which were later revised by Munger and Robinson (1991), proposing trinomial names. However, several classifications have been reported (Alefeld, 1866; Cogniaux and Harms, 1924; Pangalo, 1958; Whitaker and Davis, 1962; Filip, 1960; Grebeníčkov, 1986; Kirkbride, 1993; Pyżhenkov and Malinina, 1994; Robinson and Decker-Walters, 1999). Recently, Pitrat, Hanelt, and Hammer (2000) proposed a complete synthesis of infraspecific classification of melon. They identified the synonymous epithets used in several publications in order to propose their classification. These authors recognized 16 groups and denominated them as varieties or variety.

On the other hand, Smith and Welch (1964) and Robinson and Decker-Walters (1999) considered that Naudin’s categories are horticultural groups and not botanical varieties based in phylogeny.

Naudin’s Categories for Cucumis melo L. (Robinson and Decker-Walters 1999)

1. Cantalupensis group. Cantalope and muskmelon. Medium-size fruits with netted, warty, or scaly surface; flesh usually orange but sometimes green; flavor aromatic or musky. Fruits with abscission layer at maturity. Usually andromonoecious plants.
2. Inodorus group. Winter melons: honeydew, canary, casaba, and Crenshaw. Fruits usually larger, later in maturity, and longer keeping than those of the Cantalupensis group. Rind surface smooth or wrinkled, but not netted, flesh typically white or green and lacking a musky odor. Fruits do not detach from the peduncle when mature. Usually andromonoecious plants. However, commercial hybrid honey dew melon normally absicles when ripe.
3. Flexuosus group. Snake melon or Armenian cucumber. Fruits are very long, slender, and often ribbed. They are used when immature as an alternative to cucumber. Monoecious plants.
4. Conomon group. Makura uri and Tsuke uri (pickling melons). Small fruits with smooth, tender skin, white flesh, early maturity, and usually with little sweetness or odor. They are used for pickling, but are also eaten fresh or cooked. Andromonoecious plants.
5. Dudaim group. Pomegranate melon, chito melon, Queen Anne’s pocket melon, and mango melon. Small, round to oval fruits with white flesh and thin rind.
6. Momordica group. Phoot and snap melon. Small fruits with oval to cylindrical shape. Flesh is white or pale orange, low in sugar content. Smooth surface. Most of the cultivars are monoecious.

Alternatively, Guis et al. (1998) reported a new categorization of horticulturally important melons. These authors based their classification on a previous biosystematic monograph of the genus Cucumis (Cucurbitaceae) reported by Kirkbride in 1993, who used morphological, cytological and macro-distributional data to systematize that genus.

Guis’s Categories

1. C. melo var. cantalupensis Naud. Medium-size fruits, rounded in shape, smooth surface or warty, and often have prominent ribs and sutures, if there is netting, it is sparse. Orange-fleshed, aromatic flavor and high in sugars.
2. C. melo var. reticulatus Ser. Medium-size fruits, and netted surface. If ribs are present, they are not well marked, flesh color from green, white to red-orange. Most are sweet and have a musky odor.
3. C. melo var. saccharinus Naud. Medium-size fruits, round or oblong shape, smooth with grey tone sometimes with green spots, very sweet flesh.
4. C. melo var. inodorus Naud. Smooth or netted surface, flesh commonly white, green or orange, lacking the typical musky flavor. These fruits are usually later in maturity and longer
keeping due to reduced or no ethylene production compared to cantaloupe or reticulatus.

5. C. melo var. flexuosus Naud. Long and slender fruit, green rind, and finely wrinkled or ribbed. Green-fleshed and usually eaten as an alternative to cucumber. Low level of sugars.

6. C. melo var. conomon Mak. Small fruits, smooth surface, crisp white-fleshed. These melons ripen very rapidly and develop high sugar content but little aroma.

7. C. melo var. dudaim Naud. Small fruits, yellow rind with red streak, white to pink-fleshed.

Stepansky and Kovalski (1999) proposed an intraspecific classification of melons based on phenotypic and molecular variation. They studied a collection of 54 accessions representing diverse melon genotypes (cantaloupe, inodorus, conomon, chito, dudaim, momordica, flexuosus, agrestis, and some non-defined varieties) from more than 20 countries, building with their data a “botanical-morphological” dendrogram. Likewise, DNA polymorphism among the accessions was assessed using Inter-SSR-PCR and RAPD techniques. They concluded that the molecular phylogeny agreed, broadly, with the classification of melon into two subspecies, and it did not contradict the division into “horticultural varieties.”

Recently, Liu, Kakihara, and Kato (2004) concluded after an extensive evaluation of 72 melon accessions belonging to 6 melon varieties: cantaloupe, reticulis, inodorus, makuwa, acidulous, and saccharinus, that accessions which were previously classified in the same variety by traditional taxonomy were also located closely to each other using Principal Component Analysis (PCA) approach in 35 different morphological and physiological plant characters.

In general, both Naudin's and Guis’ categorizations have more common features than contrasting ones; therefore, both are well accepted among scientists.

Climacteric and Nonclimacteric Fruits

Fruit in general can be classified as either climacteric or nonclimacteric on the basis of their ripening pattern and autotrophic ethylene production during ripening (Tucker, 1993; Hadfield, Rose, and Bennett, 1995). Climacteric fruits, such as tomato (Lycopersicon esculentum), peach (Prunus persica), avocado (Persea americana), apple (Malus x domestica), and pear (Pyrus communis), have a respiratory burst and pronounced autotrophic ethylene production while the ripening process is proceeding. Nonclimacteric fruits, such as bell pepper (Capsicum annuum), watermelon (Citrullus lanatus), strawberry (Fragaria x ananassa), grape (Vitis vinifera), and citrus (Citrus spp.), do not show evidence of an increased ethylene evolution or respiratory rise coincident with ripening (Seymour and McGlasson, 1993). This fruit categorization might not be completely strict for all species. Within a species there could be both climacteric and nonclimacteric fruits. Usually, melon fruits were considered as a climacteric type (Seymour and McGlasson), and usually reticulatus and cantaloupe melon varieties belong to this group. However, nonclimacteric melon fruits have also been described, most of them fitting in inodorus variety (Zheng and Wolf, 2000; Péryn et al., 2002).

Therefore, it is not easy to define a set of criteria that may be used to predict the ripening-related respiratory and ethylene evolution performance of specific fruit, and then extrapolate that behavior for another fruit-related cultivar or species.

POSTHARVEST PHYSIOLOGY OF MELON

Physiological Changes During Ripening

To achieve a typical melon fruit growth pattern, pollination, satisfactory double fertilization, and a normal development of the ovules have to take place (Wien, 1997). Fruit growth patterns among melon cultivars can be similar or quite diverse. In 1971, Pratt reported that both the “Honey Dew” and “cantaloupe” types reached half of their total fruit growth almost at the same time (15 and 20 days after anthesis); however, the Honey Dew melon attained four times as much as size fruit than cantaloupe. Likewise, McCollum, Cantliffe, and Paris (1987) described a comparable fruit growth in two melon genotypes (NY and D26) for the first 14 days after anthesis, but from 21 days after anthesis to full slip, NY had greater fresh weight than did D26 fruits. McCollum, Huber, and Cantliffe (1988) measured fruit growth in “Galía” and “Noy Yizre’el” muskmelon cultivars, and reported that both fruits had sigmoid growth curves; however, differences between the cultivars were apparent; that is, Galia fruits were larger than Noy Yizre’el fruits at each stage of development and continued to grow until the time of abscission. Lester (1998b) confirmed that Honey Dew and cantaloupe fruits exhibit a sigmoidal growth pattern, but the diurnal rhythm of growth is continuous, unlike apple, pear, cherry (Prunus avium), and so forth, which have expansion during the night and shrinkage during the day.

Due to genetic diversity, melon fruits have a wide variation in ripening behavior. Fruits belonging to the reticulatus and cantaloupe varieties have a quick climacterium at, or close to the time of fruit maturity and abscission, although the abscission process is absent in some muskmelon (reticulatus) varieties (Sakata and Sugiymama, 2002). On the other hand, inodorus and saccharinus type fruits may have the climacteric process extended up to several days or it may be absent (Miccoliis and Saltveit, 1991; Aggelis, John, and Grierson, 1997; Liu, Kakihara, and Kato, 2004).

The moment of fruit maturation, as well as the beginning of fruit ripening, depends on the melon variety (Liu, Kakihara, and Kato, 2004). In reticulatus and cantaloupe varieties the abscission characteristic is one of the most practical standards to estimate harvest maturity (Pratt, Goesch, and Martin, 1977; Larrigaudiere, Guillen, and Vendrell, 1995). Other indexes of muskmelon harvest maturity include fruit color and appearance of the netted pattern. A muskmelon fruit color chart has been prepared for Galia, which categorized six different levels of maturity: 1, very dark green; 2, green; 3, light yellow with
some green areas; 4, light yellow; 5, yellow; and 6, dark yellow to orange peel (Fallik et al., 2001). Conversely, in those melon varieties, such as *inodorus*, *flexuosus*, and *saccharinus*, where an abscission layer is not formed, other characteristics are used to assess harvest maturity. For example, according to Portela and Cantwell (1998), at a commercial melon production level a variety of subtle changes in external color (green to white), peel texture (hairy to smooth), aroma at the blossom end (none to detectable), and fruit density (low to high) are used in order to assess the harvest maturity point.

Fruit ripening is a genetically determined event that involves a series of changes in color, texture, and flavor. Flavor is a multifaceted human perception, which involves taste and aroma (Shewfelt, 1993). According to Tucker (1993), fruit flavor depends on the complex interaction of sugars, organic acids, phenolics, and a wide variety of volatile compounds. In general, the quality of melon fruit is mostly associated with both an elevated sugar level and an excellent flavor in mesocarpic tissue (McCollum, Huber, and Cantliffe, 1988; Shewfelt 1993; Wylie et al., 1995). In netted melons, final fruit quality is also influenced by shading of the melon plant and the calcium supply (Nishizawa et al., 2000; Nishizawa, Kobayashi, and Aikawa, 2004). Pratt, Goeschl, and Martin (1977) reported that in California, for Honey Dew melon, a minimum of 10% soluble solids is legally required for market, but high-quality melons can reach a soluble solids content as high as 17% (Bianco and Pratt, 1977; Pratt, Goeschl, and Martin, 1977). In all varieties studied, ethylene production is low in preclimacteric fruit but increases considerably during the climacteric. Kendall and Ng (1988) measured ethylene from two netted (*reticulatus* variety) and three non-netted (*Casaba-type* *inodorus* variety) muskmelon cultivars and their hybrids immediately after harvest and found that the climacteric muskmelon fruit synthesized considerable quantities of ethylene at or close to harvest. Conversely, the nonclimacteric fruits did not produce ethylene until as late as 20 days after harvest. Hybrids were generally intermediate to the parental lines in rate and time of ethylene production. These results suggested that ethylene production in *C. melo* fruit is regulated by both genetic and developmental factors.

Because netted fruit melons produce ethylene during ripening, they do not require exogenous ethylene application after harvest (Pratt, 1971). However, inodorus fruit types may require exogenous ethylene application after harvest, in order to obtain a more uniform and rapid ripening, as well as better development of color, wax, and aroma (Gull, 1988; Suslow, Cantwell, and Mitchell, 2001). Likewise, *inodorus* fruit types must already be harvested when they have an acceptable soluble solids content, because melon fruits generally do not increase their sugar content after harvest (Bianco and Pratt, 1977).

In general, orange- or green-fleshed and netted rind fruit melons produce higher amounts of ethylene than green- or white-fleshed and smooth rind fruits (Zheng and Wolff, 2000; Liu, Kakihara, and Kato, 2004). However, exceptions to this generalization may be found in netted melons. Shiomi et al. (1999) measured the ethylene biosynthetic capacity in two netted cultivars, “Earl’s Favourite” and “Andes,” and found that ethylene production in Earl’s Favourite fruit remained low even at their commercial harvest maturity stage, whereas Andes fruit exhibited a typical climacteric pattern with a high ethylene production. They concluded that the Earl’s Favourite fruit used in that experiment behaved like a nonclimacteric fruit.

Using 63 different cultivars from eight market types of melon, belonging to *cantaloupe* and *inodorus* varieties, Zheng and Wolff (2000) were able to demonstrate a significant correlation between RFLP polymorphisms and ethylene production in the fruit. These RFLPs were associated with flesh color, rind texture, and postharvest decay characteristics in the melon genotypes examined. Low ethylene production and green- and white-flesh color were associated with the presence of a putative RFLP, MEL1 allele *Ao* (15 kb), whereas high ethylene production and orange-flesh color were associated with the allele *Bo* (8.5 kb) in the homozygous condition. Some melon cultivars, such as “Honey brew” and “HD Green flesh,” did not accumulate detectable ethylene. Likewise, Périn et al. (2002) reported that in the nonabscission melon fruit PI 161375, exogenous ethylene failed to stimulate abscission, loss of firmness, ethylene production, and expression of ethylene-inducible genes. These authors obtained a recombinant population of Charentais X PI 161375 inbred lines segregating for fruit abscission and ethylene production. Genetic analysis showed that both characters are controlled.
by two independent loci. They concluded that the nonclimacteric phenotype in fruit tissues was attributable to ethylene insensitivity conferred by the recessive allelic forms from PI 161375.

Studies with transgenic melon producing about 1% ethylene of wild-type melons have revealed that there are ethylene-dependent and ethylene-independent biochemical and physiological pathways during melon fruit ripening (Pech et al., 1999; Hadfield et al., 2000; Silva et al., 2004).

**Biochemical Changes During Ripening**

**Introduction**

Worldwide trade in melon has been limited by its highly perishable nature, sensitivity to extremes of temperature, and physical injury of the fruit due to bruising. Even though, controlled atmosphere conditions have been defined (3% O2 and 10% CO2 at 7°C) (Lester and Shellie, 2002; Shellie and Lester, 2002) for storage or shipping, they offer only moderate benefits for melons under most conditions. With extended transit times (1-28 d), naturally ripening melons are reported to benefit from delayed ripening, reduced respiration, and inhibition of molds and decay. Elevated CO2 at 10%–20% is tolerated but will cause effervescence in the fruit flesh, which, nevertheless, is lost on transfer to air. Low O2 (<1%) or high CO2 (>20%) will cause impaired ripening, off-flavors and odors, and other defects. Chilling injury typically occurs after storage at temperatures <7°C for several days. Sensitivity to chilling injury decreases as melon maturity and ripeness increase. Disease is generally not an important source of postharvest loss in comparison with physical injury.

Melon fruits have a wide variation in ripening behavior probably due to genetic diversity. Fruits belonging to the reticulatus and cantaloupenis varieties have a brief climacterium by the time of fruit maturity and abscission; although the abscission process is absent in some muskmelon (reticulatus) varieties (Sakata and Sugiyama, 2002). On the other hand, fruits from the inodorus and saccharinus varieties may have the climacteric process extended for several additional days or it may be absent altogether (Miccolis and Saltveit, 1991; Aggelis, John, and Grierson, 1997; Liu, Kakihara, and Kato, 2004). Shellie and Saltveit (1993) reported that the respiratory climacteric was observed in harvested but not in unharvested “Caravelle,” “Mission” and “Explorer” netted muskmelons, and this evidence led them to suggest that the climacterium might be an artifact of harvest and not a natural phenomenon associated with ripening of climacteric fruit. However, in contrast to those results, Hadfield, Rose, and Bennett (1995) described that “Charentais” melon attached to the plant exhibited a respiratory climacteric during ripening that is equal in magnitude to the respiratory climacteric of harvested melons from the same cultivar. Later on, Bower et al. (2002) using an antisense ACC oxidase melon line, found that the concurrent rise in respiration on climacteric melon fruits was either eliminated or significantly reduced. One possibility to reconcile these contrasting results is that there are differences in melon physiology among cultivars, which leads to variation in respiratory behavior in unharvested and harvested fruit. These differences, and others related to the levels of ethylene in attached compared to detached fruit (Shellie and Saltveit, 1993; Hadfield, Rose, and Bennett, 1995; Bower et al., 2002), only indicate the need for more detailed studies on melon postharvest physiology.

Major biochemical changes take place in fruit during maturation and ripening (Jiang and Fu, 2000; Giovannoni, 2001). The melon fruit ripening process requires a high metabolic activity, that is, synthesis and/or degradation of new structural, soluble and enzymatic proteins, novel mRNAs, changes in plant hormones levels, and DNA transcription, as well as accumulation of original pigments, organic acids and sugars, and the release of volatile compounds (Bianco and Pratt 1977; Larrigaudiere, Guillen, and Vendrell, 1995; Miccolis and Saltveit 1995; Dunlap, Slovin, and Cohen, 1996; Guillaín et al., 1998; Aggelis, John, and Grierson, 1997; Sato-Nara et al., 1999; Flores, BenAmor, et al., 2001; Villanueva et al., 2004). All these anabolic and catabolic events need both energy and a carbon- nitrogen-framework for building blocks, which are supplied via respiration. The two most important respiratory substrates found in melon fruit are sugars and organic acids (Seymour and McGlasson, 1993). Likewise, ethylene is the major plant hormone involved in the melon fruit ripening process (Bianco and Pratt, 1977; Lelièvre et al., 1997; Sato-Nara et al., 1999).

