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ARTICLE

Genetics of Flavonoid, Carotenoid, and Chlorophyll Pigments in Melon Fruit Rinds

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External color has profound effects on acceptability of agricultural products by consumers. Carotenoids and chlorophylls are known to be the major pigments of melon (*Cucumis melo* L.) rinds. Flavonoids (especially chalcones and anthocyanins) are also prominent in other fruits but have not been reported to occur in melons fruit. We analyzed the pigments accumulating in rinds of different melon genotypes during fruit development. We found that melon rind color is based on different combinations of chlorophyll, carotenoids, and flavonoids according to the cultivar tested and their ratios changed during fruit maturation. Moreover, in "canary yellow" type melons, naringenin chalcone, a yellow flavonoid pigment previously unknown to occur in melons, has been identified as the major fruit colorant in mature rinds. Naringenin chalcone is also prominent in other melon types, occurring together with carotenoids (mainly β-carotene) and chlorophyll. Both chlorophyll and carotenoid pigments segregate jointly in an F_2 population originating from a cross between a yellow canary line and a line with green rind. In contrast, the content of naringenin chalcone segregates as a monogenic trait independently to carotenoids and chlorophyll. Transcription patterns of key structural phenylpropanoid and flavonoid biosynthetic pathway genes were monitored in attempts to explain naringenin chalcone accumulation in melon rinds. The transcript levels of *CHI* were low in both parental lines, but *C4H*, *C4L*, and *CHS* transcripts were upregulated in "Noy Amid", the parental line that accumulates naringenin chalcone. Our results indicate that naringenin chalcone accumulates independently from carotenoids and chlorophyll pigments in melon rinds and gives an insight into the molecular mechanism for the accumulation of naringenin chalcone in melon rinds.

KEYWORDS: Flavonoids; carotenoids; chlorophyll; naringenin chalcone; *Cucumis melo*; transcriptional regulation

INTRODUCTION

Fruit rind color is one of the first attributes that influence consumers' choice and acceptability of the product and thus is considered an important parameter determining fruit quality. Moreover, fruit rind color differentiates between varieties and fruit types (*1*). Melon exhibits large variation in morphological and physiological characters such as fruit size, taste, and internal and external color and is an important fruit cultivated in tropical and temperate regions of the world (*1*, *2*). Rind color of melon fruit varieties includes white, yellow, orange, or green and can also be variegated or striped. The pigments conferring this external fruit color variation have been reported to be carotenoids, mainly β-carotene, and chlorophylls (*2*). Other color patterns of different plant tissues can be formed by the combination of pigments from different groups (*3*, *4*). We report here, for the first time, the identification of an additional pigmentation system of melon rind based on the flavonoid family of pigments.

Flavonoids are polyphenolic secondary metabolites ubiquitous to plants. Thus far, about 9000 flavonoids have been characterized (*5*). Flavonoids are subgrouped into chalcones, flavanones, flavandiols, flavones, flavonols, anthocyanins, pro-anthocyanidins, and aurones, among others (*6*). The flavonoid biosynthetic pathway has been studied extensively in several plants, and their biosynthetic pathway is highly conserved (*7*). In plants, flavonoids have diverse functions, acting as powerful antioxidant agents, antimicrobial compounds, UV protectants, insect protectants, pollen germination stimulantsm, and visual attractors to pollinators (*8*−*10*).

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Table 1. Properties of the Four Melon Genotypes Examined in the Study (Groups According to ref *1*)[a]

genotype	marketing type	group	fruit ripening	flesh color	skin color	
					young fruit	mature fruit
Dulce	American Shipper	*Reticulatus*	climacteric	orange	dark-green	green−orange
Noy Amid	Yellow Canary	*Inodorus*	nonclimacteric	green−white	green	yellow
Rochet	Casaba	*Inodorus*	nonclimacteric	green−white	dark-green	green−yellow
Tendral Verde Tardio	Casaba	*Inodorus*	nonclimacteric	green−white	dark-green	dark-green

[a] "Flesh" is the edible mesocarp, while "skin" is the non-edible fruit rind.

Naringenin chalcone (4,2′,4′,6′-tetra hydroxyl chalcone), the first flavonoid synthesized in the flavonoid biosynthetic pathway, is the product of the condensation of three acetate units starting from malonyl-CoA with *p*-coumaroyl-CoA catalyzed by chalcone synthase (CHS), the first enzyme fully committed to flavonoid biosynthesis. Accumulation of naringenin chalcone (a yellow colored pigment) in plant tissue is rare: chalcone rapidly isomerizes either spontaneously at a neutral pH or enzymatically by the action of chalcone isomerase (CHI) to form naringenin, a colorless flavanone (*7*). Nevertheless, there are examples of chalcone accumulation in plants mutated in CHI. Yellow corollas of *Callistephus chinensis* (China aster) (*11*) and *Dianthus caryophyllus* (Carnation) (*12*) accumulate natingenin chalcone as their major pigment and are devoid of CHI activity. Yellow onion accumulates naringenin chalcone in its bulb due to the presence of a natural premature stop codon within the *CHI* gene (*13*). Similarly, the yellow fruit cuticle of most cultivated tomato varieties is also due to the accumulation of the chalcone due to very low activity levels of CHI during fruit development (*14*).

