ENVIRONMENTAL AND GENETIC STRATEGIES TO IMPROVE CAROTENOIDS AND QUALITY IN WATERMELON

A Dissertation

by

HAE JEEN BANG

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2005

Major Subject: Horticulture

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ABSTRACT

Environmental and Genetic Strategies to Improve

Carotenoids and Quality in Watermelon.

(December 2005)

Hae Jeen Bang, B.S., KyungHee University; M.S., KyungHee University

Co-Chairs of Advisory Committee : Dr. Daniel I. Leskovar
Dr. Leonard M. Pike

The evaluation of environmental and genotypic effects on fruit physical and chemical characteristics enables assessment of the feasibility of selecting diploid and/or triploid cultivars for either specific or more diverse locations. Isolation and characterization of genes encoding enzymes in the carotenoid biosynthetic pathway provides fundamental genetic information which can facilitate breeding of watermelon cultivars having desirable flesh colors and enhanced beneficial carotenoids.

For the environmental studies, the effects of deficit irrigation on lycopene content, total soluble solids, firmness, and yield of diploid and triploid watermelon were evaluated in different locations and growing seasons. Irrigation regimes were 1.0 evapotranspiration (ET), 0.75 ET, and 0.5 ET. To investigate if there is a consistent response in cultivars across diverse locations, studies were conducted in three distinct Texas regions. Deficit irrigation reduced total marketable yield, and increased the yield of small fruits. Location and irrigation regimes had major influences on yield. Soluble solids content increased with deficit irrigation at 0.5 ET in triploids, but not in diploids.

Flesh firmness also increased in triploids compared to diploids. Lycopene content increased with maturity at all irrigation regimes and cultivars. This work confirms that deficit irrigation directly reduces yield, but does not reduce lycopene and fruit quality of the triploids used in this study. From the genetic studies, a total of eight genes encoding enzymes in the carotenoid biosynthetic pathway were isolated and characterized. Two members of the phytoene synthase (*PSY*) gene family were identified; *PSY-A* was expressed in all type of tissues, but *PSY-B* transcript was detected only in ovary, leaf, and root tissues. Gene expression of carotenoid isomerase (*CRTISO*) was not detected in salmon yellow. A color inheritance study of watermelon flesh indicated that a single gene might determine color difference between canary yellow and red without an inhibitory effect. A cleaved amplified polymorphic sequence (CAPS) marker developed from the SNP marker tagging two different lycopene β-cyclase (*LCYB*) alleles co-segregated perfectly with color phenotypes. It was concluded that color determination may be due to a reduced activity of *LCYB* enzyme in red, whereby a phenylalanine is conserved among canary yellow and valine is conserved among red watermelon.

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CHAPTER I

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum & Nakai] is one of the main vegetable crops grown in the United States. Its production for fresh market was 1.75 million tonnes with a total production value of $346 million in 2003. Harvested area was estimated 60,700 ha in the United States. The value per unit (dollars per cwt.) has gradually increased since 2001 whereas the production itself has slightly declined (USDA-National Agricultural Statistics Service, 2004).

Water demand is very critical for watermelon growth and fruit quality. However, regulations restricting water use for agriculture and competition for water with large urban sectors and recreational activities, coupled with extremely high temperatures, have placed a strain on aquifers and surface water resources. Irrigation efficiency, a measurement of the effectiveness of an irrigation system delivering water to a crop to compensate for the evapotranspiration (ET) demand, is becoming significant for the production and quality of crops in southern regions of the U.S. It is also important and necessary to examine if gains can be obtained by both environmental strategies (such as deficit irrigation) and genotypic factors related to fruit quality traits. This may lead to watermelon breeding which will enhance specific flavors or health-functional compounds and overall fruit quality utilizing deficit irrigation practices, particularly in

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This dissertation follows the style and format of the Journal of the American Society for Horticultural Science.

water-restricted regions. Ultimately, deficit irrigation practices may significantly improve water use efficiency and thus saving water without yield reduction and change in fruit characteristics.

Carotenoids are known to have various functions in plants and animals. Plant carotenoids contribute to a variety of color pigmentation, such as red, yellow, and orange, which are accumulated in chloroplasts and chromoplasts. They harvest light and protect plants against photo-oxidation. They are also precursors of abscisic acid (ABA), a growth regulator that modulates plant developmental and stress processes. For animals and humans, carotenoids provide health benefits, such as antioxidant activity, cancer and heart disease prevention and immune system enhancement.

Consumer's concern for nutritious and high quality vegetables has increased in the United States. To maximize the health-promoting benefits of carotenoids and to increase consumption of watermelons, the characterization of major carotenoids is essential. In addition, heritability and molecular genetic characterization of the carotenoid biosynthetic pathway are fundamental for breeding aimed at enhancing carotenoid-producing watermelon cultivars.

Color is a very important trait in watermelon breeding and is associated with specific phytochemicals. Red watermelon fruit is an excellent source of lycopene, containing an average of 48.7 $\mu g \cdot g^{-1}$ fw, approximately 60% higher than tomatoes (*Lycopersicon esculentum*) (Holden et al., 1999). However, the quantity of lycopene content appears to vary across maturity, genotype, and ploidy level (Perkins-Veazie et al., 2001; 2002a; 2002b). Carotenoids in non-red watermelon (e.g. canary yellow, salmon

yellow/orange, and white) and the regulatory mechanisms of carotenoid biosynthesis in watermelon have been poorly identified and characterized, whereas carotenoid biosynthesis in tomato has been studied extensively.

Color inheritance is very complex, and several loci were shown to affect watermelon fruit color inheritance. However, the identification of color determining genes has not been studied in watermelon. As color pigmentation is related to carotenoids in watermelon, we targeted structural genes in the carotenoid biosynthetic pathway as candidate genes for color determination. In this study, we hypothesized that carotenoid isomerase (*CRTISO*) might be responsible for the color differentiation between salmon yellow and red in watermelons. An additional hypothesis is that lycopene β-cyclase (*LCYB*) might be a candidate gene for determining canary yellow color.

Molecular markers generally constitute very useful and practical breeding tools. If codominant molecular markers for agronomically important traits are available, many laborious and time-consuming procedures such as progeny testing can often be avoided in breeding programs.

The purpose of this project is to investigate the environmental and genetic impacts on carotenogenesis and fruit quality of watermelons. The environmental effects will be assessed on fruit carotenoid content, quality, and yield of diploid and triploid watermelon across locations and irrigation regimes. The evaluation of environmental and genotypic effects on fruit physical and chemical characteristics will be useful to indicate the feasibility of selecting diploid and/or triploid cultivars for specific or across several

regions. The major carotenoids in different flesh colors and the regulatory mechanisms of the carotenoid biosynthesis pathway will be identified. The fundamental information about the genetics of carotenoids could facilitate breeding of watermelon cultivars having desirable flesh colors and high content of beneficial carotenoids. We expect this research to be very useful to elucidate the mechanisms of carotenogenesis in watermelon.

CHAPTER II

LITERATURE REVIEW

Deficit Irrigation and Environmental Stress on Crop Production and Quality

Irrigation efficiency is a measurement of effectiveness of water usage to produce a crop (Smajstrla et al., 2002). Irrigation efficiency is becoming crucial for vegetable and agronomic crop production particularly in the southern regions of the United States. This is due to strict pumping limitations of underground and surface water and competition for water use for agriculture, urbanization, and recreational activities. Irrigation requirements can be applied to compensate for the evapotranspiration (ET), which describes water loss from evaporation from soil and transpiration from plants to the air (Allen et al., 1998). Irrigation requirement (IR) is defined as IR = [ET_o x Kc – ER], where ET_o is the reference crop evaporation, Kc is the crop coefficient (related to the crop canopy development), and ER is the effective rainfall. Therefore, by improving irrigation efficiency we could maximize water usage and crop yield while reducing energy, and fertilizer inputs.

Water is a very important component in watermelon production, because a watermelon fruit consists of more than 93% water (Maynard, 2001). A few studies in watermelon reported that deficit irrigation during growth decreased yield significantly. Deficit irrigation to 50% of evapotranspiration resulted in approximately 50% yield reduction in south Texas (Leskovar et al., 2004). In Turkey, 50% water deficit irrigation

resulted in 64% yield reduction when it was mainly applied during the flowering period whereas the highest yield was obtained from 100% irrigation (Erdem and Yuksel, 2003, Orta et al., 2003). De Pascale et al. (1998) reported that high irrigation frequency (twice a week) and high N fertilization (400 $kg \cdot ha^{-1}$) prolonged the plant cycle from transplanting to harvest by 10-20 days due to maturity delay and increased yield per plant and root density. In wheat, carotenoids, ascorbate, and chlorophyll concentration were affected by drought stress condition. Lutein and β-carotene content increased under 40% of soil water capacity as compared to 100% regime, whereas ascorbate and chlorophyll concentration decreased (Herbinger et al., 2002).

