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New Insights into Reproductive Development in Melon (*Cucumis melo* L.)

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ABSTRACT

Melon, *Cucumis melo* L., is a high value crop prized throughout the world for sweet, flavorful, dessert-like fruits. Production of such fruits ultimately depends on a series of steps in reproductive development, flowering, fruit set, fruit growth, maturation, and ripening. New approaches, utilizing molecular, genetic, plant transformation, and genomics tools, have led to increased understanding of the underlying processes responsible for the various stages of reproductive development, and promise to further our understanding in the future. Recent studies have led to mapping, cloning, and identification of key genes associated with sex expression, and floral and fruit development; have identified transcriptional and enzymatic changes associated with various stages of development; and have furthered our understanding of the role of ethylene production and perception in floral sex determination and development, and fruit set, maturation and ripening. In this review we first examine melon flower development with an emphasis on sex expression and development of carpel-bearing flowers. We next discuss fruit development and ripening processes, including factors that contribute to production of high quality fruits such as sweetness, flavor, texture, and aroma.

Keywords: flower development, fruit development, fruit quality, fruit set, genomics, melon EST database, plant transformation, ripening, sex expression

Abbreviations: **AAT**, alcohol acyltransferase; **ABA**, abscisic acid; **ACO**, 1-aminocyclopropane carboxylate oxidase; **ACS**, 1-aminocyclopropane carboxylate synthase; **AP3**, Apetala 3; **BAC**, bacterial artificial chromosome; **CRC**, Crabsclaw; **ERS**, ethylene response sensor; **EST**, expressed sequence tag; **ETR**, ethylene response gene; **IAA**, indole acetic acid; **QTL**, quantitative trait locus

CONTENTS

INTRODUCTION

Cucumis melo L. (melon), is a highly diversified species that is widely cultivated throughout the world (Robinson and Decker-Walters 1997). The dessert melons, which are largely members of the Cantalupensis (e.g., cantaloupe, muskmelon, charantais) and Inodorous groups (e.g., honeydew, casaba, canary, crenshaw), are highly valued for sweet, flavorful fruits that exhibit a tantilizing array of colors, flavors and aromas. These high-value crops are frequently shipped to distant markets where high quality can demand a premium price. Within the melon species there is great genetic diversity for fruit characteristics including shape, flesh and skin color, surface texture, flavors, sugar and acid content, volatile profile, and ripening physiology, including both climacteric and non-climacteric cultivars (Robinson and Decker-Walters 1997; Katzir *et al*. 2006).

Melons also exhibit a diverse array of sexual phenotypes (**Fig. 1**). Flowers of most species are bisexual, producing both male (stamen) and female (carpel) reproductive organs. Cucurbit species such as melon produce varying combinations of male, female, or bisexual flowers, depending on genotype and environment. This developmental plasticity invites numerous questions in basic plant biology relative to flower development, and influences productivity in the field, as fruit production is dependent upon production of carpel-bearing flowers.

Given the great diversity in melon flower and fruit development, it is not surprising that questions of sex expression, fruit quality, and ripening characteristics are of both scientific and economic interest. In recent years, the advent of new molecular genetic and genomic tools, has

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Invited Review

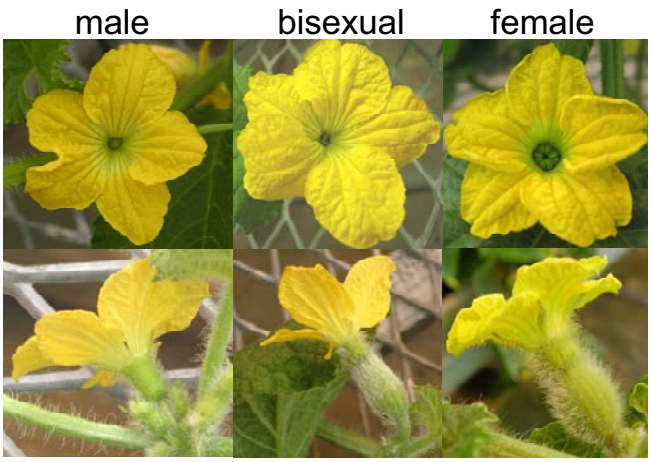

Fig. 1 Top and side view of male (cv. 'Hale's Best Jumbo'), bisexual (cv. 'Hale's Best Jumbo') and female (cv. 'Wisconsin 998') melon flowers at anthesis. Bisexual and female flowers have a pronounced inferior ovary. Pistils in the bisexual flower are largely hidden by stamens.

made it possible to apply new approaches to these questions. National and international efforts have led to the development of transformation techniques, BAC (bacterial artificial chromosome) libraries, high density maps, EST (expressed sequence tag) databases, and microarrays for melon (Papadopolou and Grumet 2002; Katzir *et al*. 2006; Monforte *et al*. 2006; Puigdomènech *et al*. 2006a, 2006b). The following review will examine new insights that have been gained in understanding reproductive development in melon.

FLOWER DEVELOPMENT AND FRUIT SET

Sex determination

One of the most striking features of reproductive development of cucurbit species is the capacity to exhibit a broad range of heritable sex phenotypes that produce varying combinations of male, female, or bisexual flowers (Roy and Saran 1990; Perl-Treves 1999). Melon is no exception. At least three sex-determining genes (Kenigsbuch and Cohen 1990), *A* (andromonoecious), *G* (gynoecious), and *M* (monoecious), contribute to sex determination in *C. melo* (Poole and Grimball 1939; Kenigsbuch and Cohen 1990). Depending on their combination, the resultant sex phenotypes include hermaphrodite (all bisexual), andromonoecious (male and bisexual on the same plant), monoecious (male and female on the same plant), or gynoecious (all female) (**Table 1**).

The presence of the *G* allele leads to production of unisexual male flowers, allowing for the occurrence of either andromonoecy or monoecy (Kubicki 1969; Kenigsbuch and Cohen 1990). The *A* locus specifies whether or not the carpel-bearing flowers are bisexual (*aa*) or unisexual female (*A*); e.g., *A* in the presence of the dominant *G* allele, converts andomonoecious plants to monoecious (Rosa 1928; Poole and Grimball 1939; Kenigsbuch and Cohen 1990). *A* in combination with *gg* converts hermaphrodites to gynoecy, but expression is influenced by the *M* gene (Kubicki 1962; Kenigsbuch and Cohen 1990). The recessive *m* stabilizes gynoecy while *aaggM-* can result in gynomonoecy (bisexual, female) or trimonoecy (all three sex types).

Table 1 Sex expression genotypes and phenotypes of *C. melo*.

Genotype[a]	Phenotype[a]	Developmental progression
ggaa (*M or m*)	Hermaphrodite	Bisexual
G-aa (*M or m*)	Andromonoecious	Male, bisexual and male
ggA- mm	Gynoecious	Female
G-A- (*M or m*)	Monoecious	Male, female and male

[a] Genotype and phenotype designations are based on Kenigsbuch and Cohen (1990), Kubicki (1966), and Poole and Grimball (1939).