In this paper, we report the analysis of the rind pigments of representatives of the highly polymorphic melon germplasm collection characterized by different rind colors and surprisingly found that the main pigment which accumulated in the rind tissue of the yellow-skinned "canary yellow" melons is the flavonoid naringenin chalcone. Naringenin chalcone was also detected in rinds of other melon cultivars. This study is part of a chemical and genetic characterization of the accumulation of pigments in melon fruit rind during fruit development as related to their effect on melon fruit external color.

MATERIALS AND METHODS

Plant Material. Four commercial melon varieties that differ in rind color, as well as in marketing type, taxonomic group, climacteric ripening, and flesh color (**Table 1**) were planted as a fall crop with 0.25 m spacing in a heated greenhouse at the Newe Yaar Research Center in northern Israel. Plants were self-pollinated. At least three fruits from each genotype were harvested at 10 and at 25 days after anthesis (DAA) as well as from mature fruits determined by experienced eyes (~60 DAA). In addition, a "Tendral Verde Tardio" ("TVT") × "Noy Amid" F_1 and F_2 hybrid population containing 25 F_1 plants and 220 F_2 plants was also grown. The hybrid population fruits were harvested as mature fruits. For rind sample preparation, the rind was removed from fruit top to bottom using a Super Duper melon rind peeler, which peels stripes of ~1 wide of the outer ~3 mm of rind. The rind was immediately frozen in liquid nitrogen, and the frozen material was ground to a fine powder in the presence of liquid nitrogen utilizing an A-11 grinder (IKA, Germany). The rind powder was divided into separate tubes for the analyses of carotenoids, chlorophyll, and flavonoids.

Carotenoids Extraction and Quantification. All chemicals and solvents were purchased from Sigma (Saint Louis, MO). Rinds for carotenoid analysis were sampled as explained in the Plant Material section. Three biological replications of every accession were analyzed, each consisting rinds of at least one fruit for which two technical replications were analyzed. All extraction steps were carried out in darkness or under gold fluorescent light to avoid possible photodegradation of carotenoids. Two aliquots of about 0.5 g were taken from each sample, representing two technical repeats of each genotype. Total carotenoids extraction were carried out according to Tadmor et al., 2005 (*15*) with minor modifications. Extraction included rigorous shaking of the powdered sample in 8 mL of hexane:acetone:ethanol (50:25:25 v/v/v) for 5 min, followed by the addition of 1 mL KOH in water (80% w/v), vortexing, and shaking for 5 min, followed by the addition of 1 mL of NaCl in water (25% w/v), vortexing, and shaking for an additional 5 min. Subsequently, 8 mL of water were added, the samples were vortexed and incubated for 10 min on the bench in darkness until phase separation was achieved. Finally, the upper phase was collected, and the lower phase was re-extracted with an additional 2 mL of hexane, and the combined extract was analyzed spectroscopically for absorbance in the wavelength range of 300−600 nm using a Varian spectrophotometer (Cary50Bio). Total carotenoids concentration was calculated as in Davis, 1965 (*16*).

Chlorophyll Determination. Rinds for chlorophyll determination were samples as explained for carotenoid analysis. All solvents were HPLC grade and were purchased from Sigma (Saint Louis, MO).

Chlorophyll extraction was performed in dimmed light to avoid possible photodegradation of chlorophyll. Chlorophyll was extracted by adding 5 mL of dimethyl sulfoxide (DMSO) to 0.5 g, vortexing and incubating in the dark at room temperature for 24 h. The extract was analyzed for absorbance in the wavelengths of 663 and 645 nm (*17*) using a Cary50Bio spectrophotometer (Varian). Chlorophyll concentration was calculated as in ref *18*.

Flavonoid Extraction and Analysis. Rinds for flavonoid analysis were sampled as explained for carotenoid analysis. All solvents were HPLC grade and were purchased from Sigma (Saint Louis, MO). Flavonoid extraction for routine HPLC analysis was essentially carried out as described in (*14*) with slight changes. Approximately 0.5 g samples were extracted in 5 mL of 100% methanol in an ultrasonic bath for 25 min. Extracts were filtered (0.45 μm poly tetrafluoroethylene filter, Acrodisc, PAL), and 40 μL samples were injected to a 2996 Waters HPLC system equipped with a Waters PDA detector 996, C18 Nova-Pak column (250 3 mm × 4.6 mm i.d.; 60 A°; 4 mm, Waters), and a Nova-Pak Sentry Guard cartridge (Waters). HPLC separation was carried out using 25% acetonitrile in 0.1% trifluoroacetic acid (TFA) under isocratic conditions at a flow rate of 1.0 mL/min. Naringenin chalcone was identified by the comparison of its retention time and its UV−visible spectra with authentic naringenin chalcone standards (Apin Chemicals, UK). A standard calibration curve was prepared and quantification was performed by integrating the peak areas of the HPLC results using Millennium chromatography software (Waters).