Carotenoids

Carotenoids are C_{40} terpenoid compounds that are synthesized through the isoprenoid biosynthetic pathway. The carotenoid biosynthetic pathway of watermelon in Fig. 1 was presumed based upon carotenoid biosynthesis of tomato (Giuliano et al., 2000; Hirschberg, 2001; Isaacson et al., 2002; 2004). More than 600 carotenoid structures have been classified so far (Britton, 1998). Extensive studies of carotenoids have been done throughout plants, animals, and humans. A variety of functions of carotenoids have been identified not only in animals and humans, but also in plants. Carotenoids have important functions in humans such as provitamin A activity, antioxidant, cell communication, and immune function enhancers. Lycopene ($C_{40}H_{56}$), one of the major carotenoids, provides various health benefits in human, such as a

reduction in the risk of cancer and cardiovascular disease (Bramley, 2000; Fish et al., 2002; Gerster, 1997; Giovannucci, 1999; Giovannucci et al., 2002; Handelman, 2001). Lutein and zeaxanthin play a protective role in macular degeneration (Semba and Dagnelie, 2003). However, animals and humans cannot synthesize carotenoids, thus uptake through a diet is essential to them.

In plants, carotenoids play a role as a light harvesting agent, protection from photo-oxidation, and are responsible for red, orange, and yellow pigmentation. Furthermore, they are one of the important parameters when evaluating fruit and vegetable quality in plants (Bramley, 2000; van den Berg et al., 2000).

Watermelon contains diverse carotenoids that are attributable to the different flesh colors such as salmon yellow/orange, canary yellow, and red. The major carotenoid in red-fleshed watermelon is lycopene containing an average of 48.7 $\mu g \cdot g^{-1}$ fresh weight (Holden et al., 1999) which is approximately 60% more than a tomato fruit. In watermelon, the amount of lycopene is variable across maturity, genotype and ploidy level. Perkins-Veazie et al. (2002b) reported that about 20% more lycopene was accumulated in fully mature watermelon than immature ones. In several studies, triploid watermelon has been shown to contain more lycopene than diploid watermelons (Perkins-Veazie et al., 2001; Leskovar et al., 2004). There are no published reports on carotenoid variability in watermelon across diverse environments.

The major pigments of orange-fleshed watermelon appear to be prolycopene and/or ζ-carotene (Tomes and Johnson, 1965). This orange watermelon might be similar to *tangerine* mutant in tomato as *tangerine* is known to contain the same major pigments

as reported by Isaacson et al. (2002). Tadmor et al. (2004) designated ‘Early Moonbeam’, a canary yellow watermelon cultivar with a very low level of carotenoids, equivalent to the *r* mutant of pale yellow tomato (Fray and Grierson, 1993). They also reported that the predominant carotenoids were prolycopene in ‘Orange Flesh Tendersweet’ and β-carotene in ‘NY162003’, although they both could be classified into the same color group of orange. In addition, each of them was related to *tangerine* mutant and *Beta* mutant in tomato (Tadmor et al., 2004).

In tomato, non-red varieties, such as tangerine, orange, or orange-red fruits, contained prolycopene and/or ζ-carotene, β-carotene or δ-carotene as the major carotenoid (MacKinney and Jenkins, 1949; Ronen et al., 2000). No substantial amount of carotenoid was identified in yellow tomato similar to yellow watermelon, therefore distinctly low total carotenoid content was observed as compared to red and orange tomato fruits (Frecknall and Pattenden, 1984; Isaacson et al., 2002; Jenkins and MacKinney, 1953; MacKinney and Jenkins, 1949; Tomes et al., 1953).

In carrot (*Daucus carota* L.), the predominant carotenoid composition has been shown to be xanthophylls, β-carotene, α-carotene, ζ-carotene, and lycopene. Total carotenoid content was extremely low in lemon yellow carrot, whereas orange or light orange showed 5-10 fold more carotenoids (Buishand and Gabelman, 1980; Imam and Gabelman, 1968; Laferriere and Gabelman, 1968; Umiel and Gabelman, 1972).

Hornero-Mendez et al. (2000) reported that the carotenoid content pattern change during maturity in red pepper (*Capsicum annuum* L.). They revealed that chloroplast pigments, lutein, and neoxanthin, decreased when fruit ripening started. β-carotene, and

antheraxanthin drastically increased and the synthesis with a remarkable increase of capsanthin, zeaxanthin, cucurbitaxanthin A, and β-cryptoxanthin was observed during fruit ripening. The synthesis of capsorubin and capsanthin-5,6-epoxide increased gradually. Red pigmentation was due to mainly capsanthin and yellow was due to zeaxanthin (Hornero-Mendez et al., 2000).

Inheritance Study of Carotenoids

Since Poole (1944) introduced the loci involved in watermelon color determination for the first time, following inheritance studies of watermelon flesh color revealed that only a few genes were associated with color determination and some of these interacted through epistasis. Based on the report by Guner and Wehner (2003) who published the latest version of a watermelon gene list, a number of loci have been shown to be involved in color determination.

Red, orange, and yellow flesh colors are controlled by *Y*, y^o, and *y*, respectively. According to Henderson (1989), red color (*Y*) is dominant to orange (y^o) and salmon yellow (*y*); orange (y^o) is dominant to salmon yellow (*y*). The *C* locus involves canary yellow flesh color determination, but *C* is inhibited by the *i* locus, which will result in red flesh. Canary yellow (*C*) is known to be dominant to red (*c*) as determined by Poole (1944). The *Wf* allele results in white flesh and is dominant to yellow (*B*) and red (*b*) (Henderson et al., 1998; Guner and Wehner, 2003). Henderson et al. (1998) proposed a modifier gene effect that produced orange-canary flesh color derived from a cross between canary yellow and orange flesh color. Another modifier gene resulting in

bicolored fruits containing both canary yellow and red color appeared to be present in the progeny derived from a cross between canary yellow and red (Henderson et al., 1998). Environmental impacts cannot be overlooked because environmental variability also has a critical impact on phenotypic expression, as Jenkins and MacKinney (1953) pointed out that tangerine tomato could be mistaken as yellow or even red due to different growing environments. Depending on pigment ratio, it could be classified into a totally different color group.

There have been numerous studies on the carotenoid inheritance in other species such as tomato and carrot. Many genes are involved in tomato pigmentation. It was proposed that sequential gene action was involved in carotenoid synthesis by Lincoln and Porter (1950). The *R* and *T* genes play an important role in producing red (*R_T_*), yellow (*rrT_*), and tangerine (*R_tt*) tomato (MacKinney and Jenkins, 1949; Jenkins and MacKinney, 1953). It was observed that *rr* decreased total carotenoid content, so only trace amounts of carotenoids were detected. On the contrary, *tt* did not affect total carotenoid content. In the presence of *R* and *T* gene, red fruit containing lycopene was obtained and *R_tt* is designated as tangerine containing ζ-carotene, poly-cis-ψ-carotenes, and prolycopene. Since *T* and *t* gene pair was named as tangerine which has prolycopene in tomatoes (MacArthur, 1934; Zechmeister, 1941), a recent study revealed that carotenoid isomerase (*CRTISO*) is the gene resulting in *tangerine* mutant. This tomato gene encoding carotenoid isomerase catalyzes the isomerization of *cis*-lycopene precursor into all-*trans* form (Isaacson et al., 2002).

Lincoln and Porter (1950) identified that the function of the *B* gene, single

dominant gene to *R* (lycopene formation), is responsible for production of β-carotene converted from lycopene. However, they proposed that the *B* gene might be incompletely dominant because they observed fruits which had intermediate β-carotene content in the F_2 progeny. Their segregation ratio appeared to be 1 high : 2 intermediate : 1 low in β-carotene when the cross was made between high and low β-carotene parents. Tomes et al. (1953) indicated that different ratios between lycopene and β-carotene could result from the lack of complete dominance of the *B* gene, but also suggested the possibility of dominance. Later, a modifier gene I^B [or Mo^B] was identified that inhibits the function of *B* reducing β-carotene content (Tomes et al., 1954; 1956). Tomes (1963) concluded that there was an alternative pathway in β-carotene synthesis by *B* or *Del* gene which was inhibited by high temperature. In comparison, β-carotene synthesis in red tomato was not inhibited by high temperature. Tangerine type (*R_tt*) contained prolycopene and ζ-carotene, whereas *beta* orange type (*BB*) contained β-carotene. High *beta* and *tangerine* strains both show orange color, which cannot be visually distinguished. Orange fruit of high beta strain resulted from predominantly β-carotene as compared to that of tangerine which results from ζ-carotene and prolycopene (MacKinney and Jenkins, 1949; Jenkins and MacKinney, 1953; Tomes et al., 1953; Tomes 1963).