The most frequent sex type of commercial melon cultivars is andromonoecious, with male and bisexual flowers produced according to a developmental gradient (Karchi 1970; Kenigsbuch and Cohen 1990; Papadopoulou *et al*. 2005). The progression along the main stem includes an initial phase of vegetative nodes, followed by a phase of nodes producing only male flowers, and then a phase of nodes producing a combination of bisexual and male flowers. In the bisexual phase, a given node can produce multiple flowers with a combination of sex types. Different andromonoecious cultivars or melon types can have varying lengths of vegetative and male phases, which contribute to variation in length of growing season needed for melon production. Lateral branches do not typically exhibit the vegetative and male phases, and generally produce bisexual flowers beginning with the first node (Rudich *et al*. 1969; Little *et al*. 2007).

Studies of floral bud development in cucumber (*C. sativus*) have contributed extensively to our understanding of the sex differentiation process in cucurbits. Cucumber flower development, from time of primordium initiation to anthesis, takes approximately 20 days (Atsmon and Galun 1960; Goffinet 1990; Hao *et al*. 2003; Bai 2004). During the first five days, all four whorls (sepals, petals, stamens and carpels) typical of a bisexual flower are initiated (Goffinet 1990). At about six days, either the stamens or carpels begin to expand rapidly; unisexuality results from specific repression of either stamen or carpel primordia. Carpel suppression in a male flower appears to occur at an earlier stage of development (stage 6 - prior to carpel elongation) than stamen suppression in a female flower (stage 7 - differentiation of the anther from the filament) (Hao *et al*. 2003; Bai *et al*. 2004). Presumably melon flowers follow the same sequence of development. Interestingly, studies of cucumber homeotic mutants demonstrated that inhibition of stamen or carpel development is dependent on whorl position, not sexual identity of the developing primordium (Kater *et al*. 2001).

Sex expression of any given developing flower bud is quite plastic, such that the ultimate sex of an individual flower at a specific node results from a combination of genetic, developmental, environmental, and hormonal factors (Roy and Saran 1990; Rudich1990; Perl-Treves 1999). Environmental conditions or exogenous hormones can cause carpel-bearing flowers to occur at earlier or later nodes, or can cause conversions among sex types of developing flower buds (e.g., female to male or bisexual; bisexual to male or female) (Rudich 1990; Perl-Treves 1999). Application of gibberellins promotes maleness, while auxins, brassinosteroids and ethylene promote femaleness (e.g., Galun 1959; Robinson *et al*. 1969; Rudich *et al*. 1969; Papadopoulou and Grumet 2005). Several studies have implicated ethylene to be the key sex-determining hormone based on comparisons to effects of auxin, gibberellins, and brassinosteroids in cucumber, zucchini, and melon (Kubicki 1969; Byers *et al*. 1972b; Tolla and Peterson 1979; Yin and Quinn 1992, 1995; Papadopoulou and Grumet 2005), and suggest that feminization by auxins and brassinosteroids occurs indirectly through induced ethylene production (Trebitsch *et al*. 1987; Papadopoulou and Grumet 2005).

Application of ethylene or ethylene-releasing compounds to developing apical meristems [e.g., 2-chloroethanephosphonic acid (etherel) or 2-chloroethylphosphonic acid (ethephon), 5-500 ppm] will cause increased or earlier production of carpel-bearing flowers, while inhibitors of ethylene production (e.g., aminoethyoxyvinylglycine, 50-200 ppm) or perception (e.g., silver nitrate or silver thiosulphate 100-500 ppm) causes increased production of male or stamen-bearing flowers (e.g., Den Nijs and Visser 1980; Rudich 1990; Owens *et al*. 1980; Perl-Treves 1999). In cucumber, ethylene production by shoot apices has been correlated with sex type, such that gynoecious lines produce approximately 2-3–fold more ethylene than monoecious or andromonoecious gentoypes (Rudich *et al*. 1972; Makus *et al*. 1975; Rudich *et al*. 1976; Trebitsch *et al*. 1987; Yama-

saki *et al*. 2001, 2003a). Other studies have demonstrated differential expression of the ethylene biosynthetic genes 1-aminocyclopropane carboxylate synthase and oxidase (*ACS*, *ACO*) in relationship to cucumber floral sex organ development (Kamachi *et al*. 1997; Kahana *et al*. 1999; Kamachi *et al*. 2000; Yamasaki *et al*. 2001, 2003a, 2003b). Transcript levels increased at the time of transition to female flowering, differed among sex types, and location of expression among the developing floral organ primordia, and could be increased or decreased by treatment with ethylene-releasing compounds or inhibitors (Kamachi *et al*. 1997; Kahana *et al*. 1999; Kamachi *et al*. 2000; Yamasaki *et al*. 2000, 2001). Ethylene treatment also induced expression of a MADS box gene (*ERA17*) that correlated with induction of female flowers (Ando *et al*. 2001); many of the key floral organ development transcription factors, are MADS box proteins (Coen 1991).

In melon, there has not been a clear correlation of sex type with ethylene evolution from the apices (Byers *et al*. 1972b). However, treatment of melon plants with hypobaric conditions to reduce ethylene levels caused increased maleness, while addition of 2 ppm ethylene into the hypobaric conditions, reversed the effect (Byers *et al*. 1972b). It has been suggested that melon sex genotypes may differ in sensitivity to ethylene, rather than level of ethylene production. More generally, it has been proposed that sex expression in a given cucurbit flower is due to a combination of ethylene production and ethylene perception. Promotion or inhibition of the stamens or carpels will depend on the sex genotype, the level of ethylene, and the threshhold sensitivity of the specific sex organ primordia (Yin and Quinn 1995; Yamasaki *et al*. 2001, 2003a). In cucumber, higher transcript levels of the ethylene receptor homologs, CS-ETR2 and CS-ERS occurred in gynoecious compared monoecious shoot apices, and, like ethylene biosynthetic genes, location of expression of the receptors varied with sex genotype, sex organ primordia, and ethylene treatments (Yamasaki *et al*. 2000, 2001).

Hormone applications indicate that there are critical stages in floral bud development during which developing stamen or carpel primorida are subject to the effects of hormone treatment. Delays of approximately ten days occur from the time of hormone application to the apical meristem to first appearance of sex converted flowers; shifting the age of treatment shifts the nodes that exhibit sex change (Robinson *et al*. 1969; Karchi 1970; Byers *et al*. 1972a). Increasing concentration can bring about changes at later stages of bud development (i.e., cause a change in sex expression at earlier nodes relative to the age of treatment) and/or increase the extent of change [i.e., conversion from male to bisexual (permit carpel development) or to female (permit carpel development and suppress stamens)] (Robinson *et al*. 1969; Karchi 1970; Byers *et al*. 1972a). Consistent with the microscopic analyses described above, indicating that differentiation of stamen primordium is a critical stage for induction of sex conversions (Hao *et al*. 2003; Bai *et al*. 2004), tissue culture studies demonstrated that cucumber buds destined to be males could be converted to females if they are removed prior to expansion of the stamen primordia (Galun *et al*. 1963). Exogenous ethylene treatments also indicate that differentiation of stamen primordium is a critical stage for induction of sex conversions (Hao *et al*. 2003; Yamasaki *et al*. 2003a, 2003b; Bai *et al*. 2004).