**Carbohydrate Metabolism**

Sweetness is the most important edible quality attribute of ripe melon fruits (Yamaguchi et al., 1977; Lester and Shellie, 1992; Artes et al., 1993). Sucrose, glucose, and fructose are the major sugars found in the mesocarp of ripe melon fruits. High levels of sucrose attribute fruit sweetness in melon (McCollum, Huber, and Cantliiffe, 1988; Hubbard, Pharr, and Huber, 1990; Burger et al., 2003; Villanueva et al., 2004). Sucrose accumulation occurs in sweet melon genotypes as a result of a decrease in soluble acid invertase (AI) and a concomitant dramatic increase in sucrose phosphate synthase (SPS) activities (Lester, Saucedo-Arias, and Gomez-Lim, 2001).

Muskmelon fruit do not store starch as some other fruits do (i.e., mango [Mangifera indica], apple, and banana [Musa spp.]) (Lester and Dunlap, 1985), therefore the fruit requires a constant supply of translocated photoassimilate from the leaf canopy for sugar use and accumulation during development and ripening (Pratt, 1971; Hubbard et al., 1989; Hubbard, Pharr, and Huber, 1990). Consequently, any factor that impairs photoassimilate translocation during fruit development will reduce sucrose content (Hubbard, Pharr, and Huber, 1990). For example, presence of viral infections, such as cucumber mosaic virus, in melon plants causes an alteration in carbon metabolism in source leaves and in resource partitioning among the various plant organs, because there is an increase in respiration and a decrease in net photosynthetic rate in infected leaves (Shalitin and Wolf, 2000; Shalitin et al., 2002).
In *C. melo*, sucrose is not the only translocated photoassimilate carbohydrate, because galactosyl-sucrose oligosaccharides raffinose and stachyose can be found in the phloem (Mitchell, Gadus, and Madore, 1992; Chrost and Schmitz, 1997; Gao et al., 1999; Feusi et al., 1999; Volk, et al., 2003). Mitchell, Gadus, and Madore, 1992; and Mitchell and Madore (1992) reported maximal amounts of sucrose (80 mM), stachyose (50 mM), and raffinose (10 mM) in sap phloem measurements.

Sugar continues to accumulate during fruit development (Pratt, 1971; Pratt, Goeschl, and Martin, 1977), beginning at early fruit enlargement and reaching its maximum at full maturity (McCollum, Huber, and Cantliffe, 1988; Seymour and McGlasson, 1993; Burger et al., 2002, 2003). The trait for sugar accumulation is controlled by a single recessive gene called *suc* (Burger et al., 2003). Therefore, sucrose accumulation is controlled through several hormones and enzymes, as well as through compartmentation processes (McCollum, Huber, and Cantliffe, 1988; Hubbard, Huber, and Pharr, 1989; Ofosu-Anim and Yamaki, 1994; Lee et al., 1997; Ofosu-Anim, Kanayama, and Yamaki, 1998; Feusi et al., 1999; Gao et al., 1999; Gao and Schaffer, 1999; Carmi et al., 2003; Volk, Haritatos, and Turgeon, 2003). Likewise, sugar accumulation is affected quantitatively by environmental and physiological factors as well, such as salinity, nutrient availability, shading, cellular size in the fruit, and available foliar area (Hubbard, Pharr, and Huber, 1990; del Amor, Martínez, and Cerda, 1999; Nishizawa et al., 2000, 2002; Kano, 2002, 2004).

Netted muskmelon and Honey Dew fruits have similar, but not identical, patterns of sugar accumulation (Seymour and McGlasson, 1993). For instance, Bianco and Pratt (1977) reported that both Honey Dew and PMR-45 fruits have a parallel pattern for sugar accumulation including total sugars, sucrose, glucose, and fructose. Likewise, McCollum, Huber, and Cantliffe (1988) reported that “Galia” and “Noy Yizre’el” fruits accumulated glucose and fructose, in nearly equal amounts, during the first 24 days after anthesis. Sucrose accumulation built up 24 days after anthesis, and it was the predominant sugar at the ripe stage. Similar results were obtained by Hubbard, Huber, and Pharr (1989), who measured the concentrations of sucrose, raffinose, stachyose, glucose, fructose, and starch in one orange-fleshed netted melon and three green-fleshed muskmelons, two of them categorized as sweet melons and one as a nonsweet type (“Birds Nest”).

In an extensive study, Stepansky et al. (1999) found considerable variation in sugar content and composition in mature flesh of melon fruits from 56 different genotypes belonging to *cantaloupensis*, *inodorus*, *conomon*, *chito*, *dudaim*, *momordica*, *flexuosus*, *agrestis*, and some nondefined varieties. Among the 14 genotypes classified as *cantaloupensis*, total sugars ranged between 40 and 100 mg/g fw, and sucrose was 50%–70% of the total sugar, although a few accessions had lower levels. Within the *inodorus* group, both low- and high-sucrose-accumulating genotypes were observed. Some genotypes reached only ∼30 mg/g fw total sugar, mostly glucose and fructose, whereas others had a high sucrose accumulation (∼50 mg/g fw). Among the six *conomon* genotypes analyzed, there were fruits with almost no sucrose (line 85–893) accumulation as well as genotypes with intermediate and high sucrose levels. In the *chito* and *dudaim* varieties, five genotypes were evaluated, four out of five genotypes accumulated less than 10 mg/g fw sucrose, but interestingly, the last one (PI 164320) had an unusual sugar pattern profile as it accumulated high levels of total sugar, due mostly to elevated glucose and fructose levels. Most members of the *agrestis* group accumulated extremely low levels of sugars; however, two accessions (PI 164493 and PI 436532) had high total sugars (41 and 58 mg/g fw, respectively). The *momordica* and *flexuosus* genotypes did not accumulate significant amounts of either sucrose or hexose. These authors also indicated that in the sweeter melon varieties, sucrose was generally the most significant component that contributed to variation in total sugars.

The physiological and biochemical aspects of sucrose accumulation in melon fruit have been extensively investigated (McCollum, Huber, and Cantliffe, 1988; Hubbard, Huber, and Pharr, 1989; Hubbard, Pharr, and Huber, 1990; Feusi et al., 1999; Gao et al., 1999; Gao and Schaffer, 1999; Burger et al., 2003; Carmi et al., 2003; Volk, Haritatos, and Turgeon, 2003; Villanueva et al., 2004). As previously stated, melon plants translocate sucrose, stachyose, and raffinose as the main soluble sugars, which are used as the carbon supply for sucrose synthesis in the fruit. Two enzymes, acid invertase (EC 3.2.1.26) and sucrose phosphate synthase (SPS) (EC 2.4.1.14), have been implicated as the determinants of sucrose accumulation in melon fruit (Hubbard, Huber, and Pharr; Stepansky et al., 1999). Both enzymes are inversely related in melon sink tissues, such as fruits (Hubbard, Huber, and Pharr, 1989; Hubbard, Pharr, and Huber; Gao et al.).

During sucrose accumulation, acid invertase activity decreases, and as a result there is less sucrose degradation. At the same time, SPS activity begins to increase significantly (Hubbard, Huber, and Pharr, 1999; Hubbard, Pharr, and Huber, 1990; Gao et al., 1999; Lester et al., 2001). In addition, SPS activity is higher in sweet melon fruit than in non-sweet genotype fruits, suggesting its function in sucrose accumulation (Hubbard, Pharr, and Huber, 1990). Moreover, Lester, Saucedo-Arias, and Gomez-Lim (2001) found that the final content of sugars in high versus low-sucrose-accumulating fruit of equal maturity is the number of downregulated acid invertase isoforms and upregulated SPS activity during fruit ripening.

In summary, as Stepansky et al. (1999) clearly stated, “the final content of sucrose in the fruit mesocarp of sweet melon is a function of two factors: the rate of sucrose accumulation, coupled with the duration of the accumulation period until abscission or harvest.”

**Organic Acids**

Organic acids are regularly found at low amounts in sweet ripe melon fruit types, such as *inodorus*, *cantaloupensis*, and *reticulatus* varieties (Yamaguchi et al., 1977; Seymour and McGlasson, 1993). On the other hand, nonsweet ripe melon
fruits (flexuosus variety) are able to accumulate higher amounts of organic acids (Pitrat, Hanelt, and Hammer, 2000; Stepsky et al., 1999). For instance, Burger et al. (2003) reported that the fruit characteristic of high organic acid content is conferred by a single dominant gene, called So, which is found only in melon varieties that do not accumulate high levels of sugars and which are used for nondessert purposes. In the recessive condition (so), melon fruits have a low organic acid attribute. Furthermore, these authors stated that the evolution of horticultural sweet melon varieties required the sequential selection of three recessive mutations: first a recessive mutation that allowed for nonbitter fruit (bij), then a recessive mutation for low-acid fruit (so), followed by a recessive mutation for high-sucrose fruit (suc) (Burger et al., 2002, 2003). Despite the fact that a low organic acid level is a genetically regulated feature, several environmental factors, such as salinity, can affect quantitatively the organic acid level in melon fruit (del Amor, Martínez, and Cerda, 1999).

Citric and malic acids are the most important organic acids found in the flesh of different melon varieties (Leach et al., 1989; Flores, BenAmor, et al., 2001; Burger et al., 2003). In 1989, Leach et al. (1989) studied the organic acid fractions from 12 melon cultivars and reported that citric acid was the major component in all melon cultivars that they analyzed. Similarly, Flores, BenAmor, et al. (2001) reported that the major organic acids found in wild-type and transgenic cantaloupe melon fruit were citric and malic acids. Artes et al. (1993) described that titratable acidity in four melon varieties varied from 0.14% in “Tendral” to 0.50% in Galia melon fruits.

Volatile Compounds

The aroma or fragrance of melon fruits, brought about by the release of volatiles, are essential quality factors strongly linked to the ripening process and genetically controlled (Yamaguchi, 1977; Wang et al., 1996; Ueda, Fujishita, and Chachin, 1997; Beaulieu and Grim, 2001; Yahyaoui et al., 2002). Unlike sugar accumulation, the aroma caused by volatile production continues after harvest (Wyllie et al., 1995); however, the profile of these compounds is altered during storage of melon fruit. Volatile ester compounds generally decrease or exhibit a transient increase before declining after 5–7 days of storage (Beaulieu, 2005). The relative percentage of acetate esters declines during storage in many cultivars, and this decrease was accompanied by simultaneous nonacetate ester increases (Beaulieu, 2005).

Upsetting the unique aroma balance through storage may negatively affect flavor and the consumer’s perception of desirable attributes, even though total volatile levels might not decrease substantially until after 5–7 days in storage. Subtle quality decreases are likely to be exacerbated with immature-harvested melons. The volatile profile, as well as the identification of the main “melon odor” substances in melon fruits, has been the subject of a considerable amount of research (Kemp, Fujishita, and Chachin, 1972; Yabumoto, Yamaguchi, and Jennings, 1977; Yabumoto, Yamaguchi, and Jennings, 1978; Buttery et al., 1982; Horvat and Senter, 1987; Leach et al., 1989; Wyllie and Leach, 1990; Homatidou et al., 1992; Wang, Wyllie, and Leach, 1996; Ueda Fujishita, and Chachin, 1997; Bauchot et al., 1998; Yahyaoui et al., 2002; Aubert and Bourger, 2004).

Early studies reported that the volatile ester pattern of ripe muskmelon (reticulatus varieties) and Honey Dew (indorus varieties) type fruit were extremely similar, except for ethyl butyrate, which was more abundant in muskmelon (Kemp, Fujishita, and Chachin, 1972; Yabumoto, Jennings, and Yamaguchi, 1977; Yabumoto, Yamaguchi, and Jennings, 1978). The volatile profile of melon fruit was made up of around 35–50 volatile compounds (Kemp, Fujishita, and Chachin, 1972; Yabumoto, Jennings, and Yamaguchi, 1977; Yabumoto, Yamaguchi, and Jennings, 1978; Aubert and Pitrat, 2006), such as solid-phase microextraction (SPME), as well as analytical and detection techniques (Aubert and Bourger, 2004, Aubert, Baumann, and Arguel, 2005), such as sniffing port analysis among others, it has been shown that the volatile compound content responsible for “melon aroma” is diverse and cultivar dependent. Indeed, Aubert and Bourger were able to differentiate statistically long-shelf-life cultivars from wild and mid-shelf-life melon cultivars, based solely on volatile compound profiles. Moreover, Beaulieu and Grim as well as Aubert and Pitrat affirmed that roughly 240 volatile compounds have been reported from muskmelon fruit.

In the particular case of “Arava” melon, which is a Galia-type melon, various volatile acetates were identified in the ripening fruit, including nine aliphatic, four aromatic, and one compound containing a sulfur moiety (Shalit et al., 2000). Benzyl acetate was the most abundant volatile compound in the headspace of this cultivar; however, hexyl acetate and 2-methyl butyl acetate were also found in considerable amounts.

The aroma and taste of most melon fruits are influenced considerably by ester compounds, as well as to a certain extent by sulfur compounds (Yabumoto, Jennings, and Yamaguchi, 1977; Wyllie and Leach, 1990; Homatidou et al., 1992). Even though, Kemp, Fujishita, and Chachin (1972) suggested that four unsaturated esters found in muskmelon fruit did not contribute significantly to the melon aroma, Yabumoto, Jennings, and Yamaguchi (1977), using three different extraction methods for melon fruit volatiles, stated that it was probable that the large quantities of volatile esters also play a critical role in the integrated flavor of melons, and that they are necessary for the strong and characteristic fruity aroma. According to Yabumoto, Yamaguchi, and Jennings (1978), the volatile ester profile of ripe reticulatus variety (PMR-45 and “Top Mark”) and indorus variety (Honey Dew and “Crenshaw”) fruit were similar, and they fit into two groups, depending on the pattern exhibited by the production of volatile esters. One group had a continuously accelerating rate of production (ethyl esters) and another increased rapidly and then reached a plateau (acetate esters).

The major compounds responsible for Honey Dew melon aroma are ethyl 2-methylbutyrate, ethyl butyrate, ethyl hexanoate, hexyl acetate, 3-methylbutyl acetate, benzyl acetate,
proteins, and aromatic compounds. Likewise, the new primary highly organized structure composed of several polysaccharides, cell types (Carpita and McCann, 2000). The plant cell wall is a cell shape and contributes to the functional specialization of cell types (Carpita and McCann, 2000). The plant cell wall is dynamic structure, which determines cell shape and contributes to the functional specialization of cell types (Carpita and McCann, 2000). The plant cell wall is a highly organized structure composed of several polysaccharides, proteins, and aromatic compounds. Likewise, the new primary cell wall comes from the cell plate during cell division, and after differentiation many cells are able to develop within the primary wall, a secondary cell wall (Carpita and McCann, 2000). According to Bennett (2002), the simplest form of the structural model of the plant cell wall can be pictured as a core structure of cellulose microfibrils embedded in two coextensive networks of pectin and hemicelluloses.

The ripening of many fruits is characterized by softening of the flesh. Fruit softening observed during ripening is associated with textural changes that are believed to result from modification and disassembly of the primary cell wall (Fischer and Bennett, 1991). Fruit softening and the underlying cell wall structural changes are complex. Softening or loss of firmness of the edible mesocarp of melon fruit starts in the middle (around 30–45 days after anthesis, depending on cultivar) of the development cycle, along with other typical changes connected with the ripening process (Lester and Dunlap, 1985). Some general events during melon fruit softening are (Bennett, 2002):

1. There is no significant change in total pectins (measured as total polyuronides) as a percentage of cell wall material; rather a substantial change in the relative solubility and depolymerization of pectin levels is observed as fruit softening proceeds, as well as a decrease in pectin molecular size (Lester and Dunlap, 1985; McCollum, Huber, and Cantliffe, 1989; Ranwala, Suematsu, and Masuda, 1992; Rose et al., 1998).
2. The polygalacturonase (PG) enzyme’s role into pectin degradation is still controversial. It seems likely that PG enzyme(s) might not be involved in that solubilization process during the early ripening stages; however, some PG-dependent developments may contribute to overall pectin disassembly at later stages (Lester and Dunlap, 1985; McCollum, Huber, and Cantliffe, 1988, 1989). On the other hand, Hadfield et al. (1998) suggested that a group of PGs, divergent from the well-characterized tomato fruit PG, might be implicated in melon fruit-ripening-associated pectin disassembly (Rose et al., 1998).
3. Other enzymes could be involved in early pectin solubilization, for instance, β-galactosidases, α-galactosidase, and/or β-galactanases (Fils-Lycaon and Buret, 1992; Ranwala, Suematsu, and Masuda, 1992; Rose et al., 1998).
4. Hemicellulose polymers undergo important modifications, such as changes in the degree of solubility and modifications from large molecular size to smaller size, and loss of specific sugars (Lester and Dunlap, 1985; McCollum, Huber, and Cantliffe, 1989; Ranwala, Suematsu, and Masuda, 1992; Simandjuntak, Barrett, and Wrolstad, 1996; Rose et al., 1998).
5. Noncellulosic neutral sugars decrease significantly in the mesocarp of ripening fruits, regularly galactose, mannose and arabinose, whereas other neutral sugars such as xylose may or may not increase during fruit softening (McCollum, Huber, and Cantliffe, 1988, 1989; Simandjuntak, Barrett, and Wrolstad, 1996).
6. There is a substantial water loss during melon fruit softening (Lester and Bruton, 1986; Rojas et al., 2001). The water loss has been associated, besides the cell wall degradation, to a cell membrane dissociation and leakage process caused by an increased lipid peroxidation (Lester and Dunlap, 1985; Lester and Bruton, 1986; Lester and Stein, 1993; Lester, 2000).