Identification of Naringenin Chalcone by LC-MS. Dry ground melon samples (50 mg) were weighed into 2 mL Eppendorf tubes and extracted with 1 mL of 80% methanol. The mixture was sonicated for 20 min at room temperature, centrifuged (15000*g*, 10 min), and filtered through a 0.22 μm PVDF membrane filter (Millex-GV 4 mm). Mass spectral analyses were carried out by the UPLC-QTOF instrument (Waters Premier QTOF), with the UPLC column connected online to a UV detector and then to the MS detector, as described in Mintz-Oron et al. (*19*). Separation of compounds was performed with isocratic elution of acetonitrile−water (containing 0.1% formic acid) buffer on the 100 mm × 2.1 mm i.d., 1.7 μm UPLC BEH C18 column (Waters Acquity). Masses of the eluted compounds (m/z range from 50 to 1500 Da) were detected with a QTOF Premier MS instrument equipped with an ESI source (performed in negative mode). MS/MS spectra were acquired at collision energy of 30 eV; argon was used as a collision gas. Naringenin chalcone in melon samples was identified by comparison of its retention time, MS spectrum, and MS/MS fragments to those of the naringenin chalcone standard, injected in the same acquisition conditions.

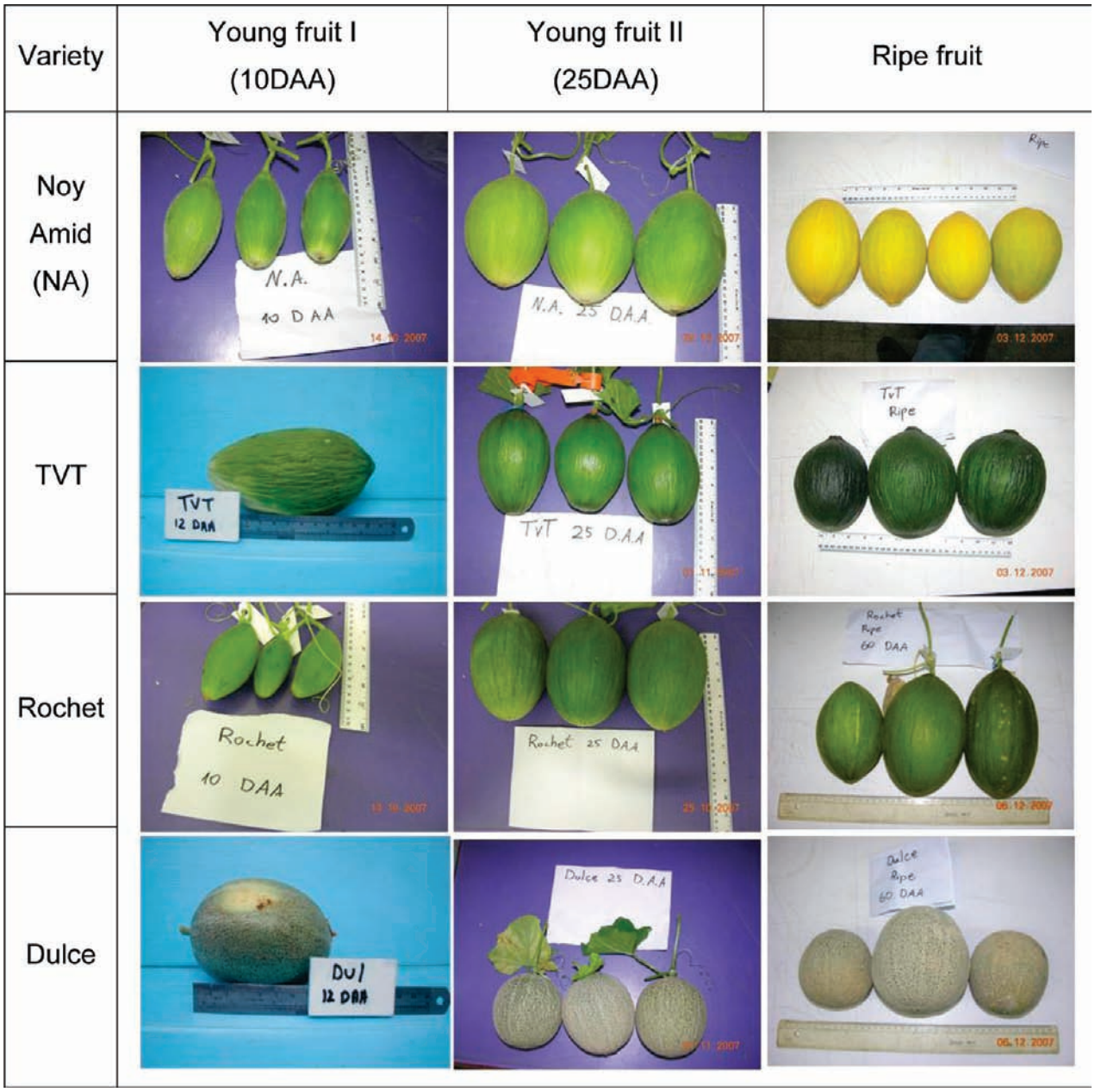

Figure 1. Developmental and varietal rind color changes in the four melon genotypes examined in this study. The gray color of matured "Dulce" fruit is due to the netting system it develops. "Dulce" fruit gets matured around 40 days after anthesis (DAA) while the other varieties get matured around 60 DAA.

RNA Extraction. Total RNA was extracted from 5 g of frozen powdered rind samples as previously described (*20*). Poly (A)+ mRNA was purified from 1 mg of total RNA by use of the Oligotex_ mRNA purification kit according to manufacturer's recommendations (Qiagen, Hilden, Germany).

Real-Time PCR Analysis. Total RNA was isolated separately from rind tissues of "TVT" and "Noy Amid" fruits at various developmental stages and used as template for cDNA synthesis. RNA samples (50 μg) were treated with RNase-free DNase I (20 units) (Epicentre, Fermentas, USA) for 15 min at 37 °C. cDNA was produced using the RNA as template with Verso cDNA Kit (Thermo Scientific) The cDNA was then diluted in a total volume of 100 μL of 3 mM Tris−HCl, pH 7.2, 0.2 mM EDTA solution. A 2 μL aliquot of cDNA was used for each real-time PCR performed with the ABI Prism7000 sequence detection system (Applied Biosystems, Foster, CA). Amplifications were conducted using the ABsoluteTMQPCR SYBR_ Green Mixes (ABgene's Inc., Epsom, UK). The following primer sequences (0.2 μm final concentration) were used:

Cinnamate-4-hydroxylase (*C4H*) (AI21D07 in ICuGI (http://www.icugi.org/))

Forward primer: 5′-CCAGAAATCCAAAGAAAGCT-3′ and reverse: 5′-GCATCGTGGAGGTTCATG-3′

4-Coumarate-CoA-ligase (*4CL1*) (FR14L23 & FR14G23 in ICuGI)

Forward primer: 5′-CGTTCTTCCGTTATTCCAC-3′ and reverse: 5′-CGCTCGAGTATGATTTGATG-3′

Chalcone synthase 1 (*CHS1*) (FR13K22 in ICuGI)

Forward primer: 5′-TTGACTGCTCGAATAATAAACC-3′ and reverse:5-′AAAGCTGAAATGTTCGTCAAG-3′

Chalcone isomerase (*CHI1*) (CI30H09 & HS35B02 in ICuGI)

Forward primer: 5′-CGGTGTTTATTTGGAGGAC-3′ and reverse: 5′-GCCACTTTCATTGCATATTG-3′

cyclophilin (a house-keeping gene, accession no. DV632830)

Forward primer 5′-GATGGAGCTCTACGCCGATGTC-3′ and reverse 5′-CCTCCCTGGCACATGAAATTAG-3′.

Thermal cycling was initiated by 15 min at 95 °C, followed by 40 cycles of 90 °C, 15 s; 60 °C, for 1 min. Real-time PCR was performed in duplicate for each primer combination and three biological samples of each developmental stage were tested. C_t values were determined by the ABI Prism 7000 SDS software. Real-time efficiencies (E) were calculated from

the slopes of standard curves for each gene (10[−1/slope]). Result analysis was performed by the $2^{-\Delta\Delta CT}$ method (*21*).

Statistical Analyses. Chi square analysis, analysis of variance, and bivariant fit correlation analysis were conducted utilizing Jump 5.0.1a software (SAS).

RESULTS

Characterization of Melon Rind Color and Pigmentation. The rinds of four different melon genotypes examined differ in external color at different stages of maturity. The rind of cv. "Noy Amid", a nonclimacteric variety of the *inodorous* group of melons, is light green when nonripe but turns into an intense "canary yellow" hue when ripe (**Figure 1**, **Table 1**). In contrast, "TVT" and "Rochet", both also nonclimacteic *inodorous* types, remain green when mature. The green color intensifies during ripening in "TVT" but obtains a green−yellow hue in the mature "Rochet". In contrast, the "Dulce" variety, a climacteric variety that is a member of the *reticulatus* group of melon is dark-green when unripe and develops a orange−green color when ripe (**Figure 1**). "Dulce" mature fruit rind color is masked by a highly developed netting system that gives its rind a gray color. To explain this variation in rind color, the rinds of the melons were analyzed for the accumulation of carotenoids and chlorophyll at various fruit developmental stages.