Recent efforts have been directed toward cloning of key sex expression genes. In cucumber, the *F* (*Female*) locus conferring gynoecy was found to encode an extra copy of the key ethylene biosynthetic enzyme, ACS (*Cs-ACS1G*), thus providing a direct connection between the sex phenotype and ethylene production (Trebitsh *et al*. 1997; Mibus and Tatlioglu 2004; Knopf and Trebitsh 2006; Mibus and Trebitsh *et al*. 2006). The promoter regions of *Cs-ACS1G* and *Cs-ACS1* were found to include potential auxin-responsive elements, consistent with the interaction between auxin and ethylene in stimulating female sex expression (Mibus and Tatlioglu 2004; Little 2005; Knopf and Trebitsh 2006). Interestingly, the distal upstream region of *Cs-ACS1G* also contains a CArG motif, which is frequently associated with binding of MADS box transcription factors (Knopf and Trebitsh 2006). Since many of the key floral organ development factors, are MADS box proteins, *Cs-ACS1G* may be a downstream target of such a factor.

Mapping efforts in melon have been directed toward location and ultimate cloning of the *A* and *G* genes. Bulked segregant and recombinant inbred analysis, and development of linkage maps have located the *A* gene on linkage group 2 and identified a SCAR marker 5.5 cM from *a* (Périn *et al*. 2002a; Silberstein *et al*. 2003; Noguera *et al*. 2005). Several ethylene related genes, including 5 *ACS*, 3 *ACO* genes, and an ethylene receptor homolog, *Cs-etr1*, and floral development genes (*AGAMOUS* and *APETELA2* homologs) also have been mapped, but these genes have not fallen in close association with the *a* locus (P**é**rin *et al*. 2002a; Siberstein *et al*. 2003; Nogura *et al*. 2005). Recent, fine mapping efforts have placed an AFLP marker 0.23 cM from *a* and approximately 1 cM on either side of *g* (Fergemy *et al*. 2006). BAC clones covering the regions of *a* and *g* have been identified, providing an important step toward cloning of these genes (Fergamy *et al*. 2006).

Transgenic approaches leading to modified endogenous ethylene production or perception also have provided new insights. Melon plants transformed to express the dominant negative Arabidopsis ethylene perception mutant, *etr1-1*, exhibited an array of phenotypes associated with inhibited ethylene perception (e.g., inhibited lateral root formation, delayed senescence and abscission, elevated ethylene production), and importantly, failed to produce carpel-bearing flowers (Little *et al*. 2007), demonstrating the critical role for ethylene perception in female sex expression in melon. Conversely, melon plants transformed to constitutively express *ACS*, exhibited increased production and enhanced femaleness with respect to earlier, and increased production of carpel-bearing flowers (Papadopoulou *et al*. 2005). The earlier production of carpel-bearing flowers was reflected in earlier fruit set in the field. These studies provide direct demonstration of the influence of elevated endogenous ethylene on sex determination of melon flowers, and may allow for future modifications to enable shorter growing seasons for melon fruit production.

Given the combined roles of ethylene production and perception, and possible varying threshhold levels that may determine outcome with respect to stamen or carpel development, it may be hypothesized that there are critical stages and locations for ethylene perception within the developing primordia. This question was addressed by introduction of the *etr1-1* gene into melon (Little *et al*. 2007) under control of the floral-targeted promoters, *APETALA3* (*AP3*; Jack *et al*. 1994), which in Arabidopsis directs expression in petal and stamen primordia, and *CRABSCLAW* (*CRC*; Bowman and Smyth 1999), which in Arabidopsis directs expression in carpel and nectary primordia. Interestingly, the observed results did not follow the prediction that inhibition of perception in the carpel primordia would prevent carpel development. *CRC::etr1-1* melons still produced carpel-bearing buds. In contrast, carpel-bearing bud production was completely abolished in *AP3::etr1-1* plants, suggesting that the critical site for ethylene perception at the time of sex determination is the stamen (or petal) primordia. These results coincide with the observations in cucumber that the time of expansion and differentiation of stamen primordium is critical for determination of sex identity, and raise the possibility of communication between developing floral primordial whorls.

In addition to the interesting developmental questions posed by sex differentiation processes, sex expression is important for several aspects of melon production. Sex expression influences breeding methods, seed production processes, fruit shape and quality, earliness and yield. Moneocy is desirable for ease of hybrid seed production, as female flowers eliminate the need for emasculation. Gynoecy, which is widely available for commercial cucumber produc-

tion, but less so for melon, allows for earlier and more uniform fruit set (Kenigsbuch and Cohen 1990; Lower and Nienhuis 1990). Fruit shape has been shown to be under polygenic control of several genes that act during ovary development, as demonstrated by the co-segregation of QTL (quantitative trait loci) markers for both fruit shape and ovary shape (Périn *et al*. 2002b). Two major QTL associated with phenotypic variation for fruit shape are linked to the flower genes *a* (*monoecious*) and *p* (*pentamerous*), which control the presence of stamens in carpel-bearing flowers and flower carpel number, respectively (Périn *et al*. 2002b). Fruits produced from bisexual melon flowers are rounder than female flowers; while fruits from female flowers have smaller blossom abscission scars, thereby reducing risk of pathogen infection and loss to disease (Périn *et al*. 2002b; Noguera *et al*. 2005).

Floral maturation and fruit set

Subsequent to sex determination, successful reproductive development requires floral maturation, pollination and fruit set. Observation of developing floral buds shows that most male buds reach anthesis (90-100% in both greenhouse and field environments; Little *et al*. unpublished). In contrast, the majority of carpel-bearing buds abort prior to anthesis (80-90% of buds on the main stem of greenhouse grown plants; 70% in the field) (Mann and Robinson 1951; Papadopoulou *et al*. 2005; Little *et al*. 2007). Interestingly, greenhouse and field trials of andromonoecious melon plants expressing an *ACS* transgene under the control of the constitutive promoter, *CaMV35S*, showed earlier and increased numbers of carpel-bearing flowers that reached anthesis than did non-transgenic control plants (Papadopoulou *et al*. 2005). In the field, approximately twice as many bisexual buds on the main stem of *35S::ACS* plants (55%) reached anthesis as on non-transgenic plants (30%) (Papadopoulou *et al*. 2005). The *35S::ACS* plants also had earlier appearance of mature bisexual flowers and increased occurrence of mature bisexual flowers on closely spaced nodes along the main stem (**Fig. 2**; Little 2005).