7. Other enzymatic activities, such as pectin methylesterase, which have been associated with pectin metabolism in other fruits (Harriman, Tieman, and Handa, 1991; Tieman et al., 1992), and/or other proteins, such as expansins, which have been proposed to disrupt hydrogen bonds within the plant cell wall polymer matrix (Rose et al., 1997; Civello et al., 1999), could also be involved in melon fruit softening. More evidence has appeared regarding the expansins’ role in early fruit softening. Brummell et al. (1999) obtained two types of transgenic tomato plants: some were suppressed and others were overexpressed in the LeExp1 protein. Tomato fruit, in which Exp1 protein accumulation was inhibited by 3%, were firmer than control fruit throughout the ripening process. Conversely, fruit overexpressing high amounts of LeExp1 protein were much softer than control fruit, even in mature green fruit before ripening had commenced.

Rose et al. (1998) and Bennett (2002) proposed a complete and understandable model of the temporal sequence of cell wall changes, pectinase activity, and PG-mRNA expression in ripening “Charentais” melon fruit at defined developmental stages. Unfortunately they did not include the role of expansins in cell wall degradation in their model.

Among the different plant hormones that are involved in fruit development, ethylene has the main role during melon fruit softening (Rose et al., 1998). Internal ethylene concentrations inside Charentais melon fruit cavity increase concomitantly with a loss of flesh firmness during ripening. Furthermore, Guis et al. (1999), using antisense ACC-oxidase transgenic melon plants to reduce ethylene production, reported that plants did not have substantial changes in pectin molecular mass observed in the wild-type fruit. Moreover, exogenous ethylene application to those transgenic fruits resumed both accelerated fruit softening and a downshift in the size of cell wall polymers. Additionally, transgenic melon plants (antisense ACC oxidase) were also used to study the role of ethylene in regulating cell-wall-degrading enzyme activities, such as endo-PG, exo-PG, pectin methyl esterase, glycosidase (beta-galactosidase and alpha-arabinosidase), and galactanase activities (Botondi, Cardarelli, and Mencarelli, 2000). In transgenic Charentais melon, the cell-wall-degradation process is regulated by both ethylene-dependent and ethylene-independent mechanisms. Pectin methyl esterase and exo-polygalacturonase enzymes were considered as ethylene-independent enzymes, whereas endo-polygalacturonase, galactanase, alpha-arabinosidase, and beta-galactosidase had a greater activity in wild-type melon fruits than in transgenic ones (Pech et al., 1999). In support of a fruit-softening ethylene involvement, it was established that the ripening-regulated expansin gene(s) in tomato was influenced directly by ethylene, and the expression of that gene parallels the pattern of xyloglucan disassembly, and early fruit softening (Rose, Lee, and Bennett, 1997).

The exact hormonal, molecular, and enzymatic mechanisms by which all these processes take place in melon-ripening fruit, and finally contribute to the fruit-softening event are not well understood. Maybe by using updated molecular and genetic techniques, such as cDNA microarrays (Fonseca et al., 2004), in order to monitor the gene expression during fruit development and ripening, there will be more evidence to understand and manipulate the melon-fruit-softening process. Moreover, as Bennett (2002) proposed: “future research should focus on using genetic strategies to assess the potential for synergistic interactions by suppression of both hemicellulose and pectin disassembly in ripening fruit.”

Pigments

The flesh color of melons is another important quality attribute for consumer appeal (Yamaguchi, 1977). In general, four basic and distinctive flesh colors can be observed in melon fruits: orange, light orange or pink, green, and white (Watanabe et al., 1991; Goldman, 2002).

According to Seymour and McGlasson (1993), the principal pigments in orange-fleshed melons are: beta-carotene (84.7%), delta-carotene (6.8%), alpha-carotene (1.2%), phytofluene (2.4%), phytoene (1.5%), lutein (1.0%), violaxanthin (0.9%), and traces of other carotenoids. Likewise, Watanabe et al. (1991) evaluated nine different melon cultivars belonging to the four basic and distinctive flesh colors. They found that the orange-flesh-colored melon cultivars “Iroquois,” “Blenheim Orange,” “Birdie Red,” “Quincy,” and “Tiffany” contained about 9.2 to 18.0 µg/g beta-carotene as the major pigment, as well as a small amount of phytofluene, alpha-carotene, zeta-carotene, and xanthophylls. They also measured pigments in light-orange-flesh-colored “Hale’s Best” melon, which contained about 4.0 µg/g beta-carotene. Phytofluene, alpha-carotene, zeta-carotene, and xanthophylls were also present but in small amounts. Finally, in the green-flesh-colored melons, Earl’s Favourite and “Fukunoka,” and the white-flesh-colored melon, “Barharman,” their main components were beta-carotene and xanthophylls. Unfortunately, Watanabe et al. did not report the chemical nature of the green pigment in green-flesh-colored melon fruits.

Chlorophyll and carotenoid changes in developing fruit muskmelon were studied earlier by Reid et al. (1970). They evaluated three melon cultivars: Crenshaw, Persian, and PMR 45. In all the fruits, chlorophyll content decreased to an intermediate level 5 weeks after anthesis, and they suggested that chlorophyll loss was probably due to dilution through growth, because chlorophyll synthesis had ceased, but an enlargement of the fruit occurred. In PMR 45 and Crenshaw fruits, however, there was a successive rapid decrease, which was concurrent with the ripening process. Carotenoid content increased steadily
3 weeks after anthesis to high levels at full maturity. The development of orange pigmentation was a gradual event, starting at the placenta and progressing outward through the mesocarp, until the flesh was uniformly orange at full maturity.

Forbus, Dull, and Smittle (1992) used delayed light emission (DLE), a nondestructive method, to study physical and chemical properties related to fruit maturity in white-green-flesh-colored Canary melons. They found that chlorophyll and yellow pigments decreased with fruit development, having a high correlation with maturity index (IM). Flügel and Gross (1982) studied pigment and plastid changes during ripening of the green-flesh-colored and yellow-skinned finely netted rind Galia muskmelon fruit. They observed that the carotenoid profile in the exocarp and mesocarp did not change during development. Also, relatively low levels of chlorophyll and carotenoids were found in the flesh. Yellowing of the exocarp was due to increased chlorophyll degradation during ripening, and a partial decrease in total carotenoids took place.

In conclusion, it seems that pigment profile accumulation and degradation in melon fruit is a cultivar-dependent characteristic, which is expressed during fruit maturity.

Lipids
As previously stated, melon fruit ripening involves texture changes, which implicate changes in cell wall metabolism and loss of membrane integrity (Lester and Stein, 1993; Lester, 1998a). Forney (1990) analyzed changes in polar lipid fatty acid composition in ripening Honey Dew muskmelons. The composition of fatty acids changed rapidly as the fruit ripened with the ratio of unsaturated-to-saturated eventually doubling. Interestingly, changes in polar lipids in the flesh were less pronounced than those in the peel, and it was suggested that this was related to the increase in chilling tolerance reported to occur with ripening or solar exposure in melons (Forney, 1990).

Much research on the role of membrane lipid metabolism in fruit ripening, senescence, and postharvest deterioration of quality has focused on the cascade of enzymes involved in phospholipid hydrolysis and fatty acid peroxidation. An irreversible increase in phospholipid catabolism is thought to predispose plant cells to membrane dysfunction and eventual cell death (Lester, 1998a). The proposed senescence cascade begins with removal of phospholipid polar head groups by phospholipase D (EC 3.1.4.4), yielding phosphatidic acid, a non-bilayer-forming phospholipid. Subsequently, phosphatidic acid is dephosphorylated to diacylglycerol by a specific phosphatidic acid phosphatase. Lipolytic acyl hydrolase then cleaves diacylglycerol, yielding free fatty acids (Brown, Lynch, and Thompson, 1987; Paliyath and Thompson, 1987). All these enzyme activities disrupt membrane structure, and linoleic and linolenic acid serve as substrates for lipoxygenase (EC 1.13.11.12) (Fobel, Lynch, and Thompson, 1987). Lipoxygenase catalyzes formation of highly reactive hydroperoxides (Vick and Zimmerman, 1987) and may also generate superoxide radicals (Lynch and Thompson, 1984). Fatty acid hydroperoxides produced by lipoxygenase can perturb the membrane bilayer directly, or break down to yield toxic volatiles and free radicals that attack additional membrane components (Thompson, Legge, and Barber, 1987). Lester (1990) reported the presence of lipoxygenase in the hypodermis of muskmelon fruit and has shown that increased lipoxygenase activity is linked with loss of plasma membrane integrity during senescence of hypodermal tissue, as well as to melon fruit softening (Lester and Stein, 1993). Accumulated evidence has indicated that senescence of mesocarp tissues in netted and non-netted muskmelon fruits is associated with increased leakage of ions across cell membranes (Lacan and Baccou, 1996) and decreased membrane H$^{+}$- and Ca$^{2+}$-ATPase activity (Lester and Stein, 1993) as a consequence of phospholipid catabolism and lipid peroxidation (Lacan and Baccou, 1996; 1998; Lester and Whitaker, 1996). Moreover, increased activities of lipoxygenase and phospholipase D have been linked with these changes in muskmelon fruit tissues (Lacan and Baccou, 1998; Lester, 1990, 2000).

In brief, the loss of membrane integrity, catalyzed by enzymes involved in phospholipid hydrolysis and fatty acid peroxidation, is one of the main events observed during texture changes in melon fruit ripening. The final outcome of these physiological/biochemical events is a loss of fruit firmness.

Gene Expression and Molecular Changes During Ripening
The studies reviewed here describe processes and enzyme activities associated with specific events of fruit ripening. The development of the capacity to isolate and analyze genes has improved so much in recent years that it is now possible to analyze gene expression at large scale and to modify specific enzyme activities to determine their effect on fruit ripening.

Melon has been the subject of intense scrutiny to understand the molecular basis of fruit ripening. In this sense, given the importance of ethylene in ripening, the key enzymes of the ethylene biosynthetic pathway have been the focus of research in a number of papers. There are ethylene-dependent and ethylene-independent biochemical and physiological pathways throughout melon fruit ripening (Pech et al., 1999; Hadfield et al., 2000; Srivastava, 2002; Silva et al., 2004), which coexist at the same time in the climacteric fruit. Likewise, besides ethylene, several plant hormones, such as IAA and ABA, are involved in melon fruit ripening (Larrigaudiere Guillen, and Vendrell, 1995; Dunlap, Slovin, and Cohen, 1996; Guillén et al., 1998; Martínez-Madrid et al., 1999; Martínez-Madrid, Flores, and Romojaro, 2002).

Ethylene biosynthesis goes from methionine, through S-adenosylmethionine (SAM), then to 1-aminocyclopropane-1-carboxylic acid (ACC), and finally to ethylene (Yang and Baur, 1969; Adams and Yang, 1979; Yang, 1980, 1982; Yang and Hoffman, 1984). Two regulatory enzymes in this pathway are ACC synthase (ACS) (EC 4.4.1.14) and ACC oxidase (ACO) (EC 1.14.17.4). The latter enzyme was formerly known as ethylene-forming enzyme (EFE) by Adams and Yang (1979), because
the reaction mechanism was not known at that time. ACC synthase is generally considered as the rate-limiting step in ethylene biosynthesis (Yang and Hoffman, 1984).

Both ACS and ACO melon enzymes are coded by multigene families (Miki et al., 1995; Yamamoto et al., 1995; Lasserre et al., 1996, 1997), therefore several isoenzymes are recognized in melon tissues. For instance, Miki et al. (1995) and Ishiki et al. (2000) isolated three cDNAs for ACS synthase from wounded mesocarp tissue of melon fruits. Lasserre et al. (1996) reported the isolation and categorization of three genomic clones, identified by screening a melon genomic DNA library with the cDNA pMEL1, corresponding to three putative members of the ACC oxidase gene family in cantaloupe melon. In addition, these authors determined the entire sequence of these genes and found that they were all transcriptionally active. One genomic clone, named CM-ACO1, presented a coding region with four exons interrupted by three introns. The other two genes, CM-ACO2 and CM-ACO3, were interrupted by only two introns, at the same positions as CM-ACO1. The degree of DNA homology in the coding regions of CM-ACO3 relative to CM-ACO1 was 75%. In contrast, the degree of DNA homology of CM-ACO2 relative to both CM-ACO1 and CM-ACO3 was 59% in their coding region.

ACS and ACO melon multigenes are differentially activated and expressed by several environmental and developmental factors (Yamamoto et al., 1995; Lasserre et al., 1996; Shiomi et al., 1999; Zheng, Wolff, and Crosby, 2002). Yamamoto et al. (1995), using tissue printing and immunoblot analysis with antibodies specific for ACO, were able to identify in which part of the fruit the accumulation of ACO protein begins in melon fruits at the early stages of ripening. They reported that the rate of accumulation of ACO protein in melon fruits increased initially in the placental tissue, then in mesocarp tissue, and finally at the rind. They also concluded that levels of ACO mRNA and protein were low in the unripe fruit stage, but became detectable in placental tissue at the preclimacteric period, and their levels increased in the mesocarp at the climacteric stage. All these results suggested that the central region of melon fruit (placental tissue and seeds) plays a major role in the production of ethylene during the early stage of fruit ripening.

A RT-PCR assay was used by Lasserre et al. (1996) to detect the differential expression of ACO melon genes (CM-ACO1, CM-ACO2, and CM-ACO3). They found that these three genes were differentially expressed during development, ethylene treatment, and wounding. CM-ACO1 was induced during fruit ripening, and also in response to wounding and ethylene treatment in leaves. CM-ACO2 was detectable at low levels in etiolated hypocotyls, whereas CM-ACO3 was expressed in flowers and was not induced by any treatment tested.

In a subsequent series of experiments, Lasserre et al. (1997) found that the regulation of the CM-ACO1 gene was connected preferentially to stress responses, whereas the CM-ACO3 gene seemed to be associated with developmental routes. Moreover, Bouquin et al. (1997), using the promoter region of the CM-ACO1 gene fused to the beta-glucuronidase (GUS) reporter gene, were capable of measuring the transcriptional activation of the CM-ACO1 gene in tobacco leaves after wounding and ethylene stimulation. Their results implied that induction of CM-ACO1 gene expression occurs via two direct and independent signal transduction pathways in response to both stimuli. Zheng, Wolff, and Crosby (2002) studied some genetic aspects of ethylene production and its relationship to the RFLPs of the ACC oxidase and ACC synthase genes in two melon cultivars. One cultivar had high ethylene production during fruit ripening (“TAM Uvalde”) and another had low levels of ethylene production (“TAM Yellow Canary”). Their results of single-copy-reconstruction assays suggested that the CMACO1 gene was present as a single copy, whereas the CMACS-1 gene was a component of a multigene family in both melon cultivars.

It has been proposed that differences in ethylene production among melon fruits might be the result of transcriptional changes in ACS and ACO genes (Shiomi et al., 1999). These authors measured the ACS-1, ACO-1, and ACO-2 mRNA expression pattern in exocarp, mesocarp, and placental tissues of Earl’s Favourite (recognized as nonclimacteric) and “Andes” (known as climacteric) fruit cultivars at different stages of maturity, finding that mRNA CMACS-1 transcripts accumulated only in the mesocarp and placenta of Andes fruit at 50 DAP (commercial harvest maturity stage). This accumulation was coincident with increases in ACS activity, ACC content, and maximum ethylene production. In contrast, CMACO-1 mRNA accumulated in elevated levels in the mesocarp and placenta of both cultivars at 50 DAP, but in Andes cultivar those transcripts were more abundant than in Earl’s Favourite fruit. In the exocarp, the CMACO-1 mRNA level was low for both cultivars. CMACO-2 mRNA was constitutively expressed in placenta and mesocarp at low levels, and was nondetectable in the exocarp. These results suggested that the difference in ethylene-forming ability between these two cultivars may result from the expression of CMACS-1 mRNA and CMACO-1 mRNA during the fruit-ripening process.

Ethylene perception is mediated by specific receptors, which have been cloned and described for several plants, such as Arabidopsis (Fuhr and Mattoo, 1996; Johnson and Ecker, 1998), tomato (Tiemann and Klee, 1999; Tiemann et al., 2000), tobacco (Terajima et al., 2001), and carnation (Dianthus caryophyllus) (Reid and Wu, 1992; Shibuya et al., 2002). In C. melo, ethylene-receptor-like homolog genes have been reported as well (Sato-Nara et al., 1999; Takahashi et al., 2002; Cui, Takada, Ma, and Ezura, 2004; Nukui, Ezura, and Minamisawa, 2004). Sato-Nara et al. (1999) isolated and characterized two cDNAs, which were described as putative ethylene receptors, from muskmelon using the Arabidopsis ethylene receptor genes ETR1 and ERS1 sequences. These authors measured the expression pattern of these cDNAs during fruit enlargement and ripening by means of Northern blot assay, finding that both clones were expressed in a stage- and tissue-specific manner. They named their cDNAs...
It is well established that ethylene-dependent and ethylene-independent biochemical and physiological pathways take place and coexist during melon fruit ripening. To investigate this further, Hadfield et al. (2000) made a differential screening of a ripe melon fruit cDNA library and identified 16 unique cDNAs corresponding to mRNAs whose accumulation was induced by ripening. This screening was carried out to characterize the changes in gene expression that go along with melon fruit ripening. Expression of 15 out of 16 cDNAs was ripening regulated, and 12 of them were fruit specific. Three patterns of gene expression were observed when the expression of cDNA clones was examined in transgenic ACC oxidase antisense melon fruit. One group of cDNAs corresponded to mRNAs whose abundance was reduced in transgenic fruit but still inducible by ethylene treatment. The second group of mRNAs was not significantly altered in the transgenic fruit and was not affected by ethylene treatment, indicating that these genes are regulated by ethylene-independent factors. The third group of cDNAs had an unexpected pattern of expression, and low levels of mRNA in transgenic fruit even remained low after ethylene treatment. These authors suggested that the regulation of this third group of genes appears to be ethylene independent, but they might be regulated by developmental signals that require ethylene at some stage during fruit development.