Imam and Gabelman (1968) investigated the carotenoid inheritance of carrot. A single gene conditions root color between light orange and orange and another single gene differentiates root color between lemon yellow and light orange. It was found that light orange was dominant to orange and that lemon was dominant to light orange. White

color is dominant to yellow and is determined by single gene action as revealed by Laferriere et al. (1968). At least three genes turned out to be involved in color determination between white and orange. However, the number of genes determining between yellow and orange were not consistent. Buishand and Gabelman (1979) concluded that the pigmentation of orange carrot is due to α- and β-carotene. The gene actions were identified in carrot; Y_2 is an inhibitor of carotenoid synthesis, *L* synthesizes lycopene, and A_1 is assumed to enhance β- and α- carotene formation (Buishand and Gabelman, 1980).

The relationships between the interaction mechanisms of these genes and the induced color phenotypes implied that most of the genes determining color might be structural genes encoding enzymes in the carotenoid biosynthetic pathway.

Genes Encoding Enzymes in the Carotenoid Biosynthetic Pathway in Plants

Most genes encoding enzymes in the carotenoid pathway have been cloned in various plant species, such as tomato, Arabidopsis (*Arabidopsis thaliana*), and pepper (Cunningham and Gantt, 1998; Sauret-Gueto et al., 2003). Cloning of carotenoid biosynthesis genes provides better understanding of the characterization of the pathway and the identification of the respective gene functions and their relationship to the inheritance of phenotypes. Furthermore, cloned genes could conceivably be used as transgenes in a variety of crops to modify the carotenoid biosynthetic pathway for the production of high levels of natural or novel carotenoids with maximum health

promoting activity (Sandmann, 2001; Giuliano et al., 2000).

Several carotenoid accumulations are known to be regulated by transcriptional or post-transcriptional levels of the carotenoid biosynthetic genes (Bartley and Scolink, 1993; Fraser et al., 1994; 1999; Hirschberg, 2001; Kato et al., 2004; Ronen et al., 1999; 2000; Sauret-Gueto et al., 2003; van den Berg et al., 2000). In tomato, phytoene synthase (*PSY*) converts two molecules of GGPP into phytoene in the first committed step of the carotenoid biosynthetic pathway. Two isoforms of *PSY* were identified in tomato (*PSY1* and *PSY2*); *PSY1* expression highly increased during fruit ripening encoding an enzyme in the tissues containing chromoplast, whereas *PSY2* was considered a vegetative gene which is abundantly expressed in mature leaves and it was not induced by fruit ripening. The expression of *PSY2* was detected in extremely low levels as the ration of *PSY2*/*PSY1* less than 0.01, only when abundant cDNA was used in PCR amplification (Bartley and Scolink, 1993; 1995; Fraser et al., 1999). Yellow fleshed fruits (*r* and r^y mutants) containing trace amounts of carotenoids resulted from the null mutation of *PSY1* with 750-bp and 50-bp shorter mRNA of *PSY1* (Fray and Grierson, 1993). Romer et al. (1993) were able to detect two *PSY* transcripts in pepper fruit.

Phytoene desaturase (*PDS*) catalyzes the desaturation steps, sequentially producing phytofluene and ζ-carotene (van den Berg et al., 2000) from phytoene. It was reported that expression of *PSY* and *PDS* started to increase at the breaker stage and slightly declined during fruit ripening (Ronen et al., 1999; Pecker et al., 1992; Giuliano et al., 1993).

ζ-carotene desaturase (*ZDS*) with carotenoid isomerase (*CRTISO*) are both

involved in the steps which sequentially convert ζ-carotene to prolycopene and to lycopene. Isaacson et al. (2002) investigated *CRTISO* to elucidate two types of mutants by map-based cloning in tomato; *tangerine*^{3183} and *tangerine*^{mic}. Both mutated fruit showed orange color, but *tangerine*^{3183} accumulates prolycopene whereas *tangerine*^{mic} accumulates prolycopene and ζ-carotene. The deletion of 24 bp in the exon and 258 bp in the intron region including splicing site of *tangerine*^{mic} were detected and an early stop codon resulted in abolishing the function of *CRTISO* gene. Mutated function of *tangerine*^{3183} was due to 348-bp deletion in promoter region of *CRTISO* gene. Recently, Isaacson et al. (2004) found that the function of *CRTISO* paralleled with that of *ZDS* to convert 7,9,9'-cis-neurosporene to 9'-cis-neurosporene and 7'9'-cis-lycopene to all-trans-lycopene.

Lycopene cyclases convert lycopene to α-, or β-carotene with a ring structure. There are two lycopene β-cyclases; *LCYB* and chromoplast-specific lycopene β-cyclase (*CYCB*) in tomato (Hirschberg, 2001). The *Beta* (*B*) mutant has β-carotene as a principal carotenoid and six additional sequence elements were detected in the promoter region of the *B* allele. Amino acid sequence analysis of the *B* gene showed 98% identity to the *b* allele in the coding region of wild type. In *old-gold* (*og*) mutant of tomatoes, a frame shift was detected in the coding region resulting in null mutation (Ronen et al., 2000). Lycopene cyclase (*CrtL-b*) was downregulated during fruit ripening in both wild type and *Beta* fruit, whereas expression of *B* increased with maturity. The expression of *B* was detected only in chromoplast-containing tissues, such as flowers and fruits as compared to *CrtL-b*, which was expressed in both leaf and fruit (Pecker et al., 1996). Its

level of expression in wild type fruit was not significant. The deduced amino acid sequence comparison showed 53% identity between *CrtL-b* and *B* and 86% between *B* and capsanthin-capsorbin synthase (*CCS*). *CCS* is also known to be expressed in chromoplast-containing tissue. It was assumed that gene duplication in the *Solanecae* family preceded before divergence of Genus by Ronen et al. (2000). They concluded *B* plays a role in β-carotene synthesis, not *CrtL-b.*

Substantially increased activity of lycopene ε-cyclase (*LCYE*) produced orange fruit by converting lycopene to δ-carotene in the *Delta* tomato mutant (Ronen et al, 1999). The deduced amino acid sequences of *LCYE* showed 36% identity to that of lycopene β-cyclase in tomato. It was also 35% identical to that of *CCS* in pepper. A noticeable feature was observed in the phylogeny tree of lycopene cyclase, which indicated that lycopene ε-cyclase seemed to have evolved before the divergence of lycopene β-cyclase and CCS (Ronen et al., 1999).

Tadmor et al. (2004) indicated that orthologous genes of *r*, *t*, *B,* and *og* in tomato might cause color differences in watermelon. However, isolation of the genes encoding enzymes in the carotenoid biosynthetic pathway in watermelon has not been investigated in contrast to tomato.

Molecular Marker Development

There are numerous molecular markers available for marker-assisted selection in breeding. Depending on the type of a marker, it has advantages and disadvantages, such

as simplicity, cost, reproducibility, reliability, codominance, and accuracy. In addition, if a molecular marker is linked to a gene of interest, recombination may occur between a marker and a gene of interest. If a marker is developed based on a causative mutation of a gene resulting in different phenotype, it will be more reliable since recombination will not occur.

In onion, several PCR-based markers were developed for allelic selection for bulb color (Kim et al., 2005a; 2005b). Bulb color is one of the most important traits in onion breeding. However, it is labor and time consuming when breeding for specific traits with desirable color because of the complex bulb color inheritance. Molecular markers were designed based on a mutation of the genes encoding enzymes in the anthocyanin biosynthetic pathway. They are direct markers resulting in different color mutants and also codominant that will allow heterozytes to be distinguished. This useful tool is important to accelerate onion breeding by allowing certain onion bulb color to be distinguished at the seedling stage.

In pepper, RFLP and specific PCR markers derived from capsanthin-capsorubin synthase (*CCS*) gene showed perfect co-segregation with phenotype. The *CCS* gene may condition red color and the deletion of the *CCS* gene might result in a yellow mutant controlled by the *y* locus (Lefebvre et al., 1998).

In watermelon, inter-simple sequence repeat (ISSR) and amplified fragment length polymorphism (AFLP) markers have been developed for genetic identification (Levi et al., 2004), but none of them are available to distinguish flesh colors.