These observations suggest that in addition to the promotion of pistil development at the time of sex determination, ethylene may have a previously unrecognized role in the sustained maturation of carpel-bearing buds. A possible role of ethylene in carpel development is not unprecedented. For example, in orchid, ethylene biosynthesis stimulated by pollination is critical for subsequent ovary maturation (Zhang and O'Neill 1993; Bui and O'Neill 1998), and down-regulation of a pistil-specific *ACO* gene in tobacco resulted in the arrest of ovule development that could be reversed with exogenous ethylene (de Martinis and Mariani 1999). Subsequent work with transgenic melon engineered to express the *Arabidopsis* mutant ethylene receptor *etr1-1*, which confers insensitivity to ethylene, also showed support for this role (Little *et al*. 2007). Bisexual buds on transgenic melon plants expressing *etr1-1* under the carpel-directed *CRC* promoter were four times more likely than buds from wild type and azygous control plants to abort at 4 mm or less (Little *et al*. 2007). A recent study of the effects of exogenous treatment of zucchini squash plants with ethylene inhibitors, also suggests involvement in floral maturation; blocked ethylene production or perception altered development of pedicel, sepals, and carpels of pistillate flowers and arrested flower maturation (Paýan *et al*. 2006).

Earlier and increased incidence of mature bisexual flowers on ACS-overexpressing melon was accompanied by earlier fruit set, increased fruit set on the main stem, and increased fruit set on closely spaced nodes (Papadopoulou *et al*. 2005; **Fig. 2**). Multiple ACS plants set four or more fruit on the main stem. This is a sharp contrast to non-transgenic plants; only 12% of the non-transgenic plants set two fruit within four nodes compared to 59% of the *35S::ACS* plants. The determining factor appeared to be presence of mature carpel-bearing flowers, as the percent mature bisexual flowers that set fruit was similar among genotypes. Consistent with earlier fruit set typically occurring on gynoecious genotypes (Lower and Neinhuis 1990), these results suggest that plant maturity is not the limiting factor in earlier fruit set.

Successful fruit set typically involves a complex interplay among several hormones (Ozga and Reinecke 2003; Srivastava and Handa 2005). Among the key hormones are auxins, gibberellins, cytokinins and abscisic acid, although the relative contributions of individual hormones can vary among species. Exogenous treatment of melon ovaries at the time of anthesis with synthetic cytokinins and auxins promote fruit set (Hayata *et al*. 2000). Cytokinins (e.g., CPPU) are particularly effective and have long been used by breeders to facilitate crossing (Munger and Lane 1983; Hayata *et al*. 2000). Pollination or cytokinin treatment, in turn, lead to increased levels of endogenous IAA in the placenta and mesocarp of carpel-bearing flowers, while unpollinated/untreated flowers show reduced levels of endogenous IAA (Lingle and Dunlap 1991; Hayata *et al*. 2002). In contrast, both pollination and CPPU treatment caused reduction in endogenous ABA in the placenta and mesocarp of pistillate flowers, while unpollinated/untreated flowers showed a rapid increase in endogenous ABA (Hayata *et al*. 2002).

Despite the increase in fruit set on the main stem of ACS-overexpressing melons, the total fruit set per plant (on main stem and lateral branches), did not differ between *35S::ACS* and control plants (Papadopoulou *et al*. 2005), suggesting a redistribution of resources and possible resource limitation to total yield. Sex expression can reflect resource availability, such that a developing fruit results in a reduction in pistillate flower production, while lack of fruit results in increased pistillate flower production (Stephenson 1981; Schapendon and Brouwer 1984; Krupnick and Weis 1998; Krupnick *et al*. 1999; Avila-Sakar *et al*. 2001). A

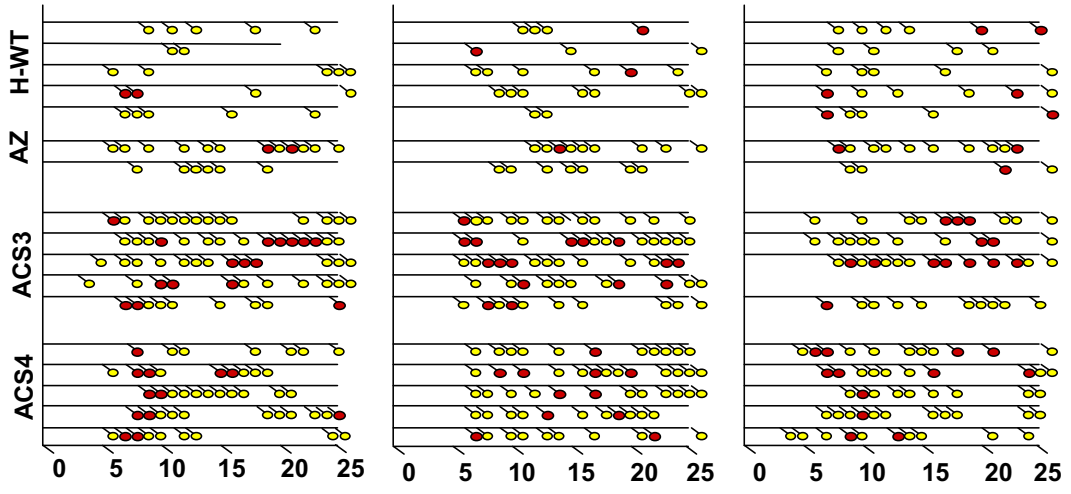

Fig. 2 Node positions of mature carpel-bearing (bisexual) flowers and fruits for non-transgenic [wild type (WT) and azygous segregant (AZ)] and transgenic ACS overexpressing (ACS3 and ACS4) melon plants in the field. Each horizontal line represents the main stem of an individual plant. Yellow circles= mature carpel-bearing flowers; red circles= fruits.

developing fruit in squash and gourd was shown to lead to reduced pistillate flower production and increased fruit abortions (Stephenson *et al*. 1988).

There is evidence to suggest that ethylene plays an important role in this integration of competing resource demands. Endogenous ethylene levels in *Cucurbita texana* were highest adjacent to tips of branches bearing fruit nearing maturity, and lowest when branches carried two or more young fruit, suggesting that the number of developing fruit and their stages of development regulate endogenous ethylene levels (Krupnick *et al*. 1999). If an analogous relationship between ethylene production and the presence of a developing fruit exists in *C. melo*, then increased production of endogenous ethylene in the *35S::ACS* transgenic lines may interfere with the typical signaling which would normally result in lower ethylene and decreased pistillate flowering on branches carrying fruit.

Additional studies have examined the relationship between source-sink balance and reproductive development in melon. Fruit removal significantly increased total number of leaves, male and pistillate flowers, pollinated pistillate flowers, and subsequent fruit set compared to control plants, demonstrating lability of floral sex expression and re-allocation of resources when a dominant sink (melon fruit) is removed (El-Keblawy and Lovett-Doust 1996; Valantin *et al*. 1999; Valatin-Morison *et al*. 2006). Although greater fruit load resulted in an increase in the fraction of assimilates allocated to the fruit, plants with unrestricted fruit load had lower seed weight, and produced fruit with reduced sweetness and firmness (Valantin *et al*. 1999; Valatin-Morison *et al*. 2006). These observations collectively demonstrate the far reaching effects of competition for assimilates between reproductive and vegetative organs, and among reproductive organs.