Pech et al. (1999) had separated ethylene-dependent events in cantaloupe Charentais melon fruits into two main groups. One group was considered as physiological changes, such as yellowing of the rind, fruit softening, volatile production, presence of climacteric respiration, abscission of the fruit, and susceptibility to chilling injury. The other group included only enzyme activities, such as galactanase, alpha-arabinosidase, beta-galactosidase, and ACC synthase (induction at onset of ripening). This classification of ethylene-dependent and ethylene-independent events provides valuable basic information, which might be used to design biochemical and/or molecular strategies with the aim of controlling melon fruit ripening.

Ethylene regulates fruit ripening by coordinating the expression of several genes (Aggelis, John, and Grierson, 1997; Aggelis, John, I., Karvouni, Z., and Grierson, 1997; Lelièvre et al., 1997; Yang and Oetiker, 1998; Jiang and Fu; 2000; Périn et al., 2002). The most common genes that are frequently regulated by ethylene during fruit ripening embrace some members of the ACS and ACO multigene families, phytoene synthase, endo-polygalacturonase, galactanase, one homolog of 3-adenosyl-L-homocysteine hydrolase (SAHH), and even a mRNA, which is ripening specific, named MEL2, and of unidentified function (Karvouni et al., 1995; Aggelis, John, and Grierson, 1997; Aggelis, John, I., Karvouni, Z., and Grierson, 1997; Hadfield et al., 1998; Pech et al., 1999, 2002; Guis et al., 1999; Périn et al., 2002).

Apart from cDNAs coding for ethylene-related processes, several cDNAs coding for enzymes involved in other fruit processes have been characterized. Complementary DNAs coding for polygalacturonase (Hadfield et al., 1998), an abscisic acid responsive gene homolog (Hong et al., 2002); cucumber, a serine protease (Yamagata et al., 1994); two unidentified proteins (Aggelis, John, Karvouni, et al., 1997); phenylalanine ammonia-lyase (Diallinas and Kanellis, 1994); an enzyme involved in betacarotene metabolism (Ibdah et al., 2006), phytoene synthase (Karvouni et al., 1995); phospholipase D-alpha and lipoxygenase (Whitaker and Lester, 2006); ascorbate oxidase (Diallinas et al., 1997; Sammartini et al., 2007); L-galactono-1,4-lactone dehydrogenase that catalyzes the last step of ascorbic acid biosynthesis (Pateraki et al., 2004); and 3-hydroxy-3-methylglutaryl coenzyme A reductase, which is involved in the synthesis of mevalonate, have been identified (Kato-Emori et al., 2001). Clones coding for enzymes involved in aroma compounds,
including alcohol dehydrogenases and alcohol acyltransferase, have also been identified (Yahyaoui et al., 2002; El-Sharkawy et al., 2005; Flores et al., 2005; Manriquez et al., 2006). Bacterial artificial chromosome libraries have been prepared and employed to isolate disease-resistance gene homologs (Luo et al., 2001; Wang et al., 2002; van Leeuwen et al., 2005).

Recently, a whole melon EST database (http://melon.bti.cornell.edu/), mostly from mature fruit and phloem, was published. The database was constructed sequencing clones from subtracted, nonsubtracted, and non-normalized libraries. Altogether, 5,531 ESTs from all these libraries were sequenced. The database also includes around 100 genes involved in major metabolic pathways (sugar, acid, and carotenoids) cloned on the basis of homology to genes from other plant families. The EST database has been mined to identify candidate genes affecting fruit ripening and fruit quality traits. Expression profiles of more than 200 genes were characterized by Northern blots and real-time PCR. Similarly, Gonzalez-Ibeas et al. (2007) accomplished eight melon cDNA libraries from several tissues at different physiological stages. More than 30,000 ESTs were sequenced and clustered into 16,637 unigenes, embracing 6,023 contigs and 10,614 singletons. Using these ESTs sequences, a melon microarray was prepared to study the melon transcriptome. On the other hand, two novel sesquiterpene synthase genes coding for proteins that are able to convert farnesyl diphasphate into (1) E,E-farnesene (CM-SS1) or (2) alpha- and delta-cadinene and alpha-copaene and a CCD-like gene able to catalyze the formation of volatile norisoprenoids from various carotenoid pigments have been isolated from melon varieties (Katzir et al., 2006). A melon cDNA microarray containing 3,066 independent unigenes and 6 negative controls is currently available. These genomic tools will be very helpful to understand the role of a number of genes in the ripening process.

### TABLE 2

<table>
<thead>
<tr>
<th>Steps in breeding</th>
<th>Conventional methods</th>
<th>Biotechnological methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Collection and evaluation of genetic resources</td>
<td>Genetic analysis of valuable traits</td>
<td>Cloning of valuable gene</td>
</tr>
<tr>
<td>2. Generation of variation</td>
<td>Intraspecific crosses</td>
<td>Genetic transformation, embryo culture, somatic cell fusion, polyploidy, anther culture, and somaclonal variation</td>
</tr>
<tr>
<td>3. Selection of desirable variants</td>
<td>Growth and evaluation</td>
<td>PCR, selectable markers, DNA markers, etc.</td>
</tr>
<tr>
<td>4. Production of fixed lines</td>
<td>Self-pollination</td>
<td>Self-pollination and haploid production by gynogenesis</td>
</tr>
<tr>
<td>5. Seed production</td>
<td>Growth under controlled conditions</td>
<td>DNA markers to test seed purity and growth under controlled conditions</td>
</tr>
</tbody>
</table>

Modified from Ezura (1999).
documented that using genetic engineering strategies is feasible to overcome most of the genetic barriers among plants, which are unsurpassable by traditional breeding techniques (Vasil, 2003).

**Improvement through Genetic Engineering**

During the 1970s, molecular biology and genetic engineering research laid the foundation for the development of transgenic plants in 1983 using the Ti plasmid from the soil bacterium *Agrobacterium tumefaciens* (Herrera-Estrella et al., 1983). This bacterium transfers a specific fragment of the Ti plasmid (T-DNA), which can be engineered to contain a selectable marker and/or genes of interest, into the plant nuclear genome under in vitro conditions. Once inserted, nontransformed plants can be killed in culture by the toxic substance to which the marker gene codes resistance. Subsequently, transformed plant cells can regenerate whole transformed plants on a plant regeneration culture medium. Within the plant biotechnology discipline, plant tissue culture methods have had an essential role, allowing the development of transgenic plants with a number of desirable agronomic, pest resistance, and food traits. It is commonly accepted that the term “plant tissue culture” refers to in vitro cultivation on nutrient media of any plant part, a single cell, tissue, or an organ under a sterile environment, leading to a whole de novo regenerated plant (Nuñez-Palenius et al., 2005).

After the milestone plant transformation achievement by Herrera-Estrella et al. (1983), a number of technological difficulties were surpassed, allowing the cloning and insertion of different genes and engineering of transgenic plants with: (1) resistance to plant viruses, fungi, and insects; tolerance to herbicides, salinity, drought, heavy metals, and low and high temperatures; (2) improved nutritional quality (proteins, oils, vitamins, and minerals among others), shelf life of fruits and vegetables, flavor and fragrance; (3) novel production of vaccines, pharmaceuticals, and therapeutic and prophylactic proteins; (4) reduced production of allergens; and (5) phytoremediation activity (James and Krattiger, 1996; Vasil, 2002, 2003; Nuñez-Palenius et al., 2005). The first transgenic commercial plant variety in the United States was released in May 1994, when Calgene marketed its Flavr-Savr™ delayed ripening tomato (James and Krattiger, 1996). At the present time, more than 60 transgenic crops have been approved for commercial planting, and at least 110 more are under field trials and/or regulatory review (Vasil, 2003, USDA-APHIS, 2007).

The influence of plant genetic engineering on commercial crop production is evident by the global increase of cultivated land with transgenic crops, also known as genetically modified crops (GMCs). This increase has happened in a relatively short time, that is, less than a decade. According to James (2006), the global land area of transgenic crops continued to grow for the 11th consecutive year in 2006, 13% or 12 million hectares (ha), compared with a 12% increase in usage in 2005. In 2006, 25% of the aggregate area of four main crops, that is, soybean (*Glycine max*), maize (*Zea mays*), cotton (*Gossypium* spp.), and canola (*Brassica napus x rapa*), totaling over one-quarter billion hectares was GMCs. The accumulated benefits during the period 1996–2005 were estimated as $27 billion ($13 billion for developing countries and $14 billion for industrial countries) (James, 2006); for instance, the total market for transgenic seed now exceeds $3.8 billion (Vasil, 2003; James, 2006).

Throughout a 10-year period, from 1996 to 2006, the land area of transgenic crops in the world increased 60 times, from 1.7 million ha in 1996 to 102 million ha in 2006 (Figure 1). Herbicide (Roundup) tolerance was the dominant trait used, followed by insect resistance conferred by *Bacillus thuringiensis* (Bt) toxin.

The highest yearly increase of global land area cultivated with transgenic crops was observed during the period 1997–1999 (Figure 2). Afterward, in 2000, a substantial decline in the rate of yearly increase took place. Nevertheless, it seems from more recent data (2001 to 2006) that global land area cultivated with GMCs will be increased, and it might be able to attain again similar levels as in previous years (James, 2001–2006).

In 2006, the United States cultivated more land area (54.6 million ha) in transgenic crops than any other nation...
In the same year, a growth rate of 8.8% in planted area of transgenic crops reflected a strong increase in both Bt toxin maize and herbicide tolerant maize, and continued growth in herbicide tolerant soybean (James, 2006). These transgenic plants, for the year 2006, were grown by 10.3 million farms in 22 different countries, 1.8 million more farms, and 1 more country than in 2005. Globally in 2006, the most common and commercialized transgenic crops were soybean (58.6 million ha), maize (25.2 million ha), cotton (13.4 million ha), and canola (4.8 million ha). Similarly, in 2006, herbicide tolerance installed in soybean, maize, canola, and cotton occupied 69.9 million ha (68%) of the global 102 million ha.

Thus, world land area planted with GMCs is increasing substantially every year. According to the Food and Agriculture Organization (FAO, 2004), global population reached 6 billion on October 12, 1999, and in view of current human population growth rates (1.5% per year), it is expected that world population will be approximately 11 billion by 2050 (Swaminathan, 1995). Doubling or tripling of the world food and fiber production by 2050 cannot be achieved using existing crop technology (James and Krattiger, 1996). Therefore, more research with plant molecular tools and transgenic crops must be accomplished in order to maintain such high human populations.

In Vitro Culture

In order to achieve a successful commercial application from biotechnology in melon, a competent de novo regeneration system from in vitro cultures is required (Guis et al., 1998). In the last 25 years, more than 40 in vitro melon regeneration protocols have been described, some using either organogenensis, somatic
embryogenesis, or both regeneration pathways (Tables 3, 4, and 5). Melon plant regeneration has been reported from adventitious buds, somatic embryos, shoot primordia, protoplasts, and axillary buds. Several biological and physical factors influence in vitro regeneration efficiency rate, and all have to be considered in order to develop a reproducible and reliable melon regeneration protocol.

Genetic Control

Due to the great genetic variability in melon (Monforte et al., 2003), genotype is the most important factor, determining regeneration potential. Melon varieties (reticulatus, cantaloupinensis, inodorus, flexuosus, etc.) and commercial cultivars have differences in their regeneration ability under the same in vitro protocol and environmental conditions (Orts et al., 1987, 1992; Gray, McColley, and Compton, 1993; Ficcadenti and Rotino, 1995; Molina and Nuez, 1995a; Kintzios and Taravira, 1997; Galperin, Patlis, et al., 2003). Likewise, organogenesis and somatic embryogenesis responses in melon cultures are also genotype dependent. For instance, Oridate et al. (1992) and Gray, McColley, and Compton (1993) reported that reticulatus varieties were more prone to produce in vitro somatic embryos than inodorus varieties.

Oridate et al. (1992) found significant differences in somatic embryogenic response from melon seeds among 18 commercial cultivars. They made reciprocal crosses between those cultivars, in order to obtain the F1 seeds and evaluate their embryogenic response. Some lines produced a large number of somatic embryos whereas others produce no somatic embryos or the response was very low. Moreover, these authors were able to transfer, by sexual crosses, the embryogenic regeneration response from superior to inferior responding cultivars, demonstrating that the capacity to de novo regenerate through somatic embryogenesis, by different melon cultivars, was under genetic control. These researchers were unable to determine the specific mode of inheritance of the somatic embryogenic capacity due to variation in the range of somatic embryogenesis from F2 seeds.

Gray, McColley, and Compton (1993) developed an improved protocol for high-frequency somatic embryogenesis from melon seeds. Using the cultivar “Male Sterile A 147,” the authors tested several factors, such as changes in plant hormone levels and combinations, type of culture media, and incubation time of explants in those media. This protocol was tested on 51 commercial melon cultivars, where all underwent somatic embryogenesis, but exhibited from 5% to 100% explant response and 0.1 to 20.2 embryos per explant, indicating again a genetic factor in melon embryogenesis response.

Plant regeneration through organogenesis is also affected by melon genotype (Orts et al., 1987; Ficcadenti and Rotino, 1995; Molina and Nuez, 1995a; Kintzios and Taravira, 1997; Galperin, Patlis, et al., 2003a). Orts et al. (1987) found significant differences in the morphogenetic response of a diverse group of melon cultivars. The percentage of calli with developed shoots ranged from 0 to 44.3 among cultivars. Variability in morphogenetic responses was found between seed lots of the same cultivar. Comparable results were obtained by Ficcadenti and Rotino (1995), who evaluated the morphogenetic response of 11 melon cultivars belonging to the reticulatus and inodorus genotypes. These authors found that melon morphogenetic response was affected by genetic background; that is, C. melo var. inodorus genotypes exhibited narrow shoot regeneration rates, whereas wide differences were noted among the reticulatus types. The number of shoots per explant ranged from 6.0 to 17.3 for reticulatus varieties and from 12.2 to 14.2 for inodorus genotypes.

A complete statistical approach was used by Molina and Nuez (1995a) to detect genotypic variability of the in vitro organogenesis (shoot regeneration) among individual melon seeds. Their results clearly evidenced the presence of highly significant differences for organogenetic response, among plants from a specific seed population. These authors used data from stochastic simulation to study the accuracy of different analyses to detect the presence of genotypic heterogeneity within a population. These analyses, together with their experimental results, allowed the separation of seed genotypes differing up to 5% in their regeneration ability. Afterwards, Molina and Nuez (1996) reported the inheritance of organogenesis response in melon cv. Charentais, by studying the distribution of the shoot regeneration frequency in F1 and F2 generations from parents representing extreme values for that in vitro organogenesis response. Their results suggested a genetic model with two genes, partial dominance, independent segregation, and similar effects for both genes. Recently, Galperin, Zelcer, and Kenigsbuch (2003) claimed that the high competence for adventitious regeneration in the BU-21/3 melon genotype was controlled by a single dominant locus, without cytoplasmic interactions.

Alternatively, Kintzios and Taravira (1997) evaluated 14 commercial melon cultivars for plant regeneration capability. Only six cultivars responded positively to a shoot induction treatment. Similarly, Galperin, Patlis, et al. (2003) screened 30 different commercial melon cultivars for shoot de novo regeneration. In 24 out of 30 melon genotypes, no detectable normal shoot growth was observed. Five of those that were able to regenerate, exhibited very low regeneration efficiency. Only the genotype BU-21, an inbred line, had profuse regeneration of multiple shoots.

In summary, melon in vitro response is under genetic control; however, other factors should be taken into account as well for melon regeneration. Among them, plant hormones have a paramount importance on the melon in vitro response.