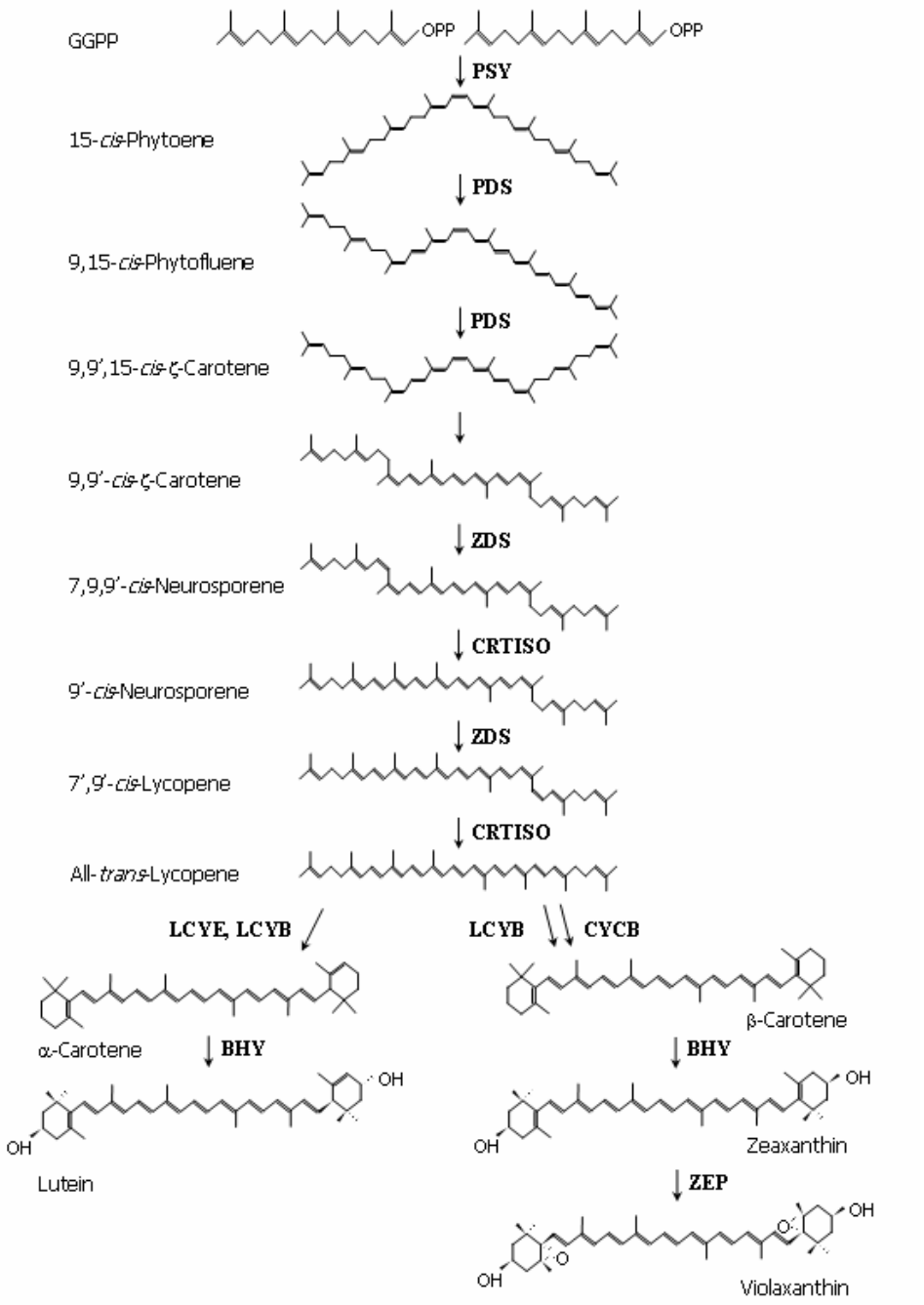

Fig. 1. The presumed carotenoid biosynthetic pathway in watermelon. GGPP: geranylgeranyl diphosphate, PSY: phytoene synthase, PDS: phytoene desaturase, ZDS: ζ-carotene desaturase, CRTISO: carotenoid isomerase, LCYB: lycopene β-cyclase, LCYE: lycopene ε-cyclase, CHYB: β-carotene hydroxylase, ZEP: zeaxanthin epoxidase.

CHAPTER III

DEFICIT IRRIGATION REGIME AND GROWING LOCATION IMPACT ON YIELD, QUALITY, AND LYCOPENE CONTENT OF DIPLOID AND TRIPLOID WATERMELONS*

Materials and Methods

Plant material

Two seeded diploid cultivars ('Summer Flavor 710' and 'Summer Flavor 800') and two seedless triploid cultivars ('Summer Sweet 5244' and 'Super Seedless 7187') were used in this experiment. Watermelon seedlings were grown in polystyrene trays (34 cm width × 67 cm length) containing 128 round cells of 3.3 cm diameter, 6.3 cm depth, and 42 mL volume (Hortiblock, Beaver Plastics Ltd., Edmonton, Canada). Seeds were sown in a transplant mix (Sunshine, Manitoba, Canada) and covered with 5 mL of diatomaceous earth (Genuine, Arizona). Trays were held in a dark room at 29 ^{o}C and 98% RH for three days and then transferred to a greenhouse at 35 ^{o}C day / 22 ^{o}C night temperature. Thereafter, seedlings were irrigated with a computer controlled overhead boom three to five times a week, depending on the growth stage. Other standard greenhouse production practices were followed (Tropical Star, Alamo, Texas).

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Locations and culture

Experiments were conducted at the Texas A&M University Agricultural Research and Extension Center located in three distinctive geographical Texas locations in 2002: Uvalde, located in the Wintergarden (29°1' N, 99°5' W) at an elevation of 276 m; Weslaco, located in the Rio Grande Valley (26°2' N, 97°6' W) at an elevation of 23 m and Lubbock, located in the High Plains (33° N, 101° W) at an elevation of 1,000 m. Three irrigation regimes were imposed at each location, 1.0 ET, 0.75 ET, and 0.50 ET. The irrigation water requirement (IR) was applied to compensate for evapotranspiration (ET). The IR for each event was calculated as follows: IR= [ET_o x Kc - ER] where ET_o is the potential evapotranspiration, Kc the crop coefficient which was estimated by the % ground cover by foliage development (Kc values were 0.2-0.4 from planting to the vining stage, 0.5-0.6 from vining to fruit set, 0.65-0.8 from fruit set to harvest), and ER is the effective rainfall (value reduced to 50% due to the polyethylene mulch). The ET_o values used were those reported by the Texas A&M University Agricultural Research and Extension Center at Uvalde, Lubbock, and Weslaco. All four cultivars mentioned before were used at Uvalde and Lubbock and two ('Summer Flavor 710' and 'Summer Sweet 5244') at Weslaco. To isolate the effect of the environment from the cultural practices, the experiments at each location followed the same field methodology except for transplanting and harvesting dates, due to regional weather conditions. Table 1 depicts weather conditions between transplanting and first harvest at each location. The main differences occurred in the high relative humidity for Weslaco and Uvalde (45% and 48%, respectively), compared with Lubbock (33%). Despite higher solar radiation at

Lubbock (24.9 $MJ \cdot m^{-2}$) than Weslaco (23.0 $MJ \cdot m^{-2}$) and Uvalde (18.15 $MJ \cdot m^{-2}$) the reference evapotranspiration (ET_o) was greater during the growing period at Weslaco (466 mm), followed by Lubbock (400 mm) and Uvalde (332 mm).

Table 1. Environmental conditions during the growth period (82, 94, and 78 days) until first harvest of mature fruits at Uvalde (29°1' N, 99°5' W), Weslaco (26°2' N, 97°6' W), and Lubbock (33° N, 101° W).

Location	Growth period (days)	ET_o (mm)	T_{max} (°C)	T_{min} (°C)	R.H. (%)	Solar radiation ($MJ \cdot m^{-2}$)	Rain (mm)
Uvalde	82	332	30.0	18.8	49	18.2	96
Weslaco	94	466	30.6	21.1	45	23.0	72
Lubbock	78	400	31.6	18.8	33	24.9	105

Five-week old seedlings were mechanically transplanted in the field at Texas A&M Agricultural Research and Extension Center at Uvalde on 27 Mar. 2002. The soil in that location is a Uvalde silty clay loam (fine-silty, mixed, hyperthermic Aridic Calciustoll, pH 7.7). Transplants were hand transplanted at Weslaco on 15 Mar. 2002 in a Hidalgo fine sandy loam soil (pH 8.5) and at Lubbock on 16 May 2002 in an Olton loam soil (pH 8.1). At Uvalde, preplant fertilizer was broadcasted ($kg \cdot ha^{-1}$; 50-38-0+2.2 Zn) and incorporated into the soil. Additional fertilizer (30-17-38) was applied through the drip system weekly for 5 weeks using urea, KNO_3 and H_3PO_4 as sources of N, P, and K, respectively. At Lubbock, preplant fertilizer was broadcasted ($kg \cdot ha^{-1}$; 48N-26P) and

incorporated into the soil. Additional fertilizer (15N-10P) was applied through the drip system. At Weslaco all fertilization was applied through the drip system ($kg \cdot ha^{-1}$; 108N-55P-130K).

Plants for each experimental plot were grown on three single raised beds on 2.03 m centers with one row per bed and 0.9 m within row spacing, giving a theoretical plant population of 5,388 $pl \cdot ha^{-1}$. Each plot was separated by a 2-m blank row, giving a 0.75 ratio of planted area (4,041 $pl \cdot ha^{-1}$). A subsurface drip system (20 cm depth) and black plastic mulch were used. Drip type used was T-Tape TSX 508 (0.2 mm wall thickness) with emitters spaced every 30 cm (T-Systems International, Inc., San Diego, California). Drip tape delivered 162, 251 and 332 $L \cdot h^{-1}$ of water per 100 m of bed at 55 kPa for the 0.5, 0.75, and 1.0 ET irrigation regimes, respectively.