Recent research in melon also has begun to identify genes associated with fertilization and fruit set (Nagasawa *et al*. 2005). Since the synthetic cytokinin CPPU is effective in the induction of parthenocarpy in melon, the comparision of gene expression between unpollinated, pollinated, and CPPU treated flowers allowed the identification of 14 cDNAs likely to be related to pericarp development, but not seed development (Nagasawa *et al*. 2005). With the exception of one cDNA, all showed similar expression patterns in pollinated and CPPU-treated fruit suggesting that mechanisms of fruit set by parthenocarpy and pollination are very similar. The high levels of expression of *8A07* and *8D10* (putative nucleiod DNA-binding-like protein and short chain alcohol dehydrogenase, respectively) concomitant with the timing of fertilization suggest a role in fruit set. Further gene expression analyses should provide more information about key changes associated with successful fruit set.

FRUIT DEVELOPMENT

The melon fruit was previously described as an indehiscent pepo or inferior berry (Whitaker and Davis 1962). The edible flesh, sucrose accumulating tissue of the fruit that is derived from the middle mesocarp (Barber 1909), was also described as the inner wall tissue (Sinnot 1939; Schaffer *et al*. 1996) or pericarp (Whitaker and Davis 1962). This is unlike the cucumber and the watermelon where the edible flesh comprises mainly the inner mesocarp or placental tissue (Schaffer *et al*. 1996; Robinson and Decker-Walters 1997). The hard lignified rind of melon is derived from the exocarp and contains a network of suberized tissue, referred to as a "net" (Robinson and Decker-Walters 1997; Keren-Keiserman *et al*. 2004). The development of a berry fruit can be divided into three phases where phase I relates to fruit set, phase II to cell division and phase III to cell expansion, followed by a ripening phase (Gillaspy *et al*. 1993). In general, this classification also fits the development and ripening of the melon fruit, although major differences between melon and tomato fruit development have been

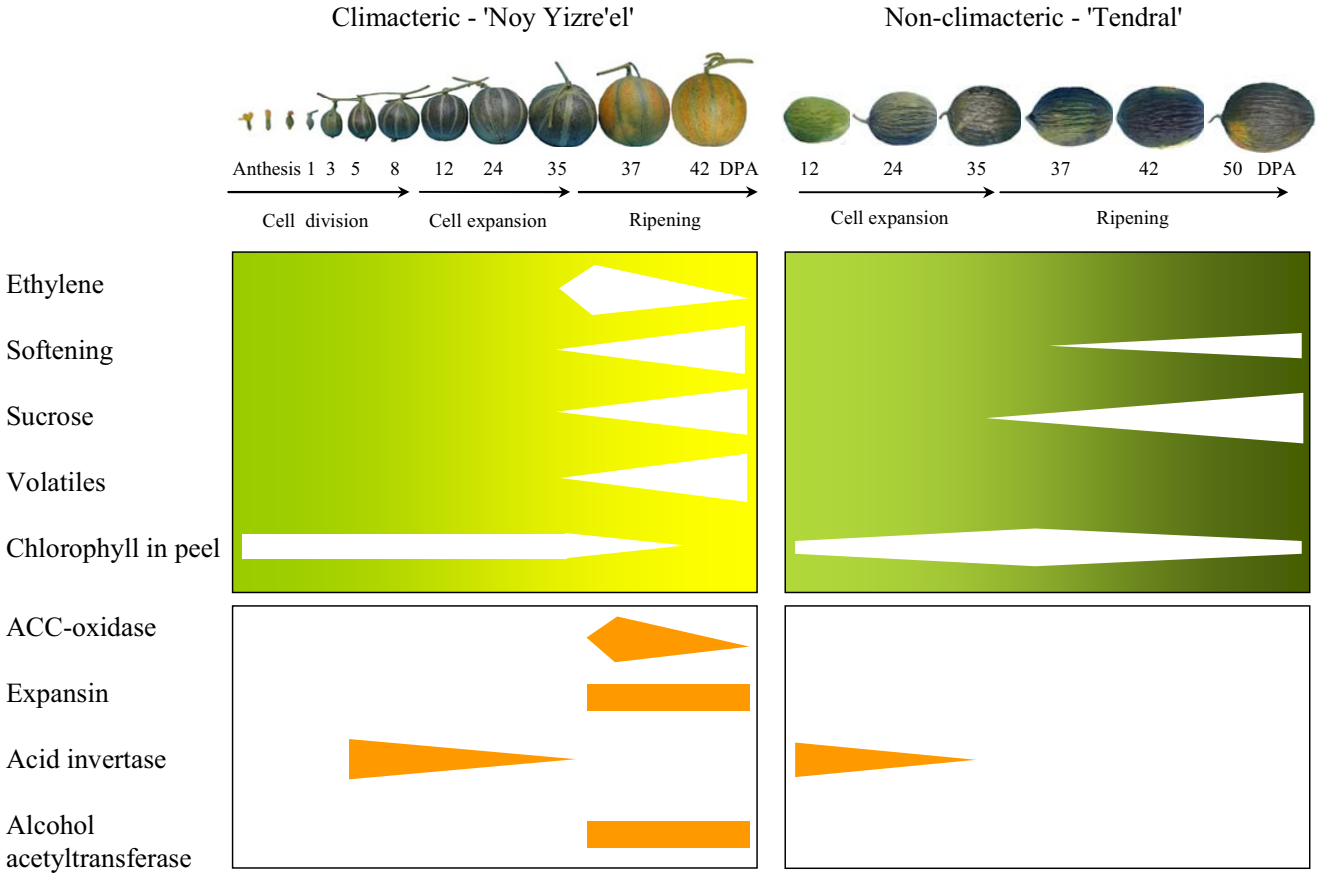

Fig. 3 Changes of physiological and molecular processes during fruit development in climacteric ('Noy Yizre'el') and non-climacteric melons ('Tendral Verde Tardif'). Changes in different traits or level of metabolites throughout melon fruit development are indicated by white diamonds. Changes in steady state levels of selected mRNAs are indicated by orange shapes.

described (e.g. flesh color development as described below). **Fig. 3** depicts the major aspects of melon fruit development in a graphic presentation using a similar format to the tomato development figure of Gillaspy *et al*. (1993).

Melon genotypes are highly diverse in terms of ripening behavior and several other fruit characteristics. Fruits differ in shape, size (affected by length of cell division period, Higashi *et al*. 1999; Kato-Emori *et al*. 2001), sutures, stripes, and color. There are wild and cultivated genotypes with fruits that accumulate various levels of soluble sugars, organic acids, starches, pigments and aroma volatiles. In addition, the fruits differ in oil content and composition of the seeds, ascorbate, secondary metabolites for bitterness and sweetness and additional quality components (Burger *et al*. 2006).