The dramatic polymorphism in external color in melon rinds is accounted for by the presence of different pigments, summarized in **Figure 2**. The dark-green "TVT" accumulated mainly chlorophyll (both a and b, not shown), and accumulation increased with fruit maturation from 200 μg/g FW 10 DAA until almost 750 μg/g FW at full maturity (~60 DAA). Carotenoids (~100 μg/g FW) were also present. A comparable pattern was found for "Dulce", a climacteric variety, that is green-skinned when unripe but orange−green when ripe at around 40 DAA (**Table 1**), and "Rochet", a nonclimacteric green variety that is green-skinned when unripe, but green−yellow when ripe. The chlorophyll levels were generally lower in these two varieties (300 μg/g FW), as compared with "TVT". Very low chlorophyll (<150 μg/g FW) and carotenoid (<20 μg/g FW) levels were found in "Noy Amid" (**Figure 1**). The absence of substantial levels of carotenoids in mature "Noy Amid" rinds prompted us to seek an alternative pigmentation system that could account for the yellow pigmentation found in this type of melons.

The main pigment found in ripe "Noy Amid" fruit rind was characterized as a flavonoid with the same retention times and UV spectrum of the unstable yellow flavonoid naringenin-chalcone (**Figure 3**). The compound identity was further confirmed as naringenin chalcone aglycone by MS-MS analysis. Naringenin chalcone was accumulated during the late stages of development in "Noy Amid" fruit rinds (**Figure 4**), generating the yellow mature rind color typical to "Noy Amid" fruit, while the chlorophyll and carotenoids concentration of "Noy Amid" fruit rind decreased to undetectable levels in mature fruit (**Figure 2**). No detectable amounts of naringenin chalcone were found in the flesh of any of the melon varieties analyzed in this study (data not shown).

Interestingly, "Rochet" fruit rind also accumulated increasing amounts of naringenin-chalcone during fruit maturation, but unlike "Noy Amid" genotype, "Rochet" fruit showed no degradation of either chlorophyll or carotenoids during its ripening (**Figure 2**). Therefore, the color of mature "Rochet" fruit rind, containing chlorophyll, carotenoids, and flavonoids pigments is dark-green with a yellow note (**Figure 1**). "TVT" melon fruit rind contained no detectable amounts of naringenin chalcone or any other flavonoid at any fruit ripening stage (not shown) while accumulating increasing concentrations of chlorophyll and carotenoids, generating a dark-green rind color (**Figure 1 and Figure 2**). "Dulce" melon fruit rind also contained no detectable levels of flavonoids during fruit development (not shown). The young "Dulce" fruit rind accumulates both chlorophyll and carotenoids, but while the chlorophyll level decreases during fruit ripening, carotenoid levels increase, resulting in a green−orange colored mature fruit rind, partially masked by the rind netting (**Figure 1 and Figure 2**), characteristic of the *reticulatus* group (*1*).