Fruit quality, yield, and *lycopene*

Watermelon fruits were harvested based on maturity. At Uvalde, immature fruits were harvested on June 7 (72 days after transplanting), mature fruits on June 17 (82 days after transplanting, DAT), overmature fruits on June 24 (overmature-1: 89 DAT) and July 9 (overmature-2: 104 DAT). At Weslaco, mature fruits were harvested on June 17 and overmature fruits on June 28. At Lubbock, only mature fruits were harvested on Aug. 12. Fruits from each experimental unit were counted, weighed, and classified by weight in the following categories: <5 kg, 5-8 kg, 8-11 kg, and >11 kg (comparable to commercial watermelon grades #6, #5, #4, and #3 fruits per 32 kg-box). Blossom-end rot, bottleneck, cracked, and misshapen fruits were considered culls. Marketable yield was

calculated by combining non-cull fruit weights for each size class.

Fruit quality characteristics were determined based on three fruits per replication. Fruits were cut in half and flesh firmness was measured with a digital force meter (DFM 10, Chaltillon, Greensboro, North Carolina) using an 11 mm diameter round-head probe (Uvalde) or a V-tip probe (Weslaco), using an average of three probes around the center of the heart tissue. Soluble solids content (SSC) was measured with a digital refractometer, using the juice extracted from the center of the heart tissue.

At each harvest and maturity stage, approximately 100 g of flesh sample was taken from the center of the fruit and stored at -80 ^{o}C in an ultracold freezer until analysis. Lycopene was measured spectrophotometrically using the Sadler's method with modification (Sadler et al., 1990). Twenty g of sample was ground with a mortar and pestle, 2 g of the puree was added to a solvent containing 50 mL hexane, 25 mL ethanol, and 25% acetone with 0.05% (w/v) butylated hydroxytoluene (BHT) and agitated with a shaker in a cold bath for 10 min. After agitation, 15 mL distilled water was added and the solution was re-agitated for an additional 5 min and then allowed to stand for 15 min for separation of polar and non-polar layers. A 1 mL sample from the top hexane layer was drawn and the absorbance was measured at a wavelength of 503 nm with a spectrophotometer (Genesys 20, Spectronic Instruments, Rochester, New York, USA).

Statistical analysis

The experiments at each of the three locations followed a split-plot design with

four replications, each consisting of three rows as previously described. Irrigation regimes, the main plots, were set up in a randomized complete-block design. Cultivars, the subplots, were randomized within each irrigation regime main plot. Yield, fruit quality characteristics, and lycopene content were subjected to analysis of variance (ANOVA) using SAS (SAS Institute, Inc., Cary, North Carolina). Means were separated by using Fisher's LSD test, $P \leq 0.05$. Each location was analyzed separately.

Results and Discussion

Yield and fruit size

There were significant differences in yield in response to deficit irrigation at Uvalde and Lubbock (Table 2). At Uvalde, 1.0 ET regime produced more total marketable and larger size fruits (> 11 kg) than deficit irrigation at 0.5 ET. As expected 0.5 ET reduced total yield and increased the percentage of smaller fruits (< 5 kg). Yield as affected by irrigation regime at Lubbock showed similar responses to Uvalde (Table 2). In this location, fruit size was not significantly different among irrigation treatments, except for an increase in the production of smaller fruits (< 5 kg) with 0.5 ET. At Weslaco, only yield data from the first harvest was obtained, due to a severe rain (43 mm) after that harvest and vine collapsed. Our result confirms previous reports that watermelon yield declines with reduced water application (Leskovar et al., 2004). It also confirms that yield was significantly affected by deficit irrigation based on the result 40% decrease at 0.5 ET when compared to 1.0 ET as well as fruit weight (Erdem and Yuksel, 2003).

Table 2. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on marketable yield of diploid and triploid watermelons.

Treatment		Percentage of fruit in size				Yield ($t \cdot ha^{-1}$)
		< 5 kg	5-8 kg	8-11 kg	> 11 kg	
Irrigation regime				***Uvalde***		
0.5 ET		9 a[y]	54 ab	34 ab	3 b	28.06 b
0.75 ET		3 b	64 a	23 b	10 a	31.75 b
1.0 ET		2 b	51 b	38 a	9 ab	44.05 a
LSD ($P \leq 0.05$)		5	13	13	6	10.90
				Lubbock		
0.5 ET		11 a	46	22	22	36.09 b
0.75 ET		4 b	47	29	21	45.24 ab
1.0 ET		2 b	40	31	27	51.18 a
LSD ($P \leq 0.05$)		4.74	NS	NS	NS	9.87
				Weslaco		
0.5 ET		0	50	42	8	10.71 b
0.75 ET		0	54	38	8	10.52 b
1.0 ET		0	35	44	21	12.55 a
LSD ($P \leq 0.05$)		NS	NS	NS	NS	1.48
Cultivar	***Ploidy***			***Uvalde***		
SF 710[z]	2*x*	2 ab	39 b	43 a	16 a	33.72 ab
SF 800	2*x*	1 b	47 b	40 ab	12 a	25.46 b
SS 5244	3*x*	8 a	74 a	17 c	1 b	39.49 a
SS 7187	3*x*	6 ab	67 a	27 bc	0 b	39.80 a
LSD ($P \leq 0.05$)		6	15	15	7	12.66
				Lubbock		
SF 710[z]	2*x*	0 b	23 b	38 a	39 a	59.38 a
SF 800	2*x*	2 b	18 b	29 ab	51 a	48.08 ab
SS 5244	3*x*	11 a	68 a	18 b	3 b	31.70 b
SS 7187	3*x*	9 a	67 a	23 b	1 b	38.85 ab
LSD ($P \leq 0.05$)		5.47	13.90	11.42	15.80	22.94
				Weslaco		
SF 710[z]	2*x*	0	88 a	8 b	3 b	9.27 b
SS 5244	3*x*	0	6 b	72 a	22 a	13.04 a
LSD ($P \leq 0.05$)		NS	22.88	27.58	18.86	1.21

[z] Summer Flavor 710, Summer Flavor 800, Summer Sweet 5244, Super Seedless 7187.

[y] Mean separation within columns by Fisher's LSD test at $P \leq 0.05$.

[NS,] Nonsignificant at $P \leq 0.05$.

Cultivar effects were more variable across environments. Triploid cultivars produced more marketable fruits than the diploid ‘Summer Flavor 800’ and more fruits than both diploids in the 5-8 kg category at Uvalde. Conversely, there was a higher percentages of fruits were higher in the 8-11 and >11 kg size categories in the diploids. Cull fruits appeared only in diploids at 0.5 ET, but data were not significantly different among irrigation treatments (not shown). The majority of culls had blossom-end-rot, a physiological disorder associated with water stress and calcium availability (Cirulli and Ciccarese, 1981).

At Lubbock, yield of the triploid ‘Summer Sweet 5244’ was significantly lower than the diploid ‘Summer Flavor 710’. The environmental characteristics of this location, particularly the higher solar radiation, in addition to the elevation (1000 m), lower humidity and lower night temperatures compared to Uvalde and Weslaco, are more conducive to enhance the yield potential of large fruited varieties such as the diploid ‘Summer Flavor 710’. This might be due to the fact that photosynthesis depends on solar energy to produce carbohydrate (Taiz and Zeiger, 1998). Similar trends were observed at that location in the statewide watermelon trials (Texas Cooperative Extension 2002 report, 2002).