In terms of ripening physiology, melon includes both climacteric and non-climacteric cultivars (Seymour and McGlasson 1993; Lelièvre *et al*. 1997, 2000; Périn *et al*. 2002c). The ripening of the climacteric melons is characterized by a rise in respiration associated with an autocatalytic ethylene burst. The induction of abscission is typical of climacteric behavior (Abeles *et al*. 1992; Seymour and McGlasson 1993; Lelièvre *et al*. 1997, 2000; Périn *et al*. 2002c). Exogenous ethylene can prematurely induce abscission, ethylene production and ripening in climacteric melons only (McMurchie *et al*. 1972; Lelièvre *et al*. 1997; Shiomi *et al*. 1999, Lelièvre *et al*. 2000; Périn *et al*. 2002c). Non-climacteric melons were suggested to be defective in their ethylene synthesis or response (Périn *et al.* 2002c; Giovannoni 2004). This is in agreement with our observations on ethylene production and expression patterns of ethylene related genes [(e.g. *ACC oxidase* (*ACO*) and *Alcohol acyltransferase* (*AAT*)] in a wide range of melon genotypes suggesting various levels of climacteric responses (Burger, Portnoy and Katzir, unpublished data). **Fig. 3** depicts a schematic presentation of traits and associated genes in climacteric ('Noy Yizre'el') versus "strong" non-climacteric ('Tendral Verde Tardif') genotypes. The termination of the ripening phase is clear-cut in climacteric melons, with a change of color and the development of the distinct abscission zone, and harder to define in non-climacteric melons.

Ethylene plays a major role in fruit ripening as well as in flower development of melon. Several members of the two gene families coding for key enzymes involved in ethylene synthesis, *AC*S and *ACO*, were cloned and characterized. Three melon *ACS* genes and their expression profiles were described: *CMe- ACS1*, *CMe- ACS2* and *CMe- ACS3* (Miki *et al*. 1995; Yamamoto *et al*. 1995; Ishiki *et al*. 2000). Balagué *et al*. (1993) cloned the first melon *ACO*-like gene, *pMEL1* from a cDNA library using a tomato ethylene-forming enzyme as a probe. The expression pattern of *pMEL1* indicated a clear association with fruit ripening and wound response. The gene was later designated *Cm-ACO1*, and proved to be the major *ACO* gene associated with melon ripening. Additional *ACO* genes, *Cm-ACO2* and *Cm-ACO3* were suggested to be associated with ethylene response in etiolated hypocotyls and in flowers, respectively (Lasserre *et al*. 1996; Bouquin *et al*. 1997). Much of the currently available knowledge concerning the effects of ethylene on melon ripening was gained through studies of an anti-sense *ACO* 1 recombinant plant, in which the capacity for ethylene synthesis was inhibited by 99% (Ayub *et al*. 1996). In the antisense fruits the ripening process was blocked both on and off the vine. It was noted, however, that the process also included ethylene independent pathways. Accumulation of soluble sugars in developing fruits and carotenoid content of the flesh were ethylene-independent while fruit softening, chlorophyll degradation and activation of the abscission zones were clearly ethylene-dependent (Ayub *et al*. 1996; Hadfield *et al*. 2000; Flores *et al*. 2001b; Nishiyama *et al*. 2007). The antisense phenotype could be reversed by exogenous ethylene treatment (Ayub *et al*. 1996). These findings were in agreement with prior studies, in which sugar and carotenoid accumulation was found to rise earlier than ethylene production (Bianco and Pratt 1977; Lingle and Dunlap 1987; Aggelis *et al*. 1997b). It also should be noted that although most orange fleshed melons are climacteric, it is possible to breed for non-climacteric orange fleshed melons (Aggelis *et al*. 1997b; Zheng and Wolff 2000). The change of flesh color, which is independent of ethylene synthesis, differs from the general scheme in tomato in which the red color is associated with ethylene synthesis and perception. The fact that sugar accumulation and the development of flesh color are independent of ethylene burst may well explain the capacity to breed for divergent, non-climacteric melon varieties.

Signal transduction of ethylene has been thoroughly studied in the model plants *Arabidopsis* and tomato. An important feature of the ethylene signaling pathway is that it comprises both positive and negative regulators, some proteins thereby serving to induce the responses while others suppress them. By identifying and cloning mutants unable to mount a normal triple response in the presence of ethylene (such as *ein2*, *etr1*, *ein3*, *ein4*, *ein5*, *ein6*, *etr2*, etc.) as well as mutants that display a constitutive triple response (such as *ctr1*, *ran1/ ctr2*, *eto1*, *eto2*, and *eto3*), a basic signal transduction cascade has been reconstructed (Guo and Ecker 2004; Klee 2004; Chen *et al*. 2005). Major components of this cascade that are associated with tomato ripening have been identified (Tieman *et al*. 2001; Alexander and Grierson 2002; Adams-Philips *et al*. 2004; Giovannoni 2004). Recently, a number of key tomato ripening genes, such as non-ripening (*nor*), ripening inhibitor (*rin*), Never-ripe (*Nr*), Colorless non-ripening (*Cnr*), and Green-ripe (*Gr*) were cloned based on studies of ripening mutants (Vrebalov *et al*. 2002; Giovannoni 2004; Manning *et al*. 2006; Barry and Giovannoni 2006), enabling better understanding of the regulation of ethylene synthesis and perception in tomato fruit, and probably in additional fleshy fruits. Melon homologs of ethylene signal transduction genes, including *Cm-ETR1*, *CmERS1* (Sato-Nara *et al*. 1999; Takahashi *et al*. 2002; Ma *et al*. 2006) and *Cmrin* (Binzel *et al*. 2007) were cloned based on conserved sequences and characterized with respect to expression profiles during fruit development.

A number of genes that are differentially expressed through ripening were cloned and several were found to code for key enzymes associated with fruit development and quality characteristics. The cDNA library used by Balagué *et al*. (1993) to isolate the *Cm-ACO1* gene was further used to isolate additional clones showing differential expression during ripening, e.g. *Mel 5* (Karvouni *et al*. 1995), *Mel 2* and *Mel 7* (Aggelis *et al*. 1997a). *Mel 5*, a phytoene synthase (PSY) homolog, was found to be expressed mainly in fruit with the highest level of PSY at the point of change of color from green to orange (Karvouni *et al*. 1995). *Mel 2* was later found to encode alcohol acetyl transferase (AAT1), a key enzyme associated with melon aroma (Yahyaoui *et al*. 2002), while *Mel 7* exhibits sequence similarity to a major latex protein (Aggelis *et al*. 1997a) whose function in ripening is yet unknown. Using a differential screening approach, Hadfield *et al*. (2000) identified 16 unique cDNAs with enhanced expression during ripening, eight of which were previously characterized, including, *Mel 1*, *Mel 2* and *Mel 7* mentioned above, and two genes associated with sulfur amino acid biosynthesis. Recently, a Melon EST Database (http://melon.bti.cornell.edu/) was developed for dissemination of approximately 4000 melon EST sequences obtained from various cDNA libraries of fruits, including SSH libraries and non-subtracted, non-normalized libraries (Giovannoni and Katzir, unpublished data). Of the genes mentioned above, *Mel 1* (ACC oxidase 1), *Mel 2* (*AAT1*) and *Mel 7* (major latex protein) were found to be most abundant in all libraries of climacteric melons (Katzir and Portnoy, unpublished data). A number of genes with sequence similarities to genes associated with ethylene synthesis and perception as well as MADs box with ripening-specific expression profiles were also identified through this database (Katzir, Portnoy and Giovannoni, unpublished data).