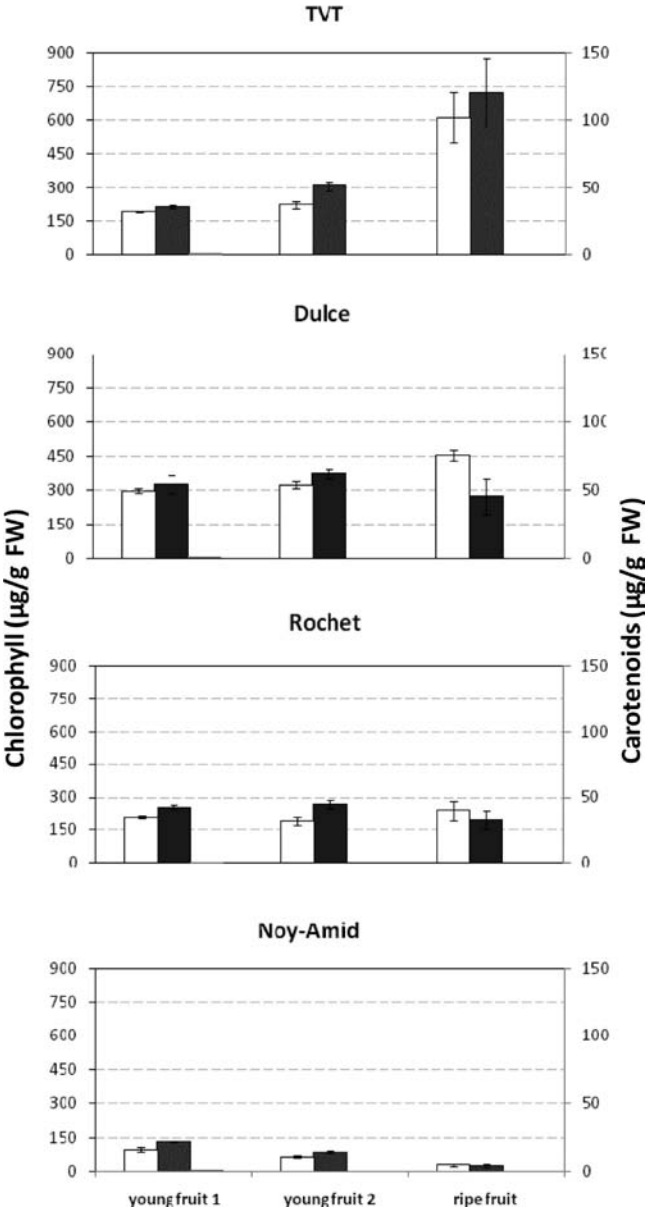

Figure 2. Carotenoid (white bars) and chlorophyll (black bars) content of melon rinds in microgram to gram fresh weight (μg/g FW) during ripening. "Young fruit I" resembles a fruit sampled 10 days after anthesis (DAA), "Young fruit II" was sampled 25 DAA. "Dulce" fruit gets ripe around 40 days after anthesis (DAA), while the other varieties get ripe around 60 DAA. Pigments were spectroscopically determined as described in Materials and Methods. Means and SE of three biological replications, each consisting of one fruit and two technical replications, are shown. Values are in microgram/gram fresh weight (μg/g FW).

Inheritance. To characterize the mode of inheritance of naringenin chalcone accumulation in the melon rind, a segregating genetic population was developed from the cross of "Noy Amid" and "TVT". All F_1 fruits accumulated naringenin-chalcone in their rind. One hundred and fifty-eight F_2 individuals had fruits

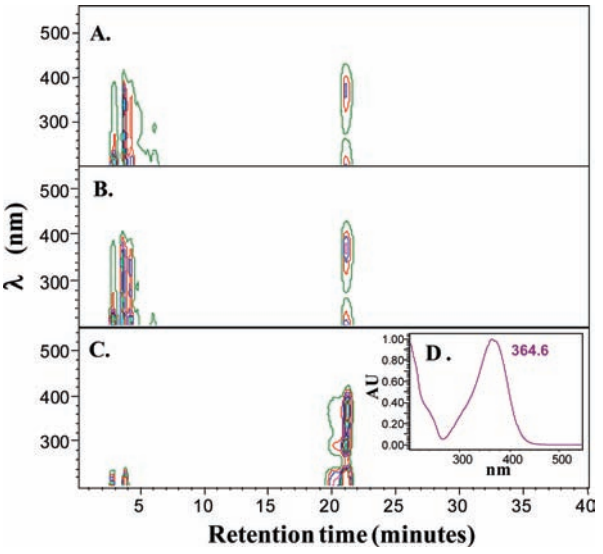

Figure 3. Identification of naringenin chalcone as the major yellow pigment of "canary yellow" melons. (**A**) UV−vis-HPLC chromatograms of flavonoids extracted from mature "Noy Amid" (**A**) and "Rochet" (**B**) fruit rinds, as compared to naringenin chalcone authentic standard (**C**). Peaks eluted at the same time (21 min) and had identical UV−vis spectra (**D**).

that accumulated detectable amounts, from a few μg/gFW to 25 μg/g FW, of naringenin chalcone, and 55 individuals did not accumulate any detectable amount of naringenine chalcone, fitting the Mendelian ratio of 3:1 ($p(\chi^2) < 0.01$) for the accumulation of naringenin-chalcone in fruit rind.

Total chlorophyll and total carotenoids concentration in the hybrid F_1 and F_2 populations were analyzed spectrophotometrically. One hundred and fifty-three F_2 individuals accumulated detectable amounts of carotenoids (from 17 to 180 μg/g FW) and 64 F_2 individuals did not accumulate any detectable amounts of carotenoids. Chlorophyll was accumulated in the rinds of 172 F_2 individuals (from 50 to 850 μg/g FW), while 56 F_2 individuals did not accumulate any detectable amounts of chlorophyll. The segregation of both traits fitted a Mendelian ratio of 3:1 ($p(\chi^2) < 0.01$) in favor of carotenoids or chlorophyll accumulating fruits. Furthermore, a significant ($p < 0.01$) bivariant fit correlation was found between chlorophyll and carotenoids accumulation in the F_2 generation. No apparent association was found between naringenin chalcone content and either chlorophyll or carotenoids accumulation, that were linked. Out of 204 F_2 plants that were analyzed both for all three pigments, 113 carried fruit that accumulated all three pigments (chlorophylls, carotenoids, and naringenin chalcone), 42 had fruit that accumulated only naringenin chalcone, and 39 accumulated only chlorophylls and carotenoids, while only 10 had white fruit that did not accumulate any visual pigment. These numbers significantly fit the expected 9:3:3:1 segregation of two independent dominant traits ($p(\chi^2) < 0.01$).