Polyploidization and Somaclonal Variation

Diploid melon plants have 24 chromosomes (haploid stage n=x=12), and a genome size of 0.94 pg (454 Mbp/1C) (Arumuganathan and Earle, 1991). A natural and spontaneous increase in the ploidy level has been observed in field melon plants (Nugent and Ray, 1992); nevertheless, this ploidy increase can also be induced in muskmelon plants using chemical compounds, such as colchicine (Batra, 1952; Kubicki, 1962). In fact, polyploidy
### TABLE 3

Melon regeneration (shoots, roots, and/or complete plants) through direct organogenesis

<table>
<thead>
<tr>
<th>Explant source</th>
<th>Cultivar</th>
<th>Induction medium (Plant growth regulators)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledon and primary leaf</td>
<td>Banana, Dixie Jumbo, Planters Jumbo, Morgan, Cavaillon Red Flesh, and Saticoy Hybrid</td>
<td>BR-1 medium: 0.1 mg/L NOA, 20 mg/L 2iP, and 0.1 mg/L CCC, HC medium: 0.05 mg/L NOA, 10 mg/L 2iP, and 0.1 mg/L CCC</td>
<td>1</td>
</tr>
<tr>
<td>Hypocotyl from 11- to 13-day-old seedlings</td>
<td>Amarillo Oro</td>
<td>4.5 mg/L IAA</td>
<td>2</td>
</tr>
<tr>
<td>Leaf (0.3–0.5 cm) from 14-day-old seedlings</td>
<td>Pusa Sharbati</td>
<td>0.22 mg/L BA and 0.2 mg/L 2iP</td>
<td>3</td>
</tr>
<tr>
<td>Cotyledons from mature seeds, and cotyledons and leaves from 5- to 7-day-old seedlings</td>
<td>Halest Best, Iroquois, and Perlita</td>
<td>NAA and BA</td>
<td>4</td>
</tr>
<tr>
<td>Cotyledons from 4-day-old seedlings</td>
<td>Superstart, Hearts of Gold, Hale’s Best Jumbo, and Goldstart</td>
<td>0.88 mg/L IAA and 1.13 mg/L BA</td>
<td>6</td>
</tr>
<tr>
<td>Cotyledons from 4-day-old seedlings</td>
<td>Not reported</td>
<td>0.2 mg/L BA</td>
<td>7</td>
</tr>
<tr>
<td>Cotyledons from 9- to 10-day-old seedlings</td>
<td>Topmark</td>
<td>1 mg/L BA</td>
<td>8</td>
</tr>
<tr>
<td>Cotyledons from mature seed, cotyledons and hypocotyl from 10-day-old seedlings, and leaf segment and petioles from 3-week-old seedlings</td>
<td>Earl’s Favorite Harukei No. 3</td>
<td>0.01 mg/L 2,4-D or 1 mg/L IAA and 0.1 mg/L BA</td>
<td>9</td>
</tr>
<tr>
<td>Cotyledons from 8-day-old seedlings</td>
<td>Charentais and Gulfstream</td>
<td>1.12 mg/L BA for Charentais and 1.12 mg/L BA and 1.75 mg/L IAA for Gulfstream</td>
<td>10</td>
</tr>
<tr>
<td>Cotyledons from 2-day-old seedlings</td>
<td>Sunday Aki</td>
<td>1 mg/L BA, 50–200 µM salicylic acid and 10 mM proline</td>
<td>11</td>
</tr>
<tr>
<td>Cotyledons from 7-day-old seedlings</td>
<td>Five inbred lines from Teziers</td>
<td>0.1 mg/L NAA and 0.5 mg/L BA</td>
<td>12</td>
</tr>
<tr>
<td>Cotyledons from immature seeds</td>
<td>Miniloup, L-14, and B-Line</td>
<td>2.25 mg/L BA</td>
<td>13</td>
</tr>
<tr>
<td>Cotyledons from mature seeds</td>
<td>Prince and Andes</td>
<td>1 mg/L BA</td>
<td>14</td>
</tr>
<tr>
<td>Cotyledons from 4-day-old seedlings</td>
<td>11 genotypes</td>
<td>0.63 mg/L BA and 0.26 mg/L ABA</td>
<td>15</td>
</tr>
<tr>
<td>3- to 4-cm expanded leaves</td>
<td>Hale’s Best Jumbo and Ananas El Dokki</td>
<td>0.87 mg/L IAA, 1.13 mg/L BA and 0.026 mg/L ABA</td>
<td>16</td>
</tr>
<tr>
<td>Cotyledons from 7-day-old seedlings</td>
<td>Pusa Madhuras</td>
<td>0.22 mg/L BA</td>
<td>17</td>
</tr>
</tbody>
</table>

(Continued on next page)
TABLE 3
Melon regeneration (shoots, roots, and/or complete plants) through direct organogenesis (Continued)

<table>
<thead>
<tr>
<th>Explant source</th>
<th>Cultivar</th>
<th>Induction medium</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledons from 2-week-old seedlings</td>
<td>14 cultivars</td>
<td>Embryogenesis: 1.98 mg/L 2,4-D and 4.99 mg/L Kin, Organogenesis: 0.01 mg/L 2,4-D and 0.059 mg/L BA</td>
<td>18</td>
</tr>
<tr>
<td>Leaves from 10 day-old seedlings</td>
<td>Védrantais</td>
<td>0.22 mg/L BA and 0.33 mg/L 2iP</td>
<td>19</td>
</tr>
<tr>
<td>Cotyledons from 2-day-old seedlings</td>
<td>Galia male and female parental lines</td>
<td>0.001 mg/L NAA and 1 mg/L BA</td>
<td>20</td>
</tr>
<tr>
<td>Cotyledons from mature seeds</td>
<td>Yellow Queen, Yellow King, and Hybrid AF-222</td>
<td>1 mg/L BA</td>
<td>21</td>
</tr>
<tr>
<td>Proximal zone of the Hypocotyl from 4-day-old seedlings</td>
<td>Revigal</td>
<td>1 mg/L BA</td>
<td>22</td>
</tr>
<tr>
<td>Cotyledons from 4- to 5-day-old seedlings</td>
<td>Some Turkish cultivars: Hasanbey I, Yuva, Kirkagac 637, Topatan, Kuscular, and Ananas</td>
<td>Medium and plant growth regulators from reference 6 (see footnote below)</td>
<td>23</td>
</tr>
<tr>
<td>Cotyledons from mature seeds</td>
<td>Thirty melon genotypes</td>
<td>1 mg/L BA</td>
<td>24</td>
</tr>
</tbody>
</table>

Note: ‡Plant growth regulators: NOA (beta-naphthoxyacetic acid), 2iP [N⁶-(2-isopentenyl) adenine], Kin [6-furfurylaminopurine (kinetin)], and CCC [chlormequatchlorid (cycocel)].


as a method of plant breeding received increased attention in the late 1930s, when it was discovered that polyploidy could be induced with colchicine treatment and the earlier demonstration that heat treatment in early embryogeny stages could also induce a chromosome doubling (Batra, 1952).

Numerous tetraploid and triploid muskmelon plants have been obtained since the 1930s (Batra, 1952; Kubicki, 1962; Ezura, et al., 1992a, 1992b; Fassuliotis and Nelson, 1992; Nugent and Ray, 1992; Adelberg, 1993; Nugent, 1994a; Nugent, 1994b; Adelberg et al., 1995; Adelberg et al., 1999). According to Ezura et al. (1992a, 1992b), Fassuliotis and Nelson (1992), and Nugent (1994b), tetraploid melon plants are characterized by having large male and hermaphrodite flowers, protruding stigmas, low fertility, thickened and leathery leaves, rounded cotyledons, highly pubescent leaves and stems, short internodes, flat fruits, a large blossom-end scar, increased number of vein tracts on the fruit, and round seeds. Nonetheless, Shifriss (1941) had previously reported that tetraploid melon plants were highly fertile and no later in maturity than the ordinary diploids. Moreover, Batra (1952) observed that the quality of melon tetraploids was superior to diploids in certain varieties and that tetraploids were sufficiently fertile to be propagated readily from seeds.

Regarding the fruit quality of tetraploid melon plants, Batra (1952) and Nugent (1994a, 1994b) reported that tetraploid fruits were superior in sugar level and firmness, and had better color than diploid fruits. However, tetraploid melon plants were less productive, because they had smaller and flatter fruits than diploids. Most cultivars depicted low fertility, and the fruits had an increased tendency to crack, therefore reducing considerably their marketable properties.

As mentioned earlier, triploid melon plants have also been produced (Ezura, Amagai, and Oosawa, 1993; Adelberg et al., 1995, 1999). Despite that the triploids plants were more vigorous than the diploids, their fruits were not as flat as tetraploids. These triploids melon plants did not have any marketable advantage over diploids, because the percentage of cracking in the triploid fruits was still greater than that in diploid fruits, and their sugar content was lower. In addition, triploid plants required adjacent diploid pollinators, because they did not set fruit when self-pollinated (Ezura, Amagai, and Oosawa, 1993; Adelberg et al., 1995).
### TABLE 4

Melon regeneration through indirect organogenesis

<table>
<thead>
<tr>
<th>Explant source</th>
<th>Cultivar</th>
<th>Callus induction medium (CIM) and shoot induction medium (SIM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledon-callus culture from 11- to 13-day-old seedlings</td>
<td>Amarillo Oro</td>
<td>CIM: 1.5 mg/L IAA and 6.0 mg/L KIN SIM: 0.01 mg/L NAA and 0.1 mg/L BA</td>
<td>1</td>
</tr>
<tr>
<td>Hypocotyl-callus culture from 7-day-old seedlings</td>
<td>Pusa Sharbati</td>
<td>CIM: 1.0 mg/L IAA and 0.5 mg/L KIN SIM: 0.5 mg/L BA and 0.5 mg/L 2iP</td>
<td>2</td>
</tr>
<tr>
<td>Cotyledon-callus from 7- to 9-day-old seedlings</td>
<td>Charentais T, Doublon, CM 17 187, and Piboule</td>
<td>CIM: 2 mg/L IAA and 2 mg/L KIN SIM: no plant growth regulators</td>
<td>3</td>
</tr>
<tr>
<td>Cotyledon-callus from 11- to 13-day-old seedlings</td>
<td>15 cultivars belonging to cantaloupensis, inodorus, and reticulatus varieties</td>
<td>CIM: 6.0 mg/L KIN and 1.5 mg/L IAA SIM: Same as CIM</td>
<td>4</td>
</tr>
<tr>
<td>Cotyledon-protoplasts from 2-week-old seedlings</td>
<td>Hong-Xin-cui</td>
<td>Protoplast Culture Medium and CIM: 0.5 mg/L 2,4-D, 0.5 mg/L Zeatin and 0.5 mg/L BA SIM: 0.3 mg/L 2,4-D, 1.0 mg/L Zeatin and 0.5 mg/L BA</td>
<td>5</td>
</tr>
<tr>
<td>Leaf segment (1.0 x 0.5 cm) from 8- to 10-day-old seedlings and petiole segment (0.4–0.8 cm) from 3- to 4-week-old seedlings</td>
<td>Cantaloupe PMR</td>
<td>CIM: 5.0 mg/L NAA and 2.5 mg/L BA SIM: no plant growth regulators</td>
<td>6</td>
</tr>
<tr>
<td>Cotyledon-protoplasts from 2-week-old seedlings</td>
<td>Charentais</td>
<td>Protoplast Culture Medium and CIM: 0.05 mg/L 2,4-D and 0.5 mg/L BA SIM: 2 mg/L BA</td>
<td>7</td>
</tr>
<tr>
<td>Cotyledon-protoplasts from 12-day-old seedlings and fully expanded leaves-protoplasts from 3-week-old seedlings</td>
<td>Charentais T and F1 hybrid cv. Preco</td>
<td>Protoplast Culture Medium and CIM: 0.75 mg/L BA SIM: 1.0 mg/L 2,4-D and 0.1 mg/L BA</td>
<td>8</td>
</tr>
<tr>
<td>Root-callus culture from 21-day-old seedlings</td>
<td>Pusa Sharbati</td>
<td>CIM: 0.61 mg/L 2iP and 0.68 mg/L BA SIM: 0.22 mg/L BA</td>
<td>9</td>
</tr>
<tr>
<td>Cotyledon and Hypocotyl from 11- to 13-day-old seedlings</td>
<td>Charentais</td>
<td>CIM: 2.5 mg/L NAA and 1 mg/L BA SIM: 0.01 mg/L NAA and 6 mg/L Kin</td>
<td>10</td>
</tr>
</tbody>
</table>

1Plant hormones: Kin [6-furfurylaminopurine (kinetin)].


When modern biotechnology, specifically plant tissue culture, was applied to *C. melo* in order to obtain reliable regeneration protocols, somaclonal variation was a common observable fact, therefore tetraploid, octaploid, mixoploid, and aneuploid melon plants were easily recovered from in vitro cultures (Bouabdallah and Branchard, 1986; Debeaujon and Branchard, 1992; Ezura et al., 1992a, 1992b; Fassuliotis and Nelson, 1992; Kathal et al., 1992; Ezura and Oosawa, 1994a; Ezura, Kikuta, and Oosawa, 1994). According to Ezura et al. (1995), somaclonal variation could be used to obtain variants lines with low-temperature germinability in melon. Changes in fatty acid patterns (Halder and Gadgil, 1984) and changes in repetitive DNA sequences (Grisvard et al., 1990) have been found in melon callus tissue. However, somaclonal variation has to be avoided in research, where genetic transformation is involved because genomic stability in transgenic plants has to be maintained in order to express the inserted transgene. In addition, regeneration of melon plants has never been achieved from long-term callus cultures of *C. melo* (Grisvard et al., 1990).

The production of tetraploid regenerated melon plants has been observed from somatic embryogenesis (Ezura et al., 1992a, 1992b), organogenesis (Bouabdallah and Branchard, 1986; Fassuliotis and Nelson, 1992; Ezura et al., 1992a), and protoplast cultures (Debeaujon and Branchard, 1992). Nevertheless, each morphogenetic pathway has a different effect on the frequency of recovered tetraploid plants, that is, somatic embryogenesis.
<table>
<thead>
<tr>
<th>Explant source</th>
<th>Cultivar</th>
<th>Induction medium (IM) and development Medium (DM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotyledons from 5-day-old seedlings</td>
<td>Hale’s Best No. 36 and Rocky Ford</td>
<td>IM: 1.0 mg/L 2,4-D and 0.5 mg/L BA; DM: 0.5 mg/L 2,4-D and 0.25 mg/L BA</td>
<td>1</td>
</tr>
<tr>
<td>Expanded cotyledons</td>
<td>Charentais T</td>
<td>IM: 4.52 mg/L 2,4-D and 0.44 mg/L BA; DM: no hormones</td>
<td>2</td>
</tr>
<tr>
<td>Cotyledons from mature seeds and cotyledons and hypocotyls from 10-day-old seedlings, leaves and petioles from 3-week-old plantlets</td>
<td>Earl’s Favourite Harukei No. 3</td>
<td>IM: 2.0 mg/L 2,4-D or 25 mg/L IAA; DM: no hormones</td>
<td>3</td>
</tr>
<tr>
<td>Cotyledons and hypocotyls from mature seeds</td>
<td>Green Pearl and Earl’s Favourite</td>
<td>IM: 4 mg/L 2,4-D, 2 mg/L NAA, and 0.1 mg/L BA; DM: no hormones</td>
<td>4</td>
</tr>
<tr>
<td>Hypocotyls from mature seeds</td>
<td>Earl’s Favourite</td>
<td>IM: 1 mg/L 2,4-D, 1 to 4 mg/L NAA, and 0.1 mg/L BA; DM: no hormones</td>
<td>5</td>
</tr>
<tr>
<td>Cotyledons from 1-day-old seedlings</td>
<td>Earl’s Favourite Haru 1</td>
<td>IM: 1 mg/L 2,4-D, 1 mg/L NAA, and 0.1 mg/L BA; DM: no hormones</td>
<td>6</td>
</tr>
<tr>
<td>Protoplasts from 12-day-old cotyledons</td>
<td>Charentais T and F1 hybrid Preco</td>
<td>IM: 1 mg/L 2,4-D and 0.1 mg/L BA; DM: no hormones</td>
<td>7</td>
</tr>
<tr>
<td>Cotyledons and hypocotyls from mature seeds</td>
<td>18 cultivars belonging to reticulatus, inodorus, makuwa and intermediated type between reticulatus and cantaloupensis varieties</td>
<td>IM: 3.0 mg/L 2,4-D and 0.1 mg/L BA; DM: no hormones</td>
<td>8</td>
</tr>
<tr>
<td>Cotyledons from mature seeds</td>
<td>52 cultivars</td>
<td>IM: 5 mg/L 2,4-D and 0.1 mg/L TDZ; DM: no hormones</td>
<td>9</td>
</tr>
<tr>
<td>Mature seeds</td>
<td>Earl’s Favourite</td>
<td>IM: 4 μg/L 2,4-D and 0.1 μg/L BA; DM: no hormones</td>
<td>10</td>
</tr>
<tr>
<td>Hypocotyls from imbibed seeds</td>
<td>Prince and Sunday Aki</td>
<td>IM: 1 mg/L 2,4-D, 2 mg/L NAA and 0.1 mg/L BA; DM: no hormones</td>
<td>11</td>
</tr>
<tr>
<td>Cotyledons from mature seeds</td>
<td>Vérdantais</td>
<td>IM: 2.2 mg/L 2,4-D and 0.11 mg/L BA; DM: no hormones</td>
<td>12</td>
</tr>
<tr>
<td>Cotyledons from mature seeds</td>
<td>Yellow Queen and Yellow King</td>
<td>IM: 5.0 mg/L 2,4-D and 0.075 mg/L TDZ; DM: no hormones</td>
<td>13</td>
</tr>
<tr>
<td>Mature seeds</td>
<td>Vérdantais and Earl’s Favourite Fuyu A</td>
<td>IM: 2.0 mg/L 2,4-D and 0.1 mg/L BA; DM: no hormones</td>
<td>14</td>
</tr>
</tbody>
</table>

plants the average stomata length was 22.1 µm and the average number of chloroplasts inside guard cells was 9.4 in diploid melon cells to generate in vitro shoots was greater than tetraploid cells. The ability of tetraploid cells to differentiate into somatic embryos was greater than diploid cells. These same authors reported that the ability of somatic embryos to develop into plantlets decreased in the following order: diploid > tetraploid > octaploid. Ezura and Oosawa (1994a) and Kathal, Bhatnagar, and Bhojwani, (1992) reported the longer melon cells are kept under in vitro conditions, the greater the possibility to increase the ploidy levels in those cells. The frequency of chromosomal variation leading to aneuploid (hyperploid and hypoploid) plants at diploid, tetraploid, and octaploid levels also increases.

Melon plant ploidy levels can be determined by cytological methods, such as counting the chromosome number using root squash tips (Ezura et al., 1992a, 1992b; Kathal, Bhatnagar, and Bhojwani, 1992; Ezura, Amagai, and Oosawa, 1993; Ezura, Kikuta, and Oosawa, 1994; Adelberg et al., 1995) or young tendrils (Yadav and Grumet, 1994). These methods are very laborious and time consuming, because melon chromosomes are smaller in comparison with other plants, which complicate chromosome observation. Unconventional and indirect techniques have been developed in order to determine the ploidy level of regenerated melon plants, although they are not as reliable as chromosome counting methods (Fassuliotis and Nelson, 1992; Adelberg et al., 1994, 1995). Among them, pollen grain shape and stomata length, as well as the chloroplast number in guard cells from stomata have been commonly used. Diploid plants have pollen grains with typical triangular-appearing shape and are tripolar, whereas tetraploid and mixoploid regenerants from mature cotyledons, whereas explants from apical meristems produced fewer or no tetraploid plants (Adelberg et al., 1994, 1995, 1999).