The major metabolic pathways associated with fruit ma-

turation include cell wall, carbohydrate, color and aroma metabolism. While each of the above-mentioned pathways deserves a separate review, current knowledge on each is briefly provided below.

Cell wall metabolism

The process of cell wall disassembly in melon, and the genes and enzymes involved has been studied extensively (e.g., Hadfield *et al*. 1998; Rose *et al*. 1998; Nishiyama *et al*. 2007). Ethylene plays a major role in enhancing this process (Ayub *et al*. 1996; Guis *et al*. 1997; Hadfield *et al*. 2000; Flores *et al*. 2001a) and consequently, the activities of these enzymes differ widely between climacteric and non-climacteric varieties. Ethylene-suppressed lines of melon exhibited delayed and reduced fruit softening (Ayub *et al*. 1996; Guis *et al*. 1997; Flores *et al*. 2001a; Nishiyama *et al*. 2007). In addition, recent analyses by Nishiyama *et al*. (2007) of a diverse range of cell wall related genes, including those for polygalacturonases, xyloglucan endotransglucosylase/hydrolases, expansin, and β-galactosidases, identified specific genes within single families that could be categorized as ethylene-dependent, ethylene-independent, or partially ethylene-dependent. Their results support the hypothesis that while individual cell wall-modifying proteins from each family contribute to cell wall disassembly accompanying fruit softening, other closely related family members are regulated in an ethylene-independent manner and apparently do not directly participate in fruit softening (Nishiyama *et al*. 2007).

Sugar

The major components of the soluble sugar fraction of the ripe melon fruit are sucrose, glucose and fructose. The increase in sugar content during ripening is primarily a function of the accumulated sucrose, while glucose and fructose levels fluctuate much less, if at all (Rosa 1928; Pratt 1971; Hughes and Yamaguchi 1983; Lester and Dunlap 1985; Schaffer *et al*. 1987; McCollum *et al*. 1988; Hubbard *et al*. 1989; Schaffer *et al*. 1996). The fruit undergoes a phase of developmental transition in which the early period of growth is characterized by the absence of sucrose and followed by a period of sucrose accumulation, which continues until abscission or harvest. Because the melon fruit contains no starch reserves (Rosa 1928) there is no net increase in sugar content after harvest. Therefore, the accumulation of sucrose is a most important variable in determining melon fruit quality.

Variation in sucrose levels accounts for the genetic differences in total sugar contents and for the natural variability within a particular cultivar due to environmental differences (Stepansky *et al*. 1999; Burger *et al*. 2002, 2006). While a number of genes participate in the sucrose metabolism and affect sucrose content, a single recessive gene was suggested to control the ability to accumulate sucrose in the sweet melons (Burger *et al*. 2002).

Studies have demonstrated that the metabolic transition from the stage of fruit growth to that of sucrose accumulation is characterized by a developmental loss of soluble acid invertase (AI) activity (Schaffer *et al*. 1987; McCollum *et al*. 1988; Hubbard *et al*. 1989; Ranwala *et al*. 1991; Iwatsubo *et al*. 1992; Lester *et al*. 2001). A key role for sucrose phosphate synthase (SPS) activity in sucrose accumulating melon fruit was proposed by Hubbard *et al*. (1991) who had demonstrated that sucrose accumulation was characterized by a developmental rise in SPS activity, in addition to the loss of AI activity. Lester *et al*. (2001) confirmed the importance of the loss in AI activity and the rise in SPS activity in two sweet melon cultivars and emphasized particularly the necessity for SPS activity to be higher than that of AI. The activities of both sucrose synthase (SuSy, Giaquinta 1979; Schaffer *et al*. 1987; Moriguchi *et al*. 1990, 1992; Suzuki *et al*. 1996) and neutral, or alkaline invertase (NI, Ricardo and ap Rees 1970; Glasziou and Gayler 1972; Kato and Kubota 1978) also have been implicated in sucrose accumulation. However, the role of these enzymes in sucrose accumulation is not obvious, since SuSy is generally associated with sucrose cleavage rather than synthesis, and NI catalyzes the hydrolysis of sucrose.

Although primary photosynthate production plays a role in determining the availability of assimilate supply, the accumulation of sucrose appears to be controlled by the metabolism of carbohydrates in the fruit sink itself (Hubbard *et al*. 1989; Schaffer *et al*. 1996, 2000; Lester *et al*. 2001). Sucrose and the galactosyl-sucrose oligosaccharides, raffinose and stachyose, are translocated from the source to fruit sink in the *Cucurbitaceae* family, including *C. melo* (Mitchell *et al*. 1992; Chrost and Schmitz 1997). Yet, the absence of raffinose and stachyose in the fruit flesh points to the rapid hydrolysis and metabolism of these translocated sugars in the fruit, or adjacent to it (Hughes and Yamaguchi 1983; Hubbard *et al*. 1989; Pharr and Hubbard 1994; Chrost and Schmitz 1997). Key genes associated with sucrose and galactose metabolism have been recently cloned (Carmi *et al*. 2003; Dai *et al*. 2006; melon EST database).

Organic acids and pH

The sweet melon varieties of the *reticulates*, *inodorus* and *cantaloupensis* groups are unique in that organic acid levels play little role in determining their quality, which is determined by sweetness alone (Yamaguchi *et al*. 1977). Sweet melons have a low content of organic acids, of which citrate is the major acid (Seymour and McGlasson 1993). Other *C. melo* groups have fruit with high acidity in the mature fruit (Kubicki 1962; Mallick and Mausi 1986; Stepansky *et al*. 1999; Pitrat *et al*. 2000; Burger *et al*. 2003). These fruits are typically consumed when young, prior to the decrease of pH, similar to a cucumber. High acidity in melon fruit is controlled by a single dominant gene, *So* (*Sour*) (Kubicki 1962; Danin-Poleg *et al*. 2002; Burger *et al*. 2003).

In addition, the high-acid groups do not accumulate the high levels of sugar characteristic of the sweet melon groups. The combination of high sugar and high acid has been reported recently (Burger *et al*. 2003). Developmental studies show that the accumulation of acid and sucrose are temporally separated. Acid accumulation precedes sucrose accumulation, which only begins when acid accumulation is completed (Burger *et al*. 2003).

Aroma

The aroma of melons is the result of complex mixtures of volatile compounds. Aroma development in melons is strongly associated with climacteric ripening and represents a major characteristic in the overall quality of the fruit (Wang *et al*. 1996; Beaulieu and Grimm 2001; Jordan *et al*. 2001; Shalit *et al*. 2001). In recent years melon volatiles have been extensively investigated (Shalit *et al*. 2001; Yahyaou *et al*. 2002; Aubert and Bourger 2004; Aubert and Pitrat 2006; Ibdah *et al*. 2006; Manriq'uez *et al*. 2006). In aromatic melon varieties, volatile esters, mainly acetate derivatives, are prominent, together with lower amounts of lactones, sulfur compounds, sesquiterpenes, norisoprenes, short-chain alcohols and aldehydes (Shalit *et al*. 2001; Yahyaoui *et al*. 2002; Aubert and Bourger 2004; Aubert and Pitrat 2006; Ibdah *et al*. 2006; Manriq'uez *et al*. 2006). Non-climacteric varieties (e.g., 'Rochet') often have much lower levels of total volatiles, and especially lack the volatile esters (Shalit *et al*. 2001). In contrast, volatile aldehydes and alcohols are most abundant in the non-climacteric variety 'Rochet' (Shalit *et al*. 2001). The ACC-oxidase antisense melons exhibited a considerable reduction in total volatile production (Flores *et al*. 2002). In hybrids developed from the ACC-oxidase antisense melon, the total volatiles were 60-85% lower than in non-transformed hybrids (Bauchot *et al*. 1998; Flores *et al*. 2002). In agreement with these studies, a considerable reduction in total volatiles

(49-87%) was observed in long shelf life cultivars of Charentais melon, compared with shorter shelf life Charentais melons (Aubert and Bourger 2004).