Transcription Pattern of Flavonoid Pathway Genes. Differences in expression patterns of genes known to be involved in phenylpropanoid and flavonoid biosynthesis were identified between "Noy Amid" and "TVT" during fruit rind development. The chalcone accumulating "Noy Amid" showed increased expression of genes encoding for cinnamate-4-hydroxylase (*C4H*), 4-coumarate-CoA (*4CL*), and chalcone synthase (*CHS*), compared to the rind of "TVT" (**Figure 5**). On the other hand, very low expression of chalcone isomerase (CHI) was observed in both genotypes.

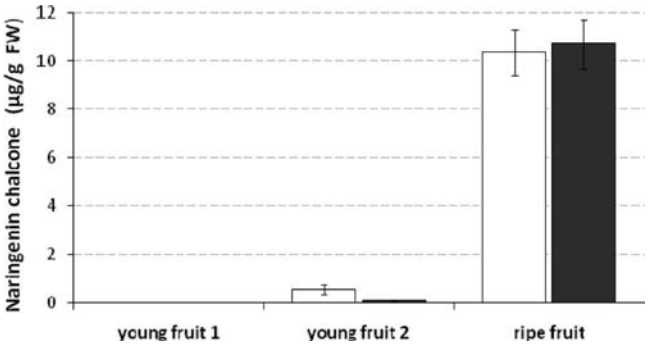

Figure 4. Naringenin chalcone levels (microgram/gram fresh weight ((μg/g FW)) in melon "Noy Amid" (white bars) and "Rochet" (black bars) rinds during fruit development. "Young fruit I" resembles a fruit sampled 10 days after anthesis (DAA), "Young fruit II" was sampled 25 DAA, while "ripe fruit" were sampled around 60 DAA. Averages and SE of three independent biological replications each consists one fruit and two technical replications. No naringenin chalcone was found in "TVT" and "Dulce" rinds.

DISCUSSION

We show here that the developmental and varietal differences in melon fruit rind coloration is governed by three pigmentation systems that consist of three metabolite groups, chlorophylls, carotenoids, and flavonoids. Chlorophylls are the major fruit rind pigments of dark-green melon fruits represented here by "Rochet" and "TVT" (**Figures 1** and **2**). All chlorophyll accumulating fruits also contain carotenoids characteristic of chloroplasts. Accordingly, in both "Rochet" and "TVT", the ratio of chlorophyll to carotenoid content is similar (5:1). In contrast, the yellow flavonoid pigment naringenin-chalcone is present in the rind of some melon genotypes. "Canary yellow" melon fruits, represented here by "Noy Amid", accumulate naringenin-chalcone as their major yellow pigment and appear lemon-yellow in the absence of the green chlorophylls or yellow−orange carotenoids. Within the dark-green varieties analyzed, green fruited "Rochet" accumulates naringenin-chalcone in addition to the chlorophylls while the green fruited "TVT" does not accumulate naringenin-chalcone. This indicates that the biosynthesis of chalcone is independent from the biosynthesis of chlorophylls. Additionally, our findings indicate that the yellow naringenin chalcone, as well as the chloroplast carotenoids, are visually masked in the background of dark-green fruits. "Dulce", on the other hand, accumulates carotenoids concomitants with the breakdown of chlorophyll, characteristic of many chloroplast-chromoplast transformations which occur in cucurbit fruit (*22*), while only "Rochet" accumulates naringenin chalcone in addition to the two other pigments, further supporting that naringenin-chalcone biosynthesis is independent from the biosynthesis of carotenoids. This notion is also supported by the 9:3:3:1 independent segregation of chlorophyll accumulation and naringenin chalcone accumulation in the F_2 population.

Each of the three pigment types (chlorophyll, caotenoids, and flavooids) segregated as monogenic traits in the F_2 population. This could be explained by the segregation of two independent dominant genes in the analyzed population, one which regulates naringenin chalcone accumulation while the other likely regulates chloroplast biogenesis or maintenance and thus the accumulation of the chloroplast pigments, both chlorophylls and carotenoids. Nevertheless, quantitative variation in all three pigmentation systems was found. This variation can be explained by either the existence of additional minor genes that quantitavely regulate the accumulation of these pigments or to biological variability