Soluble solids content and firmness

There was a significant irrigation and cultivar interaction for SSC and fruit firmness at Uvalde. Soluble solids content did not change in ‘Summer Flavor 800’ and slightly decreased at 0.5 ET for ‘Summer Flavor 710’ (Fig. 2). However, deficit irrigation (0.5 ET and 0.75 ET) increased SSC in both triploids at 0.5 ET regime. In a subsurface

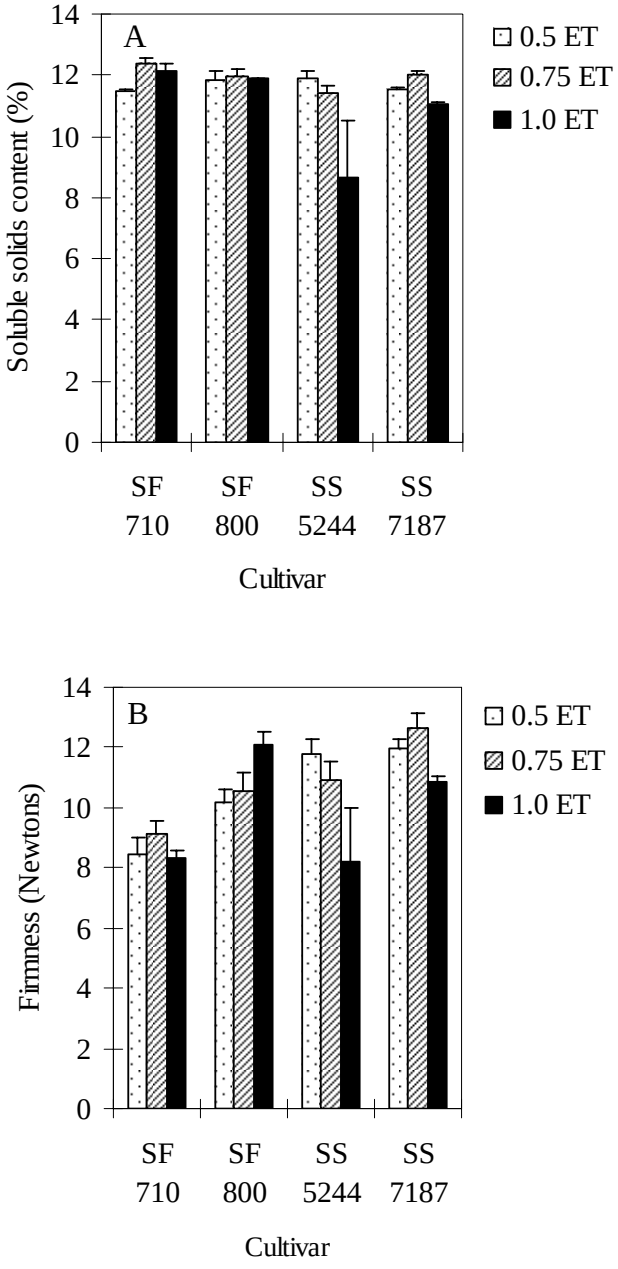

Fig. 2. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on soluble solids content (A) and firmness (B) of diploid (SF 710 and SF 800) and triploid (SS 5244 and SS 7187) watermelon at Uvalde. Bar represents standard error (± SE).

trickle irrigation study, deficiencies of soil water did not affect SSC of the watermelon diploid (Pier ad Doerge, 1995). SSC was not changed by different N-treatments (40, 130, and 270kg·ha-1).

Fruit flesh firmness was significantly affected by irrigation and cultivar. Triploid fruits were firmer with 0.5 ET regimes as compared to the 1.0 ET (Fig. 2). 'Summer Flavor 710' had much softer flesh (8-9 Newtons) than the other three cultivars, while 'Summer Sweet 7187' was the firmest (10.9-12.7 Newtons). At Weslaco, flesh firmness for the triploid cv. 'Summer Sweet 5244' fruits at the mature and overmature stages was higher (10.30±0.84, 7.00±0.70 Newtons) than for diploid cv. 'Summer Flavor 710' (6.29±0.35, 4.88±0.22 Newtons, respectively). These responses were consistent with those at Uvalde. The firmness values of Weslaco were lower than those of Uvalde, because a V-tip probe rather than a round tip probe was used in that location.

Lycopene

At all three locations, there were no significant irrigation and cultivar interactions for lycopene content. Across all cultivars, lycopene increased as fruit matured at all irrigation regimes (Fig. 3A). Deficit irrigation (0.75 and 0.5 ET) did not reduce lycopene at the mature and overmature stages. In fact, fruits from the 0.5 ET regime had higher lycopene levels than at 1.0 ET regime. A previous study reported that fully mature fruits had higher lycopene content than underripe and overripe fruits (Perkins-Veazie et al., 2002b). In a recent work with nine cultivars at Uvalde, lycopene content increased from 55.8 to 60.2 $\mu g \cdot g^{-1}$ fw for the mature and overmature stages, respectively (Leskovar et al.,

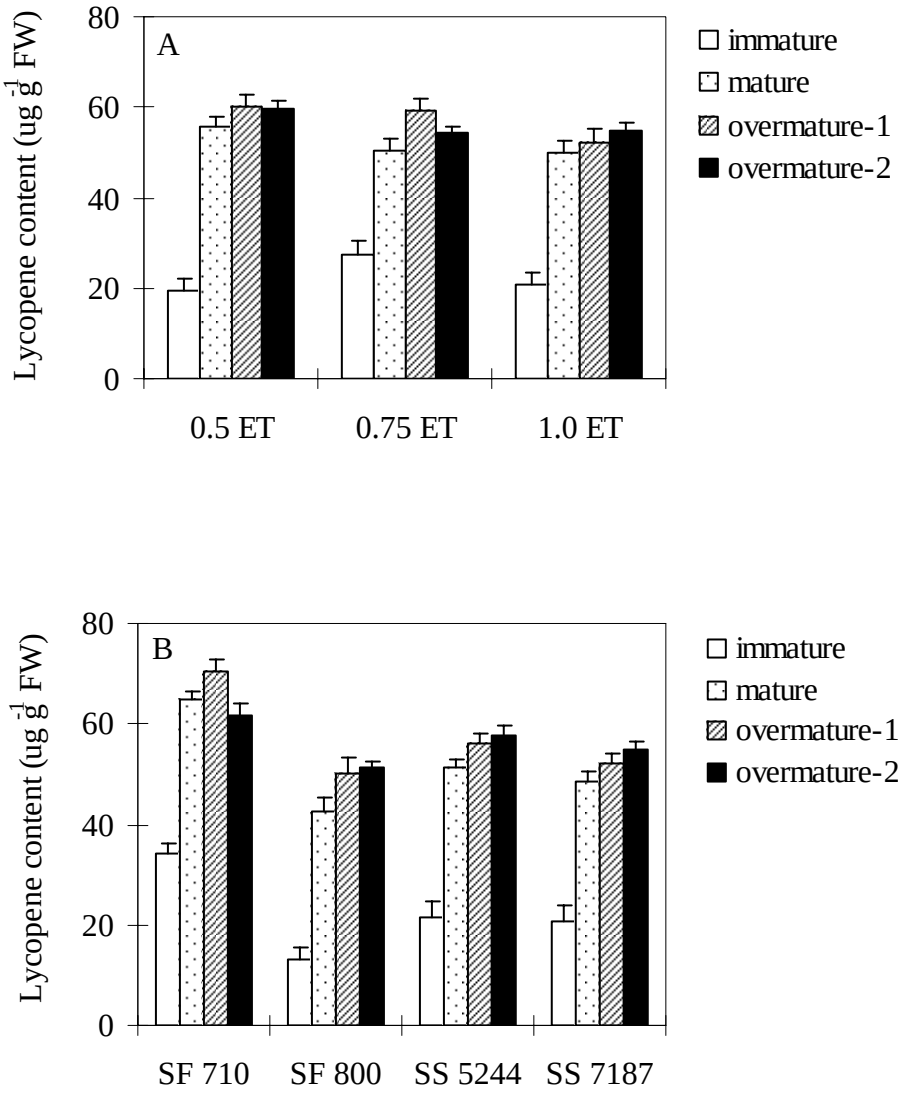

Fig. 3. Effect of deficit irrigation regime based on evapotranspiration (ET) rate (A) and cultivar (B) on lycopene content at different maturity stages of diploid (SF 710 and SF 800) and triploid (SS 5244 and SS 7187) watermelons at Uvalde. Immature stage is 7 d before mature, overmature-1, and overmature-2, 7 and 22 d after mature. Bar represents standard error (± SE).

2004). Lycopene content did not decrease for fruits in advanced stage of maturity, such as overmature-2 or 22 days after ripening. This finding may have practical applications, since overmature fruits still attached to plants but left in the field as waste, may constitute an additional source for this potent antioxidant.

Across all irrigation regimes, more lycopene was found in the diploid 'Summer Flavor 710' than the diploid 'SF 800' or the triploid cultivars at all irrigation regimes (Fig. 3B). Regarding maturity for each cultivar, lycopene sharply increased from immature to mature and overmature-1 stages in all cultivars. 'Summer Flavor 710' was the only cultivar that had a decline from overmature-1 to overmature-2 stage. However, 'Summer Flavor 710' had the highest lycopene content at the mature and overmature stages.

At Weslaco, like Uvalde, lycopene was not reduced by limited irrigation at either mature or overmature stage (Table 3). In fact, lycopene content was significantly higher at 0.5 ET compared to 1.0 ET regime for the overmature fruit stage. In wheat, drought stress increased carotenoid content including lutein and β–carotene up to 25% (Herbinger et al., 2002). At Weslaco the lower values at the overmature stage were due to a 20% decrease of lycopene in the diploid cv. 'Summer Flavor 710', but not the triploid 'Summer Sweet 5244'. At Uvalde, lycopene content for 'Summer Flavor 710' did not change from mature to overmature-1, but decreased in the overmature-2 stage (Fig. 3). At Lubbock, lycopene content at the mature stage was not reduced at 0.75 ET compared to 1.0 ET (Table 3). At this location, the cv. 'Summer Flavor 800' had the lowest lycopene content, a response that was similar at Uvalde.