Recently, biochemical and molecular characteristics of the enzymes involved in aroma production have been extensively studied, mainly those of AATs (Shalit *et al*. 2001; Yahyaou *et al*. 2002; El-Sharkawy *et al*. 2005). In addition, alcohol dehydrogenase (ADH, Manriq'uez *et al*. 2006), carotenoid cleavage enzymes (Ibdah *et al*. 2006) and sesquiterpenes synthase genes were described (Benyamini *et al*. submitted).

Color

As described above, the development of flesh color is an ethylene-independent trait. In contrast, the change of rind color during fruit ripening is affected by ethylene synthesis. This change is a result of two processes: chlorophyll degradation and pigment (carotenoids and others) exposure and/or accumulation, where chlorophyll degradation is the ethylene-dependent component (Ayub *et al*. 1996; Guis *et al*. 1997; Flores *et al*. 2001a, 2001b). This is in agreement with change in rind colors of climacteric varieties that occur during fruit development, due to chlorophyll degradation, from dark green to yellow-orange (e.g. Galia or American muskmelon types) or from white-green to cream yellow (e.g. Charantais type). The non-climacteric varieties that begin dark green (e.g. green cassaba varieties such as 'Tendral' and 'Piel de Sapo') stay dark green throughout fruit development due to lack of chlorophyll degradation, while pale green fruits (e.g. Yellow Canari type) become yellow due to pigment accumulation.

CONCLUDING REMARKS

Successful flower and fruit development are the result of a highly orchestrated interplay of developmental, hormonal, genetic, and environmental cues. In melon, extreme diversity in sex expression and fruit characteristics provide additional layers of complexity to these processes. Recent studies have led to mapping, cloning, and identification of key genes associated with sex expression, floral development and fruit development; have produced gene expression analyses to identify enzymatic and regulatory changes associated with various stages of development; and have furthered our understanding of the role of ethylene production and perception in floral sex determination and development, and fruit set, maturation and ripening.

Floral development in *Cucumis*, especially with regard to the differential development of stamens and carpels, is in many ways a story of ethylene. Ethylene biosynthetic and receptor homolog genes in developing cucumber flowers exhibit highly regulated location and timing of expression with respect to the development of floral sex organs (Kamachi *et al*. 1997; Kahana *et al*. 1999; Kamachi *et al*. 2000; Yamasaki *et al*. 2000, 2001, 2003a, 2003b). Differentiation of stamen primordia has been identified as a critical stage for sex determination in cucumber (Hao *et al*. 2003; Bai *et al*. 2004) and ethylene perception by melon stamen primordia appears to be necessary to stimulate development of carpels (Little *et al*. 2007), suggesting ethylene-mediated cross talk between the developing primordia. In cucumber, the *F* locus conferring femaleness encodes a second copy of the key ethylene biosynthetic gene, *ACS* (*CsACS1G*), thereby providing a means to increase the endogenous level of ethylene and promote carpel development (Trebitsh *et al*. 1997; Mibus and Tatlioglu 2004; Knopf and Trebitsch 2006). In melon, it has been suggested that sex expression genes may influence ethylene sensitivity (Byers *et al*. 1972b). Recent molecular mapping and cloning efforts have been directed toward the melon *A* and *G* sex expression genes (Périn *et al*. 2002a; Silberstein *et al*. 2003; Noguera *et al*. 2005; Fergamy *et al*. 2006). The molecular identity of these genes will be of great interest in further understanding the sex determination process.

Ethylene also appears to be important for maturation of the carpel-bearing flowers to anthesis and to play a role in resource allocation to reproduction (Papadopolou *et al*. 2006; Little *et al*. 2007). Increased number of mature flowers on ACS-overexpressing melons led to increased fruit set on closely spaced nodes (Papadopoulou *et al*. 2006). Typically, presence of a developing melon fruit will inhibit further flower and fruit production (El-Keblawy and Lovett-Doust 1996; Valantin *et al*. 1999; Valatin-Morison *et al*. 2006). In *Cucurbita texana* it has been observed that the presence of young developing fruits is associated with reduced endogenous ethylene levels (Krupnick *et al*. 1999). A two-step, ethylene-mediated regulation of carpel-bearing flower development raises the possibility of two stages of control of resource allocation: initiation of carpel-bearing flowers based on developmental stage of the plant; and maturation or termination of carpel-bearing flowers depending on current fruit set status. Recent studies also have begun to identify melon gene expression associated with fertilization and fruit set and to compare mechansims of fruit set associated with parthenocarpy vs. pollination (Nagasawa *et al*. 2005).

Fruit development and especially fruit ripening are additional facets of development for which ethylene plays a key role. Climacteric ripening is characterized by a rise in respiration, associated with an autocatalytic ethylene burst. Major metabolic pathways in the fruit are associated with this ethylene burst, including cell wall disassembly, abscission zone formation, synthesis of aroma volatiles and chlorophyll degradation which affects rind change of color. Recent genomic studies have begun to identify key genes associated with ripening processes and quality parameters (e.g. Balagué *et al*. 1993; Aggelis *et al*. 1997a, 1997b; Hadfield *et al*. 2000; El-Sharkawy *et al*. 2005; Ibdah *et al*. 2006; Manrìquez *et al*. 2006; Binzel *et al*. 2007) and transgenic studies have helped to differentiate between ethylene dependent and independent processes (Ayub *et al*. 1996; Guis *et al*. 1997; Flores *et al*. 2001a, 2001b; Nishiyama *et al*. 2007). The metabolic pathways that are ethylene-independent include the accumulation of sucrose, which is the major soluble sugar of sweet melons and the accumulation of carotenoids which determines flesh color (Bianco and Pratt 1977; Lingle and Dunlap 1987; Aggelis *et al*. 1997b). Genes associated with these pathways have been cloned (Karvouni *et al*. 1995; Carmi *et al*. 2003; Dai *et al*. 2006; and melon EST database). However, little is known about the genes that control the ethylene burst and the potential mutations that result in non-climacteric physiology characterizing specific melon groups.

In summary, analysis of reproductive development in melon provides numerous questions of fundamental interest and economic importance. Recent studies have yielded new insights into key developmental processes and have raised new questions to be explored with regard to sex expression, floral development, fruit set and development, and climacteric and non-climacteric ripening processes.

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