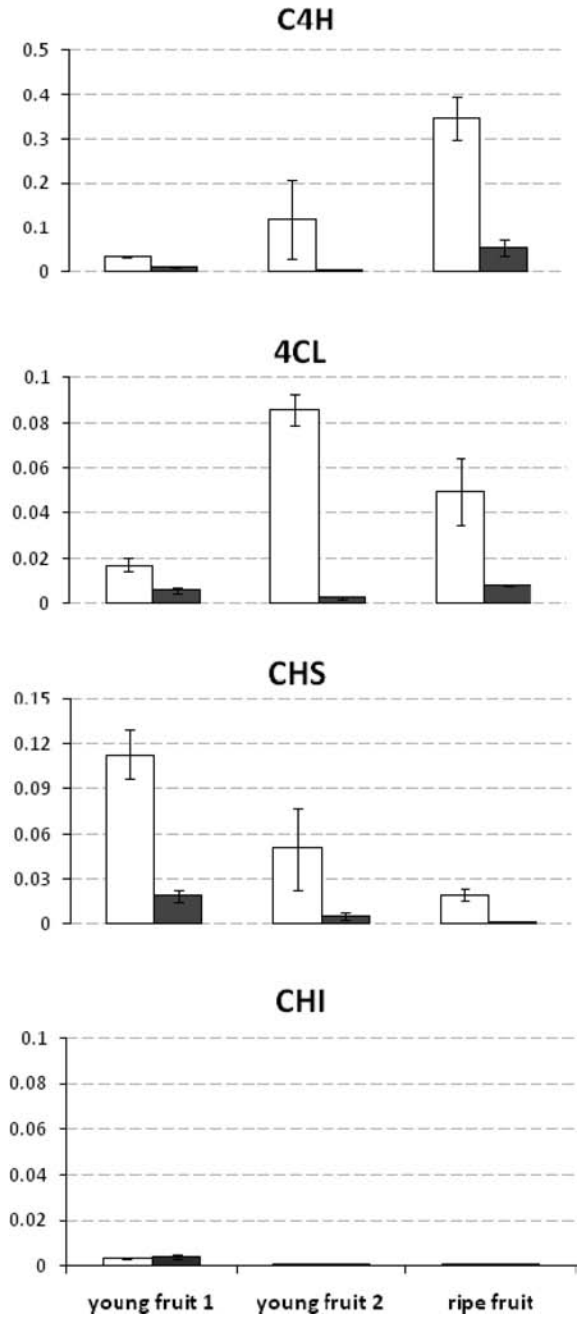

Figure 5. mRNA transcript expression levels of *C4H*, *4CL*, *CHS*, and *CHI* (left to right and top to bottom) in "Noy Amid" (white bars) and "TVT" (black bars) melons rinds at different fruit developmental stages. "Young fruit I" resembles a fruit sampled 10 days after anthesis (DAA), "Young fruit II" was sampled 25 DAA while "ripe fruit" were samples around 60 DAA. Values are relative to *cyclophilin*. Averages and SE of three independent biological replicates.

due, in part, to unsynchronized fruit maturation in the segregating F_2 population.

The genotypes "Noy Amid" and "TVT" differ in the phenylpropanoid and flavonoids biosynthetic pathway gene expression; "Noy Amid" is characterized by high expression of *C4H*, *4CL*, and *CHS*, while these genes are only weakly expressed, or nonexpressed, in "TVT". The pattern of increasing expression levels of the phenylpropanoid genes *C4H* and *4CL* in "Noy Amid" (**Figure 5**) is in accordance with the timing of naringenin chalcone accumulation in fruits (**Figure 4**). These results suggest a potential regulation of naringenin chalcone accumulation in melon rinds at the level of transcription. A single dominant gene, perhaps a transcription factor, may control this phenomenon, suggested by the monogenic inheritance of naringenin chalcone accumulation. Regulatory genes that control expression of the structural genes of the anthocyanin biosynthetic pathway have been identified in many plants. These transcription factors influence the intensity and pattern of anthocyanin biosynthesis and generally control expression of many different structural genes (*7*). Logical candidates for such a transcription factor are members of the R2R3-MYB-type transcription factor family, which have been shown to play roles in controlling flavonoid metabolism in plants (*23*), for example, MYB12 in *Arabidopsis* (*24*), MYB PA1 in grapevine (*25*), SlMYB12 in tomato (*26*, *27*), and the MYB homologue *P* gene in maize (*28*). It remains to be determined whether naringenin chalcone accumulation in melon rinds is similarly regulated by a MYB transcription factor.

Our expression data indicate that CHI is hardly expressed in both the naringenin chalcone accumulating cultivar, Noy Amid, as well as in TVT that does not accumulate detectable amount of naringenin chalcone. A major flavonoid accumulating in the peel of tomato fruit is naringenin chalcone (*29*). This is most probably due to very low activity levels of CHI during fruit development (*14*). It has been shown that production of colored anthocyanins is made possible in tomato via the introduction of the appropriate transcription factors, either by cross breeding (*30*,*31*) or by genetic engineering (*32*, *33*). Our results indicate that naringenin chalcone is accumulated in the rind of melon, it is yet to be discovered whether this finding can lead to new colors in melon fruits and thus the potential of utilizing this trait for the breeding and development of novel fruit color patterns remains to be realized.

In summary, the accumulation of naringenin-chalcone in melon fruit rinds, reported here for the first time, indicates that at least in some genotypes of melon the first steps of flavonoid biosynthesis occurs in the fruit rinds. The understanding of the regulation and the genetics of the flavonoid pathway in melon will contribute to the development of novel pigmentation pattern melons.

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