Table 3. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on lycopene content at mature and overmature stages of diploid and triploid watermelons at Weslaco and Lubbock.

Treatment		Lycopene content (µg·g^{-1} fw)		
		Mature (Weslaco)	Overmature (Weslaco)	Mature (Lubbock)
Irrigation regime				
0.5 ET		66.58[y]	63.99 a	51.59 b
0.75 ET		67.54	60.65 ab	57.12 a
1.0 ET		64.28	54.05 b	56.85 a
LSD (P≤0.05)		NS	8.92	3.38
Cultivar	***Ploidy***			
SF 710[z]	2*x*	72.70 a	58.35	57.37 a
SF 800	2*x*	-	-	50.48 b
SS 5244	3*x*	59.57 b	60.78	56.94 a
SS 7187	3*x*	-	-	55.96 a
LSD (P≤0.05)		6.96	NS	3.90

[z] Summer Flavor 710, Summer Flavor 800, Summer Sweet 5244, Super Seedless 7187.

[y] Mean separation within columns by Fisher's LSD test at P≤0.05.

[NS,] Nonsignificant at P≤0.05.

CHAPTER IV

CHARACTERIZATION OF CAROTENOID CONTENT AND FRUIT QUALITY OF RED, ORANGE, AND YELLOW FLESH WATERMELONS EXPOSED TO DEFICIT IRRIGATION

Materials and Methods

Plant material and culture

Three seeded diploid cultivars ‘Summer Flavor 710’ (red), ‘Orange Flesh Tendersweet’ (orange), and ‘Summer Gold’ (salmon yellow) and three seedless triploid cultivars ‘Summer Sweet 5244’ (red), ‘Sunshine’ (orange), and ‘Amarillo’ (canary yellow) were used in this experiment in 2003. Standard seedling production practices followed the same methodology as described in Chapter III.

Experiments were conducted at Texas A&M University Agricultural Research and Extension Center, Uvalde in Texas. Two irrigation regimes, 1.0 ET and 0.50 ET, were imposed. The irrigation water requirement (IR) was applied to compensate for evapotranspiration (ET). The IR for each event was calculated as follows: IR= [ET_o x Kc - ER] where ET_o is the potential evapotranspiration, Kc the crop coefficient which was estimated by the % ground cover by foliage development (Kc values were 0.2-0.4 from planting to the vining stage, 0.5-0.6 from vining to fruit set, 0.65-0.8 from fruit set to harvest), and ER is the effective rainfall (value reduced to 50% due to the polyethylene mulch). The ET_o values used were those reported by the Texas Agricultural Experiment

Stations at Uvalde.

Five-week old seedlings were mechanically transplanted in the field of Texas A&M Agricultural Research and Extension Center at Uvalde on 27 Mar. 2003 for the standard growing season and 21 May 2003 for the late growing season. The soil in that location was a Uvalde silty clay loam (fine-silty, mixed, hyperthermic Aridic Calciustoll, pH 7.7). Preplant fertilizer was broadcasted ($kg \cdot ha^{-1}$; 50-38-0+2.2 Zn) and incorporated into the soil. Additional fertilizer (30-17-38) was applied through the drip system weekly for 5 weeks using urea, KNO_3 and H_3PO_4 as sources of N, P, and K, respectively. Table 4 depicts weather conditions between transplanting and first harvest.

Table 4. Environmental conditions during the growth period until harvest of mature and overmature fruits at Uvalde ($29^o1'$ N, $99^o5'$ W).

Growing season [z]	Growth period (DAT [y])	ET_o (mm)	Rainfall (mm)	T_{max} (^oC)	T_{min} (^oC)	T_{avg} (^oC)	RH_{max} (%)	RH_{min} (%)
Standard	85	373	132	30.5	18.6	24.2	89	42
Late	70	283	295	32.3	21.8	26.8	93	46

[z] Standard growing season : 3/27/03 - 6/20/03; Late growing season : 5/21/03-7/30/03.

[y] DAT, days after transplanting for overmature fruit

Plants for each experimental plot were grown on a single raised bed on 2.03 m centers with one row per bed and 0.9 m within row spacing and each plot was separated by a 2 m blank row. A subsurface drip system (20 cm depth) and black plastic mulch

were used. Drip type was T-Tape TSX 508 (0.2 mm wall thickness) with emitters spaced every 30 cm (T-Systems International, Inc., San Diego, California).

Fruit quality, yield and carotenoid content

Individual flowers were tagged at anthesis and watermelon fruits were harvested based on maturity. Both mature fruits and overmature fruits were harvested on 20 June 2003 for early planting (85 DAT for overmature) and 30 July 2003 for late planting (70 DAT for overmature). Fruits were classified as marketable and culls (blossom-end rot, bottleneck, cracked, and misshapen) based on maturity, then counted, and weighed. Fruit quality characteristics of three fruits per replication (total n=12) were measured. Fruits were cut in half and flesh firmness was measured with a digital force meter (DFM 10, Chaltillon, Greensboro, North Carolina) using an 11 mm diameter round-head on three spots around the center of the heart tissue. Soluble solids content (SSC) was measured with a digital refractometer, using the juice extracted from the center of the heart tissue. At each harvest and maturity stage, approximately 100 g of flesh sample was taken from the center of the fruit and stored at -80 ^{o}C in an ultracold freezer until analysis.

Carotenoid content was analyzed by HPLC and spectrophotometrically. The frozen sample was pulverized using a rubber mallet and 4-5 g sub sample was taken and placed in a 50-mL tube and homogenized with 20 mL acetone. The homogenate was poured in a funnel with filter paper and washed with acetone until it became colorless. The extract was collected in a 500 mL bottle and 50 mL hexane was added and shaken for mixing. Two hundred mL of water was added to separate the hexane layer from the

extract. Absorbance of the hexane layer was measured at 503 nm using a spectrophotometer at 503 nm for lycopene of red flesh and 435 nm for carotenoids of yellow flesh.

The hexane extract was evaporated in a nitrogen stream and resuspended with acetone. The sample was injected into an HPLC system for separation of carotenoids. The system includes a pump (Perkin Elmer Series 200, Norwalk, CT), a series 200 autosampler, a UV-Vis or a series 200 diode array detector, and data collection computer. The Spherisorb-ODS2 column (25 x 0.46 cm, Alltech) and a mobile phase of 30% ethyl acetate in acetonitrile-water (9:1 + 0.1% triethyl amine) were used. Flow rate was 2 mL/min and detection. Forty μL of sample was injected and the sample was run for 30 min to complete the analysis. Individual carotenoids were separated by preparative column and quantified by using extinction coefficients for the standards. Spectrum of each peak was analyzed by using the diode array detector to confirm the identity of the compounds and unknown samples were quantified by using the purified standard carotenoid compounds.

Statistical analysis

The experiments for both growing seasons followed a split-plot design with four replications, each consisting of three rows as previously described. Irrigation regimes, the main plots, were set up in a randomized complete-block design. Cultivars, the subplots, were randomized within each irrigation regime main plot. Yield, fruit quality characteristics, and carotenoid content were subjected to analysis of variance (ANOVA)

using SAS (SAS Institute, Inc., Cary, North Carolina). Means were separated by using Fisher's LSD test, $P \leq 0.05$.

Results and Discussion

Yield and fruit size

Total marketable yield reduction was not significant at 0.5 ET regardless of growing season (Tables 5 & 6), though yield tended to slightly decrease under deficit irrigation regime. This result is probably because most of the rainfall (83%) occurred during June until harvest, the period of most critical for the reproductive development and fruit growth. Therefore, the differential irrigation rate didn't seem to make an impact in yield. Total marketable yield of the late growing season was drastically reduced compared to the standard growing season. Overall, individual fruit size and weight slightly decreased when watermelons were grown during late season. This suggests that reduction in yield may be mainly due to a decrease of the number of fruit harvested, not due to fruit size. Tables 5 and 6 emphasize that the standard growing season had a 4.8 fold increase over the late growing season (e.g. 74.49 vs. 15.56 $t \cdot ha^{-1}$ at 1.0 ET, respectively). This yield decline for the later planting was expected due to the temperatures and vine collapsed that resulted from excess rainfall (124%) during fruit set and fruit development stage. Erdem and Yuksel (2003) indicated that water deficit during flowering may result in yield reduction. Vines also collapsed when watermelon was grown at Weslaco in our previous study and resulted in serious problem in

Table 5. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on yield and fruit characteristics of diploid and triploid watermelons during standard growing season.