Ezura and Oosawa (1994a, 1994b) reported that the capacity of diploid melon cells to generate in vitro shoots was greater than tetraploid cells. The ability of tetraploid cells to differentiate into somatic embryos was greater than diploid cells. Therefore, when a callus stage is involved in the regeneration process, the likelihood to augment the ploidy level in the regenerated plant is increased as well. In addition, explant origin affects the frequency of tetraploid plants from melon tissue cultures (Adelberg et al., 1994). Immature cotyledons produced more tetraploid regenerants than mature cotyledons, whereas explants from apical meristems produced fewer or no tetraploid plants (Adelberg et al., 1994, 1995, 1999).

In order to avoid somaclonal variation and regenerate mostly diploid plants from melon in vitro cultures, several strategies have been proposed. Among them, the induction of shoot primordium aggregates from shoot-tips, then cultivating them in liquid medium shaken at low speed, has been proposed (Ezura, Kikuta, and Oosawa, 1997). Using this protocol, the frequency of tetraploids and mixoploids regenerated plants was less than 8% after 4 years of culture. The cryopreservation of shoot primordia cultures at low temperatures (liquid nitrogen) using a slow prefreezing procedure has given excellent results as well (Niwata et al., 1991; Ogawa et al., 1997). A reliable system for transformation of a cantaloupe Charentais-type melon leading to a majority of diploid regenerants was developed (Guis et al., 2000). Unfortunately, this regeneration system did not generate completely developed transgenic shoots for other commercial melon cultivars (Nuñez-Palenius, Cantliffe, and Klee, 2002; Gaba, 2002, personal communication); again, a genetic factor is involved at some stage in melon culture in vitro response.

In summary, it is particularly important to avoid in vitro conditions that produce polyploid melon plants or other induced somaclonal variations, in order to maintain commercial marketability of the new genotype.

Vitrification

Woody and herbaceous explants are prone to suffer anatomical, morphological and physiological abnormalities when they are cultivated in vitro. Several terms have been used to describe these abnormalities, such as vitrification, translucency, hyperhydration, succulency and glassiness (Paques and Boxus, 1987, Ziv, 1991). Vitrification is the most often used term to describe physical changes in leaves and roots of cultured explants (Paques, Boxus, and Dulos, 1987).

In vitro melon cultures are very sensitive to undergo spontaneous vitrification, even if explants are cultured on non-inductive media or conditions (Leshem, Shaley, and Izhar, 1988; Leshem, Werker, and Shaley, 1988b). Different factors have been proposed to induce and maintain an explant in a vitrified state, such as, high relative humidity inside the in vitro container, high water potential of the media, low agar level, deficiency in Ca²⁺ level, high NH₄ concentration, presence of ethylene within the flask, and a high level of cytokinins (mostly N⁶-benzyladene, BA) (Leshem, Shaley, and Izhar, 1988a; Leshem, Werker, and Shaley, 1988b; Paques and Boxus, 1987; Paques, Boxus, and Dulos, 1987; Ziv, 1991). Leshem, Shaley, and Izhar studied the development of vitrification in melon shoot tips cultured in solid and liquid media. These authors found that on solid medium the vitrification process gradually increased with time, whereas on liquid medium it was an “all-or nothing” effect. Cytokinins had the major effect on vitrification induction on melon buds as well (Leshem, Werker, and Shaley, 1988b). Paques, Boxus, and Dulos (1987) and Kathal, Bhatnagar, and Bhojwani (1994) reported that vitrification process was an inducible and reversible
physiological event. If the tissues are frequently subcultured, vitrification may be avoided; however, this may induce somaclonal variation.

The following modifications in the culture media have been suggested to avoid or prevent vitrification: increasing the agar concentration; diminishing chloride ions; reducing potassium; increasing calcium; adding cobalt; modifying the plant hormone balance by reducing the amount or type of cytokinins; suppressing the use of casein hydrolysate and adenine sulfate; and adding pectin, phoroglucinol, or phloridzin (Paques and Boxus, 1987; Paques, Boxus, and Dulos, 1987; Ziv, 1991). In vitro environmental conditions can also be altered to reduce vitrification, including a cold treatment to the plants before in vitro culture, reducing culture room temperature, increasing the daily dark period, increasing the container-environment gas exchange, and reducing the relative humidity within the flask (Paques and Boxus, 1987; Paques, Boxus, and Dulos, 1987; Ziv, 1991).

Regeneration by Organogenesis

In general, a cytokinin/auxin ratio greater than 1 is used in order to induce de novo bud formation; however, auxins are not always a prerequisite to achieve that goal (Tables 3 and 4), and cytokinins alone are able to induce bud formation. Among cytokinins, BA is the most frequently used in high levels (1 mg/L or higher) to induce bud formation. BA concentration is lowered (0.5 mg/L or lower) to allow shoot elongation. Elongated shoots are then transferred to a plant growth regulator-free medium or with low-auxin level [1-naphthaleneacetic acid (NAA) or indole-3-acetic acid (IAA)] to induce the rooting process. If indirect regeneration is used, a two- or even a three-step method has to be utilized. First, an induction callus growth is stimulated on the explant by applying strong cytokinins [thidiazuron (TDZ)] and auxins [2,4-dichlorophenoxy-acetic acid (2,4-D)] to the medium culture. Second, those calli, which have green nodules are transferred to a low-level plant hormone medium to induce shoot differentiation. Third, differentiated shoots are cultured in a low-cytokinins medium to induce shoot elongation (Table 4). In general, indirect regeneration is longer in time than direct regeneration, to recover a whole regenerated plant. This is due to the several subcultures required for shoot elongation and 2 or 3 months are needed for rooting using the indirect method (Moreno, Garciasogo et al., 1985; 1985b; Kathal, Bhatnagar, and Bhojwani, 1986; Kathal, Bhatnagar, and Bhojwani, 1994). Other plant hormones, such as gibberellins, and abscisic acid (ABA) (Kathal, Bhatnagar, and Bhojwani, 1986; Niedz et al., 1989; Piccadenti and Rotino, 1995), and/or plant additives, such as proline, proline analogues, ornithine, salicylic acid, aspirin, and fish protein hydrolysates (Shetty et al., 1992; Milazzo et al., 1998, 1999), calcium antagonists (Leshem and Lurie, 1995), and silver nitrate (Niedz et al., 1989; Rousan, Latche, and Fallot, 1992; Yadav, Saleh, and Grumet, 1996) have been used to increase in vitro shoot regeneration frequency. Nevertheless, the obtained results using both types of components (plant hormones and plant additives) are either melon genotype dependent or are not consistent among reported results.

Alternatively, environmental factors, such as light, temperature, nature of the culture media gelling agent, and relative humidity within the culture flask, influence the efficiency of regeneration method (Niedz et al., 1989; Piccadenti and Rotino, 1995; Yadav, Saleh, and Grumet, 1996; Kintzios and Taravira, 1997; Curuk et al., 2003). For instance, Niedz et al. studied the effect of temperature (22°C, 25°C, and 29°C) and light (0, 5, 10, 30, 60, and 3,000 µmol/m2/s) on the percentage of bud initiation in cotyledonary explants of “Hale’s Best Jumbo” melon. The greatest bud initiation value was obtained when explants were cultured at 29°C under a range of light intensities of 5 to 30 µmol/m2/s. Conversely, lower temperatures (22°C and 25°C), darkness, and higher light intensities (60 and 3,000 µmol/m2/s) reduced bud initiation. Similarly, Kintzios and Taravira (1997) tested two levels of light intensity, 50 and 250 µmol/m2/s, on shoot and root induction in 14 different melon cultivars. As expected, lower light intensities induced a greater root induction in many melon cultivars. Also, higher photosynthetic photon flux density (PPFD) (250 µmol/m2/s) values adversely affected shoot induction from cotyledonary explants. Interestingly, Curuk et al. (2003) recently described that shoot
regeneration from hypocotyls (proximal part to the cotyledons) of *Cucumis* species does not require light.

High relative humidity within the culture flask might induce ethylene accumulation, which affects shoot regeneration in melon cotyledons (Roustan, Latche, and Fallot, 1992). These authors added several levels of silver nitrate (60-120 µM AgNO₃) into culture media to inhibit ethylene action. They were able to obtain a twofold increase in shoot regeneration by using silver. Furthermore, a transgenic antisense ACC oxidase line, which had little ethylene production, displayed a 3.5-fold increase in regeneration frequency compared to a wild-type line (Amor et al., 1998).

As pointed out previously, the nature of medium gelling agent also has an important role in melon regeneration (Ficcadenti and Rotino, 1995; Yadav, Saleh, and Grumet, 1996). Ficcadenti and Rotino (1995) reported that using agar, instead of "gelrite," they were able to attain a better cotyledon organogenetic response. Likewise, Yadav, Saleh, and Grumet preferred "phytagel," as a substitute of agar for organogenesis from leaf explants.

**Regeneration by Somatic Embryogenesis**

In addition to organogenesis, somatic embryogenesis is an alternative de novo morphogenetic pathway that can be used to recover whole plants (Liu and Cantliffe, 1983). Somatic embryogenesis was described in melon explants before organogenesis and used to regenerate complete melon plants Table 5 (Blackmond, Reynolds, and Postek, 1981b). In general, cotyledonal tissue has been the most efficient explant for the induction of melon somatic embryogenesis (Table 5).

Embryogenic response in melon is affected by the nature of explant and genotype. Gray, Mccolley, and Compton (1993) reported significant differences in the frequency of embryogenic muskmelon explants, when they compared two commercial sources of the same melon cultivar. High regeneration frequency, up to 100%, may be attained from cotyledon explants with an average number of 20.2 embryos per explant (Gray, Mccolley, and Compton, 1993). However, not all melon cultivars achieve such a high regeneration frequency, some as low as 5% with 0.1 embryos per explant (Gray, Mccolley, and Compton, 1993). The conversion rate of somatic embryos to plantlets might be a limiting step in some melon genotypes, for example, Trulson and Shahin (1986) were able to recover only five melon plants from hundreds of somatic embryos, whereas Branchard and Chateau (1988) reported a 12% conversion rate, and Homma, Sugiyama, and Oosawa (1991) recorded a conversion rate from 7% to 61%, depending on explant type.

Tabei, Kanno, and Nishio (1991) concluded that cotyledons were the best explant to induce somatic embryogenesis in the melon cultivar Earl’s Favourite Harukei No.3 using high concentrations of 2,4-D. Homma, Sugiyama, and Oosawa (1991) tested the effects of explant shape on the production of melon somatic embryos, finding that the most reproducible results were obtained with explants that consisted of radicle, hypocotyls, and a proximal part of cotyledon. Oridate et al. (1992) found significant differences in somatic embryogenesis capability from 18 different melon cultivars from four genotypes. These authors concluded that genetic differences in somatic embryogenic formation capacity existed among cultivars rather than among genotypes.

Debeaujon and Branchard (1993) published a complete and extensive review on somatic embryogenesis in Cucurbitaceae, including *C. melo*, where they concluded that even though somatic embryogenesis and plant recovery have been obtained from numerous plant sources including protoplasts, the best results were observed with explants coming from seedling parts, especially cotyledons and hypocotyls. These authors also reported that the genetic constitution of donor plants seemed to play a key role in the success of somatic embryogenesis.

**Medium Composition and Environmental Factors**

Media composition (mostly plant growth regulators) has a great effect on melon somatic embryogenesis. The embryogenic pathway involves a two-stage protocol: first, explants are cultured onto an "induction" medium, to which auxins have been added; and second, "induced" explants are transferred to development media, where full and complete normal embryo development takes place in the absence of the induction hormone. Auxins are prerequisite for induction of somatic embryogenesis (Tabei, Kanno, and Nishio, 1991; Oridate, Atsumi, Ito, and Araki, 1992; Debeaujon and Branchard, 1993; Gray, Mccolley, and Compton, 1993; Guis, Latche, et al., 1997; Nakagawa et al., 2001).

In general, the most common and efficient auxin to induce somatic embryogenesis in melon explants is 2,4-D (Oridate and Oosawa, 1986; Debeaujon and Branchard, 1993). However, other auxins can be used, such as IAA and NAA, although NAA at high concentrations can induce abnormal embryo growth (Tabei, Kanno, and Nishio, 1991). Likewise, Tabei, Kanno, and Nishio reported that IAA was the most efficient auxin to induce somatic embryogenesis in "Earl’s Favourite Harukei No.3" melon. Auxins can be used in combination with cytokinins, such as BA and TDZ, and/or other hormones, such as ABA (Trulson and Shahin, 1986; Tabei, Kanno, and Nishio; Homma, Sugiyama, and Oosawa, 1991; Debeaujon and Branchard, 1992; Gray, Mccolley, and Compton, 1993; Guis, Latche, et al., 1997; Nakagawa et al., 2001). Hormones are removed to mature the embryos (Table 5); nevertheless gibberellins can be added to the culture medium (Tabei, Kanno, and Nishio, 1991). ABA (10 mg/L) was supplemented into the culture medium in order to control the desiccation process and to increase the survival rate of somatic embryos before cryopreservation (Shimonishi et al., 1991).

The type and concentration of carbohydrate in the media plays a role in somatic embryogenesis in melon (Oridate and Yasawa, 1990; Debeaujon and Branchard, 1992; Gray,
Haploid Plants and Zygotic Embryo Culture

Mccolley, and Compton, 1993; Guis, Latche, et al., 1997). Oridate and Yasawa (1990) reported that a complex combination of different sugars, such as sucrose, glucose, fructose, and galactose, led to the highest rate of somatic embryogenesis. Similarly, Gray, Mccolley, and Compton (1993) concluded that the sucrose concentration in embryo induction and development media had a profound effect on somatic embryogenesis; that is, 3% sucrose produced a greater explant response than lower or higher levels of the carbohydrate. These authors found that sucrose concentration also exerted an effect on the relative percentage of somatic embryo stages recovered and on abnormal embryo development and precocious germination. Guis, Latche, et al. (1997, 1999) tested the effects of several levels of sucrose, glucose, and maltose on inducing somatic monosomic embryogenesis. Glucose enhanced the embryogenic response by almost twofold, whereas maltose at any level totally reduced somatic embryogenesis. Nakagawa et al. (2001) reported that the addition of mannitol to the initial media increased the frequency of somatic embryogenesis in “Prince” melon.

Several physical factors can affect melon somatic embryogenesis, among them, presence and quality of light, and physical state of media culture being the most important. In order to induce somatic embryogenesis, melon explants are cultured in light (Trulson and Shahin 1986; Branchard and Chateau, 1988; Homma, Sugiyama, and Oosawa, 1991; Shimonisashi et al., 1991; Tabei, Kanno, and Nishio, 1991; Debeaujon and Branchard, 1992; Ezura et al., 1992a; Oridate et al., 1992; Debeaujon and Branchard, 1993; Hosoi et al., 1994; and Nakagawa et al., 2001). Somatic embryo formation has been augmented by pretreatment in a dark period, usually 1 or 2 weeks before placing in light (Gray, Mccolley, and Compton, 1993; Guis, Latche, et al., 1997). Culturing on solid medium (Branchard and Chateau, 1988) is better than using liquid medium. Different gelling agents have been used for this purpose, obtaining improved developed embryos by using “gelrite” and/or “phytigel” as a substitute for agar (Branchard and Chateau, 1988). Nevertheless, recently Akasaka-Kennedy, Tomita, and Ezura (2004) reported that somatic embryos from “Vedrantais” and “Earl’s Favourite Fuyu A” melon cultivars underwent development without vitrification, if agar was used instead of gelrite. Thus different melon cultivars give diverse responses under similar in vitro conditions. Kagayama, Yabe, and Miyajima (1991); and Moreno, Garcia-Gómez et al. (1985) reported that the vitrification state of regenerated plants is increased if liquid cultures are used during the initial steps of somatic embryogenesis. Kagayama, Yabe, and Miyajima reported that consecutive washing of somatic embryos with hormone-free MS medium, with 0.5% activated charcoal, increased twofold the number of somatic embryos.

Haploid Plants and Zygotic Embryo Culture

Hybrid cultivars represent the F1 progeny of crosses that may involve inbred lines, clones, or populations (Fehr, 1987). The most common type of hybrid cultivar is produced by crossing two or more inbred lines, which have to be homozygous for certain important traits (Fehr, 1987). The production of inbred lines in C. melo requires several generations, taking more than 7 years of inbreeding in order to obtain homozygosis (Yashiro et al., 2002). Through using a plant biotechnology approach, such as production of haploid melon plants, it is possible to reduce the time required to obtain inbred melon lines. Double-haploid plants can be induced by chromosome doubling agents, such as colchicine or oryzalin (Yetisir and Sari, 2003; Lotfi et al., 2003). This approach has been used to obtain plants tolerant to diseases, such as virus or powdery mildew (Kuzuya et al., 2000, 2002, 2003; Lotfi et al., 2003).

According to Guis et al. (1998), using androgenesis and gynogenesis has not been successful in producing haploid melon plants. Using either gamma- or soft X-ray-irradiated pollen will induce in situ gynogenetic haploid parthenogenesis in melon (Sauton and Dumax de Vaulx 1987; Cuny et al., 1992, 1993; Yanmaz, Elliali-Toğlu, and Taner, 1999; Kuzuya et al., 2000). Sauton and Dumax de Vaulx (1987) developed an in vitro technique (commonly named embryo rescue or embryo culture) to recover muskmelon haploid plants. These authors obtained haploid plants after pollination of hermaphrodite flowers with irradiated (Coγ-rays) pollen and subsequent in vitro culture of ovules or immature embryos. They also developed a new culture medium to allow further development of these embryos into plants, resulting in an average number of 2.5 haploid embryos per 100 seeds. Sauton and Dumax de Vaulx’s embryo culture technique has been applied not only to induce and rescue haploid melon plants but also to culture diploid embryos in numerous melon cultivars with excellent results (Sauton, 1988; Kuzuya et al., 2000; Oliver et al., 2000; Kuzuya et al., 2002; Kuzuya et al., 2003; Lotfi et al., 2003; Yetisir et al., 2003; Nuñez-Palenius, Klee, and Cantliffe, 2006b).

The rate of melon haploid production is affected by genotypic factors and environmental growth conditions of donor plants (Sauton, 1988, Cuny et al., 1992, 1993; Yanmaz, Elliali-Toğlu, and Taner, 1999). Sauton (1988) studied haploid embryo production on seven melon cultivars, belonging to five melon types, that is, reticulatus, cantaloupeensis, inodorus, comonon, and acidulus. Gynogenetic haploid embryo production among those melon genotypes ranged from 0 to 1.7%. Cuny et al. (1993) reported significant differences in haploid embryo production between two melon cultivars: “Vedrantais” which produced an average number of haploid embryos of 3.5%, whereas “F1.G1” formed 1.7%. Sauton (1988) found that “Arizona” muskmelon produced the highest number of haploid embryos (3%) if melon plants were grown during the summer season. Cuny et al. (1992) reported the effect of season planting on haploid embryo induction. These authors found that haploid embryo induction was greater in “Vedrantais” melon fruits obtained from plants grown in summer than in those harvested in autumn.

Unlike other plant systems, such as carrot (Daucus carota) and tobacco (Nicotiana tabacum), melon pollen is able to tolerate high gamma-irradiation doses (up to 3,600 Grays) and still...
germinate—in vitro as well as in vivo—and inducing partheno-
carpic haploid plant production (Cuny et al., 1992, 1993; Yan-
maz, Elliiitioliou, and Taneer, 1999). However, a significant
reduction in pollen tube length has been observed using high
radiation doses. This reduction was proportional to the amount
of gamma-radiation used (Cuny et al., 1992, 1993; Yanmaz,
Elliiitioliou, and Taneer, 1999). High gamma-irradiation doses
can induce an increase in the percentage of necrotic haploid
embryos. The most common gamma irradiation doses are be-
tween 250 and 350 Grays (Sauton and Dumax de Vaulx, 1987;
Cuny et al., 1992, 1993; Beharav and Cohen, 1995b; Yanmaz,
Elliiitioliou, and Taneer, 1999; Lotfi et al., 2003; Yetisir and Sari,
2003), and for soft-X-rays the doses are between 65 and 130 kR

Niemierowicz-Szcztt and Kubicki (1979) demonstrated that
strong incompatibility events occurred in the intergeneric and
interspecific crosses within the Cucurbitaceae family, avoid-
ing sexual hybridization among C. melo. Thus, in vitro em-
broyo culture has been utilized to recover melon haploid plants
and to save valuable diploid plant material through zygotic
embryo culture and somatic embryogenesis (Fassuliotis and
Nelson, 1988). Different diploid plant material has been recov-
ered, such as hybrid plants obtained after interspecific cross of
C. melo (PI140471) × C. metuliferus (PI 29190) (Norton, 1981),
C. melo (PI 292190, PI 202681, 3503, and 701A) × C.
anguria (PI 233646) (Fassuliotis and Nelson, 1988), and C.
melo (“Cantaloupe Charentais”) × C. anguria L. var. longipes
(Dabausa et al., 1998). On the other hand, interspecific crosses
between C. melo (“Gylan” gynoeocious E6/10) × C. metuliferus
(“Italia”) (Beharav and Cohen, 1995a), and C. melo (“Can-
taloupe Charentais albino mutant”) × C. myriocarpus (Bordas
et al., 1998) failed to produce viable hybrid plants.

In summary, the potential of in vitro zygotic embryo culture
has led to reduced time to obtain inbred melon lines. In addition,
the improvement of this technique as well as the cointegration
to marker-assisted selection (MAS) (Varshney Mohapatra,
and Sharma, 2004) may eventually allow the transfer of disease re-
sistance and/or other important horticultural traits from other
cucurbits into C. melo species.

Genetic Transformation

Two main natural and artificial genetic transformation pro-
cesses have been used to obtain melon transgenic plants;
Agrobacterium tumeafaciens and particle gun bombardment
(Table 6). Successful transformation of melon with A. rhizo-
genest has not been reported. Gaba, Kless, and Antignus (1992)
and Gonsalves et al. (1994) used particle gun bombardment to
transform melon explants and recover transgenic plants through
organogenesis. Gray et al. (1995) used the same transformation
protocol and recovered plants from embryogenic materials.
Gonsalves et al. (1994) and Gray et al. (1995) reported that both A.
tumeafaciens and microprojectile gene transfer produced almost
the same percentage of transgenic plants. Gray et al. (1995)
produced stable normal plants via particle bombardment, whereas
embryos from Agrobacterium-mediated transformation were ab-
normal. Mefoxine was used to stop Agrobacterium growth, so it
may have caused this.

Transformation success via Agrobacterium or particle gun
bombardment is genotype-, explant source-, and in vitro cul-
ture conditions dependent (Fang and Grumet, 1990; Dong et al.,
1991; Yoshioka et al., 1992; Gonsalves et al., 1994; Vallées and
Lasa, 1994; Gray et al., 1995; Bordas et al., 1997; Clendenen
et al., 1999; Ezura et al., 2000; Nuñez-Palenuis et al., 2002;
Akasaka-Kennedy, Tomita, and Ezura, 2004). The Agrobac-
terium strain, vector structure, and co-cultivation with acetosy-
rings have an influence on melon transformation efficiency
(Dong et al., 1991; Yoshioka et al., 1992, 1993; Vallées and

Fang and Grumet (1990) tested several factors, such as
kanamycin concentration, Agrobacterium inoculum level,
length of inoculation, period of co-cultivation, and the use
of tobacco nurse cultures, on melon transformation efficiency
rate. These authors found that 75 mg/L kanamycin, fresh
overnight grown bacteria at a concentration of 107–108 bac-
teria/mL (OD600 = 0.8), 10 min of inoculation, 3 days of co-
cultivation, and not using tobacco nurse culture were the
best conditions to attain an efficient transformation rate.
Unfortunately, these transformation conditions were only tested
in one melon cultivar, “Hale’s Best Jumbo.” A similar approach
was described by Dong et al. (1991). These authors tested the
sensitivity of melon cotyledons to kanamycin and methotrex-
ate concentrations, co-cultivation time, and different selection
schemes. These were as follows: (1) no selection pressure,
(2) explants placed in selection medium immediately after co-
cultivation, and (3) explants placed under selection 2 weeks after
co-cultivation. The highest transformation frequency was ob-
tained when 75 μg/L methotrexate and 100 mg/L kanamycin,
5–6 days of co-cultivation period, and immediate selection pres-
sure were routinely used. These experiments were carried out
on “Orient Sweet, F1 Hybrid” melon cultivar. Vallées and
Lasa (1994) reported that 2 days of co-cultivation with A. tumefac-
iciens cotyledons of “Amarillo Oro” melon were necessary
to reach an efficient transformation process. If they used a longer
period of co-cultivation, no transgenic shoots were recovered.

Different bacterial and plant genes, which provide tolerance
or resistance to several selectable chemical agents, have been
used to delay or completely inhibit the growth of nontransformed
buds and shoots of melon during the selection process. Among
them, nptII, which provides tolerance to aminoglycoside an-
tibiotics (Fang and Grumet, 1990; Dong et al., 1991; Yoshioka
et al., 1992, 1993; Vallées and Lasa, 1994; Gonsalves et al.,
1994; Gray et al., 1995; Ayub et al., 1996; Bordas et al., 1997;
Clendenen et al., 1999; Akasaka-Kennedy, Tomita, and Ezura,
2004); dfr, which gives resistance to methotrexate (Dong et al.,
1991); and CP4syn, which offers tolerance to glyphosate herbicide
(Nuñez-Palenuis, Cantliffe, and Klee, 2002; Nuñez-Palenuis, Cantliffe,
2006) have been used. When typical concentrations (75–150
mg/L) of kanamycin are not able to inhibit the nontransgenic bud
<table>
<thead>
<tr>
<th>Transgene</th>
<th>Phenotypic trait</th>
<th>Explant used and morphogenetic pathway</th>
<th>Cultivar</th>
<th>Transformation method</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td>anpt-II gene</td>
<td>Selectable marker</td>
<td>4- to 5-day-old cotyledons</td>
<td>Hale’s Best Jumbo</td>
<td>A. tumefaciens LBA4404 strain</td>
<td>1</td>
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<tr>
<td>npt-II, Dihydrofolate reductase, and luciferase gene</td>
<td>Selectable markers and reporter gene</td>
<td>Mature cotyledons Organogenesis</td>
<td>Orient Sweet, F1 Hybrid</td>
<td>A. tumefaciens GV3111SE strain</td>
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<tr>
<td>CMV-coat protein gene</td>
<td>Virus resistance</td>
<td>Mature cotyledons Organogenesis</td>
<td>Prince and EG360</td>
<td>A. tumefaciens LBA4404 strain</td>
<td>3</td>
</tr>
<tr>
<td>uida gene</td>
<td>GUS Reporter gene</td>
<td>3-day-old cotyledons Organogenesis</td>
<td>Galia</td>
<td>Particle bombardment</td>
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</tr>
<tr>
<td>ZYMV-coat protein gene</td>
<td>Potyvirus resistance</td>
<td>4- to 5-day-old cotyledons</td>
<td>Hale’s Best Jumbo</td>
<td>A. tumefaciens LBA4404 strain</td>
<td>5</td>
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<td>uida gene</td>
<td>GUS Reporter gene</td>
<td>5-day-old cotyledons Organogenesis</td>
<td>Amarillo Oro</td>
<td>A. tumefaciens LBA4404 strain</td>
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<tr>
<td>CMV-white leaf coat protein gene</td>
<td>Virus resistance</td>
<td>3-day-old cotyledons Organogenesis</td>
<td>Burpee Hybrid, Hale’s Best Jumbo, Harvest Queen, Hearts of Gold, and Topmark</td>
<td>A. tumefaciens C58Z707 strain</td>
<td>7</td>
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<tr>
<td>ZYMV, WMV, and CMV coat protein genes</td>
<td>Virus resistance</td>
<td>Leaves Organogenesis</td>
<td>Don Luis, Galleon, Hiline, Mission, Harvest Queen, Hearts of Gold, and Parental inbred</td>
<td>A. tumefaciens</td>
<td>8</td>
</tr>
<tr>
<td>npt-II gene</td>
<td>Selectable marker</td>
<td>Cotyledons Organogenesis</td>
<td>Eden Gem</td>
<td>A. tumefaciens and Particle bombardment</td>
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<td>ACC oxidase antisense gene from melon</td>
<td>Improved fruit quality</td>
<td>5-day-old cotyledons Organogenesis</td>
<td>Védrantais</td>
<td>A. tumefaciens LBA4404 strain</td>
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<td>HAL I gene</td>
<td>Halotolerance</td>
<td>7-day-old cotyledons and 2-week-old leaves Organogenesis</td>
<td>Pharo and Amarillo Canario</td>
<td>A. tumefaciens LBA4404 strain</td>
<td>11</td>
</tr>
<tr>
<td>ACC synthase antisense gene</td>
<td>Improved fruit quality</td>
<td>Cotyledonary explants</td>
<td>Asgrow western shipper cantaloupe inbreds</td>
<td>A. tumefaciens</td>
<td>12</td>
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<tr>
<td>CMV, ZYMV and WMV-2 coat protein genes</td>
<td>Resistance to CMV, ZYMV and WMV-2</td>
<td>Cotyledonary explants</td>
<td>Asgrow western shipper cantaloupe inbreds</td>
<td>A. tumefaciens</td>
<td>13</td>
</tr>
<tr>
<td>SAM hydrolase gene</td>
<td>Improved fruit quality</td>
<td>NR</td>
<td>NR</td>
<td>A. tumefaciens</td>
<td>14</td>
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</table>

(Continued on next page)
### Table 6

Genes transferred to melon by plant genetic transformation (Continued)

<table>
<thead>
<tr>
<th>Transgene</th>
<th>Phenotypic trait</th>
<th>Explant used and morphogenetic pathway</th>
<th>Cultivar</th>
<th>Transformation method</th>
<th>Reference</th>
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<tr>
<td><strong>HAL1 and TPS1</strong></td>
<td>Salt and drought tolerance</td>
<td>Cotyledon and leaf explants</td>
<td>Pharo</td>
<td>A. tumefaciens</td>
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<td><strong>ACC oxidase antisense gene from melon</strong></td>
<td>Improved fruit quality</td>
<td>10-day-old leaves</td>
<td>Védrantais</td>
<td>A. tumefaciens LBA4404 strain</td>
<td>16</td>
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<tr>
<td><strong>SAM hydrolase gene</strong></td>
<td>Improved fruit quality</td>
<td>NR</td>
<td>NR</td>
<td>A. tumefaciens</td>
<td>17</td>
</tr>
<tr>
<td><strong>Ribozyme genes</strong></td>
<td>Potyvirus resistance</td>
<td>0 to 4-day-old cotyledons</td>
<td>US Patent 5,422,259</td>
<td>A. tumefaciens</td>
<td>18</td>
</tr>
<tr>
<td><strong>Bar gene</strong></td>
<td>Resistance against herbicides</td>
<td>Fully expanded cotyledons</td>
<td>Arava</td>
<td>Zucchini yellow mosaic potyvirus-vector</td>
<td>19</td>
</tr>
<tr>
<td><strong>Polyribozyme genes</strong></td>
<td>Protection against potyviruses</td>
<td>U.S. patent 5,422,259</td>
<td>NR</td>
<td>NR</td>
<td>20</td>
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<tr>
<td><strong>uidA and gfp genes</strong></td>
<td>GUS and GFP Reporter genes</td>
<td>2-day-old cotyledons</td>
<td>Galia male and female parental lines</td>
<td>A. tumefaciens ABI strain</td>
<td>21</td>
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<tr>
<td><strong>ACC oxidase antisense gene from apple</strong></td>
<td>Improved fruit quality</td>
<td>NR</td>
<td>NR</td>
<td>A. tumefaciens LBA4404 strain</td>
<td>22</td>
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<tr>
<td><strong>uidA and hpt genes</strong></td>
<td>GUS and selectable marker genes</td>
<td>Mature seeds</td>
<td>Védrantais and Earl’s Favourite Fuyu A</td>
<td>A. tumefaciens C58C1Rif® strain</td>
<td>23</td>
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<tr>
<td><strong>At1 and At2 genes</strong></td>
<td>Resistance against Downy Mildew</td>
<td>Cotyledons from mature seeds</td>
<td>BU21/3 line</td>
<td>A. tumefaciens LBA4404 or EHA105 strains</td>
<td>24</td>
</tr>
<tr>
<td><strong>ACC synthase (gene from petunia)</strong></td>
<td>Increased carpel-bearing flower</td>
<td>4-day-old cotyledons</td>
<td>Hale’s Best Jumbo</td>
<td>A. tumefaciens EHA105 strain</td>
<td>25</td>
</tr>
<tr>
<td><strong>ACC oxidase antisense gene from melon</strong></td>
<td>Improved fruit quality</td>
<td>2-day-old cotyledons</td>
<td>Galia male parental line</td>
<td>A. tumefaciens ABI strain</td>
<td>26</td>
</tr>
</tbody>
</table>

ZNR: not reported.


or shoot growth (Dong et al.; Guis et al., 1998), other antibiotics, which are also detoxified by the neomycin phosphotransferase protein (NPTII), may be added to melon in vitro cultures in order to improve the selection efficiency. Gentamycin, hygromycin, and paromomycin have been used in melon cultures as alternative to kanamycin with excellent results (Nuñez-Palenius et al., unpublished results; Ezura, personal communication).

Genetic transformation efficiency rate in most melon systems is normally lower than that in other plant species (Fang and Grumet, 1990; Dong et al., 1991; Gaba, Kless, and Antignus,
Several transformation rate and efficiency values have been reported according to transformation protocol and melon cultivar used, among them, average efficiencies such as 3%–7% (Fang and Grumet, 1990), 4%–6% (Dong et al., 1991), 1% (Gaba, Kless, and Antignus, 1992), 0.0%, 0.9%, and 1% (Gonsalves et al.), 0.7%–3% (Bordas et al., 1997, 1998), 2.4% (Guis et al., 2000), 10% (Nuñez-Palenius, Cantliffe, and Klee, 2002), and 2.3% (Akasaka-Kennedy, Tomita, and Ezura, 2004) have been depicted. Unfortunately, in many cases most of the recovered transgenic plants had somaclonal variation especially ploidy changes (tetraploids [75%, Ayub et al., 1996], octaploids, mixoploids) or had morphogenetic altered characteristics, which were expressed in the T0 and T1 generation (Gonsalves et al., 1994).

**Improvement of Disease Resistance**

*C. melo* is attacked by numerous viral, bacterial, mycoplasmal, and fungal organisms, which cause severe diseases (Zitter, Hopkins, and Thomas, 1996). These diseases can affect melons at any plant developmental stage, causing enormous economic losses. According to Zitter, Hopkins, and Thomas (1996), definitive disease control is reached by using genetic-resistant melon cultivars. More than 30 viruses are able to induce disease symptoms in melon plants. Cucumber mosaic virus (CMV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus (WMV) are the most prevalent (Zitter, Hopkins, and Thomas, 1996; Gaba, Zelcer, and Gal-On, 2004).

The first virus-resistant transgenic melon plants were obtained by Yoshioka et al. (1992). These authors transferred and overexpressed the gene for CMV coat protein via *Agrobacterium tumefaciens* using “Prince,” “EG360,” and “Sunday Aki” melon cotyledons. Those transgenic melon plants, which overexpressed the CMV-CP gene, grown under greenhouse conditions, were found to be resistant to infection after inoculation with a low dose of CMV (Yoshioka et al., 1993). Transgenic plants did not develop symptoms of disease during a 46-day observation period, whereas control plants had mosaic symptoms 3 days after inoculation. When the virus-dose was increased by 10-fold, only a delayed appearance of symptoms was observed in transgenic plants (Yoshioka et al., 1993). After Yoshioka’s achievement, different authors were able to obtain transgenic melon plants overexpressing the CMV-CP gene for other melon cultivars, such as “Burpee Hybrid,” “Hales Best Jumbo,” and “Topmark” (Gonsalves et al., 1994), and “Don Luis,” “Galleon,” “Hiline,” “Mission,” and a distinct inbred line (Clough and Hamm, 1995). In addition, transgenic plants overexpressing either CMV-CP for specific viral strains (White Leaf strain; Gonsalves et al., 1999) or multivirus resistance (CMV, WMV, and ZYMV) (Clough and Hamm, 1995; Fuchs et al., 1997) were described. Gonsalves et al. found strong resistance to CMV-White Leaf strain in 5 of 45 transgenic melon plants. High-level resistance to ZYMV, as measured by lack of symptom development and virus accumulation for a 30-day period in the greenhouse, also was achieved with the ZYMV-CP gene (Fang and Grumet, 1993). Gaba, Zelcer, and Gal-On stressed that CP-protection gave effective field resistance, but not 100% protection.

Field trials were conducted to determine whether transgenic plants would retard the spread of the aphid nontransmissible strain C of CMV or they were completely resistant to virus diseases (Tabei et al., 1994; Clough and Hamm, 1995; Fuchs et al., 1997, 1998; Reynolds et al., 1997). Clough and Hamm (1995) tested the level of resistance of five transgenic melon varieties to WMV and ZYMV. Transgenic melon plants had little or no virus infection, whereas more than 60% of the control plants developed virus symptoms. Fuchs et al., who evaluated transgenic melon resistance under high disease pressure, achieved by mechanical inoculations, and/or natural challenge inoculations by indigenous aphid vectors, achieved similar results. After five different trials, two or three viruses did not infect more than 90% of the homozygous and 75% of the hemizygous plants, whereas 99% of the wild-type melon plants had mixed virus infections. Moreover, control plants were severely stunted (44% reduction in shoot length) and had poor fruit yield (62% loss), and most of their fruits were unmarketable (60%) compared to transgenic melon plants.

Regarding the resistance to fungal diseases, transgenic melon (BU21/3 line) plants were obtained by Taler et al. (2004). These transgenic melons overexpressed the enzymatic resistance (*eR*) genes *At1* and *At2* and displayed enhanced activity of glyoxylate aminotransferases and noteworthy resistance against *Pseudomonas cubensis* (Downy Mildew disease causal agent). These cloned *eR* genes will make available a new reserve for developing downy mildew-resistant melon varieties.

**Improvement of Tolerance to Abiotic Factors**

Several environmental factors, such as high or low temperature, salt accumulation, low sun irradiance, drought, and flooding, seriously affect melon field cultivation and production (Robinson and Decker-Walters, 1999). Only one report on transgenic melon providing tolerance to one environmental factor has been published (Bordas et al., 1997). The **HAL1** gene, which encodes a water-soluble protein and provides halotolerance in yeast, was inserted using *A. tumefaciens*-transformation protocol to “Pharo” and “Amarillo Canario” melon cultivars. In vitro shoots from transgenic and control plants were evaluated for salt tolerance after 16 days of incubation on medium containing 10 g/L sodium. The frequency and intensity of root formation were higher in **HAL1**-positive plant populations compared to wild-type plants. However, no differences in vegetative fresh weight and number of leaves between transgenic and control plants were scored. Moreover, greenhouse and field evaluations of transgenic plants were not reported.

**Improvement of Postharvest Characteristics**

According to Perishables Group Research (Anonymous, 2002), price, firmness, and appearance are among the top criteria
for consumers when deciding to purchase melons. Appearance includes color, and signs of damage or disease are the top criteria for consumers when purchasing melons. Customers are interested in knowing nutritional and ripening information in store displays (Anonymous, 2002). Extended shelf life in melon fruit is an important quality attribute because it provides the opportunity to commercialize melon commodities.

The first transgenic melon plants carrying genes involved in fruit ripening process were obtained by Ayub et al. (1996). Using the Agrobacterium-mediated transformation system and cotyledons of the Charantais type Cantaloupe melon cv. “Védrantais,” these authors were able to transfer the 1-aminocyclopropane-1-carboxylic-acid oxidase gene (ACC oxidase from melon under the control of a constitutive promoter) in antisense orientation to reduce the level of ethylene production. Different ripening parameters were evaluated in transgenic melon fruits, such as internal and gas space ethylene production, total soluble solids, titratable acidity, flesh pigment content, flesh firmness, rind and flesh color, harvest maturity (timing from anthesis to full slip), and reversion to wild-type behavior by exogenous ethylene treatment (Ayub et al., 1996; Guis, Botondi, et al., 1997).

Ayub et al. (1996), and Guis, Botondi et al. (1997) found that in wild-type fruit attached to the vine, the internal ethylene concentration rose at 39 days after pollination and reached maximum (120 ppm) values at 42 days. In antisense fruit the internal ethylene concentration on the vine remained at lower levels (0.6 ppm), even at late fruit development stages (60 days after pollination). When wild-type fruits were detached from the plant there was a significant increase in the internal ethylene concentration, producing 180 ppm 48 h later. Detached transgenic fruit also had an increase in ethylene production, but reached only 10 ppm 12 days after harvest. Compared to wild-type fruit, antisense fruit did not undergo significant rind yellowing and flesh softening at maturity. Transgenic fruit remained attached to the vine for a longer period of time (65 days after pollination) compared to control plants (38 days after pollination). Exogenous ethylene treatment (50 ppm) of transgenic fruits allowed the recovery of the wild-type behavior and phenotype. There were no significant differences in carotenoid content (flesh color) and total soluble solids (°Brix) content in wild-type and transgenic fruit at any stage of ripening.

Clendennen et al. (1999) utilized the product of the S-adenosylmethionine hydrolase (SAMase) gene (from T3 bacteriophage) to catalyze the degradation of SAM, the initial precursor of ethylene. Unlike the T-DNA construct used by Ayub et al. (1996), Clendennen et al. (1999) used a fruit-specific promoter (chimeric ethylene-responsive E8/E4 promoter) aimed to overexpress the SAMase gene in two American Cantaloupe (i.e., netted muskmelon) lines, which were proprietary inbred lines from Harris Moran Seed Company, Inc. These authors evaluated several postharvest fruit quality parameters, such as fruit size and weight, firmness, decay susceptibility, external and internal color, soluble solids, harvest maturity (timing from anthesis to full slip, measured as heat units), and ethylene production, in wild-type and transgenic melon fruits from plants grown under field conditions. Transgenic melon fruit from both lines “A” and “B,” did not differ in horticultural traits from wild-type fruits, except for the intended goal of SAMase expression on ethylene biosynthesis and related events. In lab experiments, transgenic fruits produced half of the ethylene accumulated by wild-type fruits. However, in field trials, the onset of maturity, measured on four different dates, was not significantly delayed in transgenic fruit compared to wild-type, but transgenic fruits ripened more uniformly in the field. Firmness was also measured on transgenic and wild-type fruits from three different field trial locations. Significant differences were found in fruit mesocarp firmness between transgenic and wild-type melons, but only from one location. Clendennen et al. (1999) claimed that by expressing SAMase in a regulated manner by a fruit-specific promoter, transgenic fruits produced less ethylene than non-transgenic fruit ensuing in a modified ripening phenotype.

Silva et al. (2004) obtained transgenic Cantaloupe melon plants cv. Védrantais by inserting and overexpressing ACC oxidase from apple and not from melon as in the Ayub et al. (1996) protocol. These authors reported the characterization of ripening melon fruits, and their experimental comparison between transgenic and control fruit provided very similar results in almost all the evaluated parameters, such as harvest maturity, total soluble solids content, rind color, and internal ethylene production, to those previously reported by Ayub et al. and Guis, Botondi, et al. (1997).

Unlike Ayub et al. (1996), Guis, Botondi, et al. (1997) and Silva et al. (2004), who applied the RNA antisense technology to target the ACC oxidase on a cantaloupensis melon variety, Nuñez-Palenius et al. (2007) used a reticulatus melon cultivar, “Krymka,” to reduce the ethylene production from fruits by introducing a single copy of the ACC oxidase gene in antisense orientation (Nuñez-Palenius, Cantliffe, et al., 2006). Transgenic ACC oxidase antisense Krymka fruit had a greater firmness than wild-type fruit, GUS-transgenic, and azygous counterparts at full-slip developmental stage. Likewise, ethylene production and ACC oxidase activity in half-slip antisense fruit were almost 10 times lower, than those from wild-type, azygous, and GUS-transgenic fruit.

In Charantais type Cantaloupe melon (cv. Védrantais), climacteric respiration, yellowing, and carotenoid content of the rind, chilling injury, and formation of the peduncular abscission zone are strong ethylene-dependent events (Pech et al., 1999; Flores Martinez-Madrid, et al., 2001), whereas fruit softening, volatiles synthesis, and membrane deterioration are partially dependent on ethylene (Bauchot et al., 1998; Bauchot et al., 1999; Guis et al., 1999; Flores, Martinez-Madrid et al., 2001; Flores et al., 2002). In Galia male parental line (cv. Krymka), fruit weight and size, titratable acidity, seed number, mesocarp size, and total soluble solids (TSS) are ethylene-independent characteristics, whereas, yellowing of the rind and softening are ethylene dependent (Nuñez-Palenius et al., 2007). According to Guis, Botondi, et al. (1997), Pech et al. (1999), and Silva...
et al. (2004) sugar accumulation and the increase in carotenoid content in the flesh are ethylene-independent events. Nuñez-Palenius et al. (2007), who described that TSS accumulation was an ethylene-independent event, reported similar events. There is still some controversy about organic acid metabolism and loss of acidity during melon fruit ripening; that is, Guis, Botondi, et al. (1997), Pech et al. (1999), and Nuñez-Palenius et al. (2007), suggested that the organic acid metabolism was ethylene independent, whereas Silva et al. (2004) recently implied that organic acid metabolism is an ethylene-dependent process. It is likely that these differences in conclusions might be related to a different ethylene concentration reached by each transgenic fruit. In Silva’s report, transgenic fruit attained a lower internal ethylene level (<0.09 μL/L) than found in Guis, Botondi, et al.’s results (<0.5 μL/L). This difference in ethylene concentration could have obvious outcomes such as at the specific levels of perceiving that plant hormone (Srivastava, 2002; Silva et al., 2004).

All fruit-quality-oriented transgenic melon plants have been obtained using just four melon cultivars, such as Védrantais belonging to cantaloupe (Ayub et al., 1996; Silva et al., 2004), and lines A and B (Clendennen et al., 1999) and Galia male parental line, “Krymka,” which are reticulatus melon varieties (Nuñez-Palenius et al., 2007). Considering that there are seven commercial and horticultural important melon varieties (cantaloupe, reticulatus, saccharinus, inodorus, flexuosus, conomon, and dudaim) (Kirkbride, 1993; Guis et al., 1998), and hundreds of melon cultivars, much more work could be accomplished. There still remains a need to improve in vitro regeneration and transformation protocols (Guis, Latche, et al., 1997; Akasaka-Kennedy, Tomita, and Ezura, 2004; Gaba, Zelcer, and Gal-On, 2004).

Controlling Sex Expression

The majority of commercial melon cultivars are andromonoecious, producing both male and bisexual flowers (Kenigsbuch and Cohen, 1989), although several modern cultivars are monoecious as well. Plant development follows a typical pattern of vegetative nodes, followed by nodes bearing male flowers, and then nodes bearing a combination of male and bisexual flowers. As only carpel-bearing flowers can set fruit, the time of transition to bisexual flower production is important with respect to yield, time to harvest and market, and length of growing season. Numerous studies have shown that sexual determination in cucurbit species, including melon, is influenced by hormones, with ethylene playing the predominant role (Perl-Treves, 1999). Application of ethylene or ethylene-releasing products increases femaleness as measured by number of carpel-bearing ( bisexual) flowers and time of transition to production of carpel-bearing flowers.

Transgenic melons were produced to constitutively express the ethylene biosynthetic enzyme, 1-aminocyclopropane-carboxylate synthase (ACS) (Papadopoulou et al., 2005). ACS overexpressers exhibited two floral development phenotypes: an increase in the earliness of number of carpel-bearing flowers and an increase in the earliness and percent of carpel-bearing flowers that reached maturity. When tested in the field, the ACS overexpressors set fruit earlier than did control genotypes, indicating that fruit set was controlled by time of development of mature carpel-bearing flowers, rather than of vine size, and can be manipulated by expression of ACS. Fruit on the ACS overexpressors also matured earlier, but effects of earlier fruit set cannot be separated from possible ethylene-mediated effects hastening ripening.

CONCLUDING REMARKS AND FUTURE CHALLENGES

Melon has been the subject of intense research for decades. This is a reflection of the importance of this cucurbit as a species. Melons are grown worldwide as a profitable crop. Much about melon fruit biology and crop cultivation is known. Even though melon fruit and plant improvement by traditional hybridization has led to a generation of improved melon varieties, this method of new plant development is relatively slow and limited to a restricted gene pool. For that reason, biotechnological approaches have been successfully applied in melon, although, not in all known melon cultivars. Genetic manipulation of melon is now a reality, and several aims have been achieved, from tolerance to biotic and abiotic stresses to the control of sex expression. The recent application of functional genomics to melon suggests that we will understand, in the not too distant future, how specific genes control definite physiological/biochemical processes in melon. One area that will particularly benefit from this is the production of aroma compounds by the fruit. There are still many gaps in our understanding as to how several volatile compounds are produced and released. The availability of methods to transfer genes into different melon varieties will allow the detailed analyses of particular genes in specific genetic backgrounds with the aim to improve the crop. New insight will be available on the genetic control of melon fruit development and ripening, when the molecular techniques, such as a complete genome sequence protocols, which have produced so much information from model systems, such as Arabidopsis, rice (Oryza sativa) and tomato, might some day be applied to different melon genotypes. Moreover, that information could also be retrieved from wild-type and landrace melons, in order to find valuable genes to be transferred to commercial varieties. It is not unreasonable to consider that in the near future, new melon cultivars in which either the floral sex can be precisely controlled, or with resistance to pests and diseases, or with improved fruit quality traits, such as shelf life extension and reduced incidences of enteric bacteria, by avoiding their attachment to melon fruit skin, might be obtained. This is certainly an exciting time for melon researchers.

REFERENCES

Adelberg, J. 1993. Tetraploid Melon from Tissue Culture and Their Triploid Hybrids. Ph.D. Clemson University, Clemson, SC.


Martínez-Madrid, M. C., Martínez, G., Pretel, M. T., Serrano, M., and Romojaro, F. 1999. Role of ethylene and abscisic acid in...


Whitaker, B. D., and Lester, G. E. 2006. Cloning of phospholipase Dα
and lipoxigenase genes CmPLDa1 and CmLOX1 and their expres-
sion in fruit, floral, and vegetative tissues of ‘Honey Brew’ hybrid

Whitaker, T. W., and Davis, G. N. 1962. *Cucurbits. Botany, Cultiva-

Wien, H. C. 1997. The cucurbits: cucumber, melon, squash and pump-
kim. In: *The Physiology of Vegetable Crops*, pp. 345–386. Wien, H.C.,

Wyllie, S. G., and Leach, D. N. 1992. Sulfur-containing compounds in
food and evidence of RFLP polymorphisms linked to ethylene genes in


Yang, S. F. 1980. Regulation of ethylene biosynthesis. *HortScience* 15:
238–243.


(*Cucumis melo L.* var. *flexuosus* Naud.). In: *First International
Symposium on Cucurbits*, pp. 307–310. Abak, K., and Büyükalaca, S.,
Eds., ISHS, Adana, Turkey.

Yoshioka, K., Hanada, K., Nakazaki, Y., Minobe, Y., and Oosawa, K.
1993. Virus-resistance in transgenic melon plants that express the
cucumber mosaic-virus coat protein gene and in their progeny. *Jpn.
J. Breed.* 43: 629–634.

Yoshioka, K., Hanada, K., Nakazaki, Y., Minobe, Y., Yakuba, T.,
and Oosawa, K. 1992. Successful transfer of the cucumber mosaic-

lene biosynthesis and restriction fragment length polymorphisms
(RFLPs) of ACC oxidase and synthase genes in melon (*Cucumis melo

evidence of RFLP polymorphisms linked to ethylene genes in

Cucurbit Diseases.* APS Press, St. Paul, MN.

Ziv, M. 1991. Vitrification: morphological and physiological disorders of
in vitro plants. In: *Micropropagation. Technology and Applica-