Treatment		Yield ($t \cdot ha^{-1}$)	Fruit characteristics		
			Length (cm)	Diameter (cm)	Rind thickness (cm)
Irrigation regime					
0.5 ET		63.87	29.8 b	22.5	1.17
1.0 ET		74.49	32.1 a	23.0	1.09
LSD (P≤0.05)		NS	1.97	NS	NS
Cultivar	***Ploidy***				
Summer Flavor 710	2*x*	80.11 a	35.9 ab	23.7 a	1.06 ab
Orange Flesh Tendersweet	2*x*	67.56 ab	37.5 a	23.4 ab	1.28 a
Summer Gold	2*x*	70.90 ab	34.8 b	22.6 bc	1.27 a
Summer Sweet 5244	3*x*	78.90 a	27.6 c	22.4 c	1.09 ab
Sunshine	3*x*	61.48 ab	25.5 d	22.6 bc	0.86 b
Amarillo	3*x*	56.13 b	24.4 d	22.1 c	1.24 a
LSD (P≤0.05)		21.58	1.73	1.02	0.24
Maturity					
Mature			29.8 b	22.1 b	1.11
Overmature			32.1 a	23.4 a	1.16
LSD (*P*≤0.05)			0.99	0.42	NS
Irrigation		NS	*	NS	NS
Cultivar		NS	***	*	**
Irrigation*Cultivar		NS	NS	NS	NS
Maturity			***	***	NS

[z] Mean separation within columns by Fisher's LSD test at *P*≤0.05.

[NS, *, **, ***] Nonsignificant or significant at *P*≤0.05, 0.01, or 0.001, respectively.

Table 6. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on yield and fruit characteristics of diploid and triploid watermelons during late growing season.

Treatment		Yield ($t \cdot ha^{-1}$)	Fruit characteristics		
			Length (cm)	Diameter (cm)	Rind thickness (cm)
Irrigation regime					
0.5 ET		14.29	29.2 a [z]	21.2	1.38
1.0 ET		15.56	28.1 b	21.3	1.39
LSD (P≤0.05)		NS	0.87	NS	NS
Cultivar	***Ploidy***				
Summer Flavor 710	2*x*	23.65 a	32.9 b	22.1 a	1.24 cd
Orange Flesh Tendersweet	2*x*	9.67 c	35.5 a	21.3 a	1.68 a
Summer Gold	2*x*	10.04 c	29.3 c	19.7 b	1.19 d
Summer Sweet 5244	3*x*	19.92 ab	26.6 d	21.7 a	1.38 bc
Sunshine	3*x*	14.58 bc	23.9 e	21.4 a	1.33 bcd
Amarillo	3*x*	11.70 c	23.7 e	21.4 a	1.46 b
LSD (P≤0.05)		6.85	1.75	1.08	0.17
Maturity					
Mature			28.9	21.3	1.42
Overmature			28.4	21.2	1.34
LSD (*P*≤0.05)			NS	NS	NS
Irrigation		NS	*	NS	NS
Cultivar		***	***	**	***
Irrigation*Cultivar		*	*	NS	NS
Maturity			NS	NS	NS

[z] Mean separation within columns by Fisher's LSD test at *P*≤0.05.

[NS, *, **, ***] Nonsignificant or significant at *P*≤0.05, 0.01, or 0.001, respectively.

muskmelon production in the Rio Grande Valley (Leskovar et al., 2004; Mertly et al., 1991).

Both diploid and triploid red cultivars produced more fruits than non-red cultivars in both standard and late growing seasons. Fruit size (weight, length, and diameter) increased as fruit matured whereas rind thickness was not affected by either fruit maturity or irrigation regime during standard growing season.

Individual fruit weight under deficit irrigation decreased during standard growing season (data not shown). The size of overmature fruit during the standard growing season was larger than mature fruit but there was no difference in size during late growing season (data not shown). This indicates that fruits continued to develop until overmature stage during the standard growing season. However, excess rainfall during mainly in the late season seemed to cause the lack of difference in fruit size across irrigation regime and across maturity. The impact of growing season clearly varied among cultivars. The three diploid cultivars used in this study were more unstable showing relatively more fruit weight decrease compared to the triploids (Fig. 4).

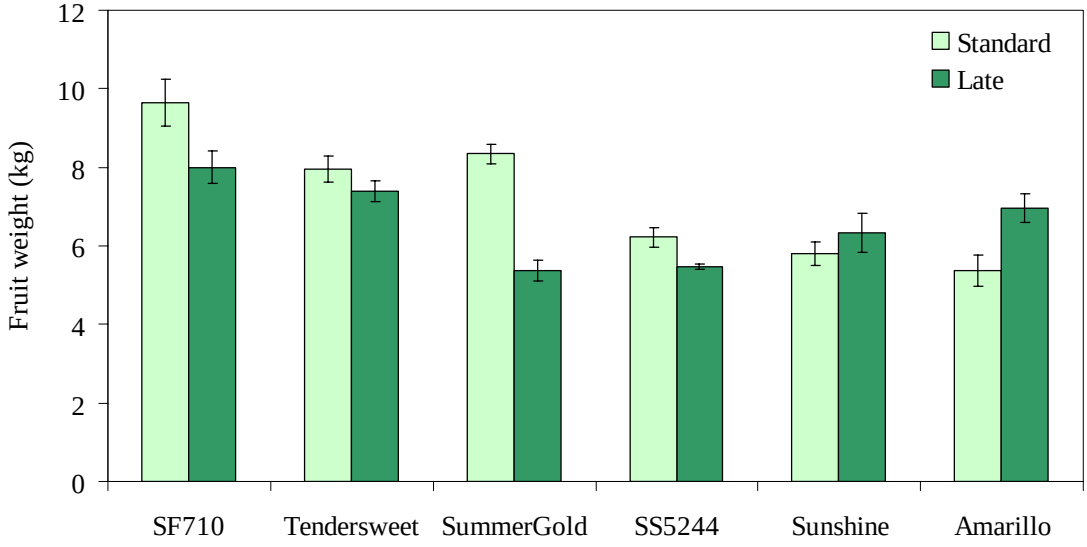

Fig. 4. Effect of growing season and cultivar on fruit weight of mature diploid and triploid watermelons. Bar represents standard error (± SE). Diploids were Summer Flavor 710 (red), Orange Flesh Tendersweet (orange), and Summer Gold (salmon yellow) and triploids were Summer Sweet 5244 (red), Sunshine (orange), and Amarillo (canary yellow).

Firmness and soluble solids content

As it was expected, triploids were much firmer compared to diploid (Table 7). It was consistent with the previous report by Leskovar et al. (2004). However, firmness of 'Amarillo' considerably decreased ranging from 18.22 to 11.86 Newtons when they were grown at the late season. This suggests that fruit quality based on firmness for the cultivar 'Amarillo' is more sensitive to climatic conditions, being more unstable when it is grown under abnormal high temperature/rainfall environments. Mature fruit was

Table 7. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on firmness and soluble solids content of diploid and triploid watermelons.

Treatment		Firmness (Newton)		Soluble solids content (^{o}Brix)	
Growing season		Standard	Late	Standard	Late
Irrigation regime					
0.5 ET		13.60	12.31	11.15	9.97
1.0 ET		12.72	12.44	11.01	9.75
LSD (P≤0.05)		NS	NS	NS	NS
Cultivar	***Ploidy***				
Summer Flavor 710	2*x*	12.38 bc	13.11 ab	11.42 a	10.15 a
Orange Flesh Tendersweet	2*x*	9.66 d	8.87 c	10.23 b	8.98 b
Summer Gold	2*x*	11.41 cd	11.05 b	11.25 a	8.77 b
Summer Sweet 5244	3*x*	12.83 bc	14.17 a	11.31 a	10.50 a
Sunshine	3*x*	14.45 b	15.19 a	10.93 a	10.27 a
Amarillo	3*x*	18.22 a	11.86 b	11.33 a	10.49 a
LSD (*P*≤0.05)		2.47	2.09	0.57	0.62
Maturity					
Mature		13.67 a	12.94 a	10.78 b	9.84
Overmature		12.65 b	11.81 b	11.38 a	9.88
LSD (*P*≤0.05)		0.93	0.85	0.24	NS
Irrigation		NS	NS	NS	NS
Cultivar		***	***	**	***
Irrigation*Cultivar		NS	NS	NS	NS
Maturity		*	*	***	NS

[z] Mean separation within columns by Fisher's LSD test at *P*≤0.05.

[NS, *, **, ***] Nonsignificant or significant at *P*≤0.05, 0.01, or 0.001, respectively.

significantly firmer than overmature fruit. Although there was a numerical increase in firmness for the 0.5 ET regime, this difference was not statistically different when

compared to 1.0 ET for both standard and late growing seasons.

Soluble solids content (SSC) was not reduced by different irrigation regimes in both standard and late growing seasons. A previous study reported that deficit irrigation reduced total sugar content (Erdem and Yuksel, 2003). SSC varied across cultivars and increased with maturity during the standard growth season. The difference of SSC between mature and overmature fruit was smaller under 1.0 ET, when compared to 0.5 ET (Fig. 5). In Table 7, SSC during late season was lower compared to standard season, probably due to excess rainfall during fruit development. Overall SSC and firmness of watermelons were not affected by different irrigation regimes in this experiment, again probably due to the excess rainfall during fruit development.

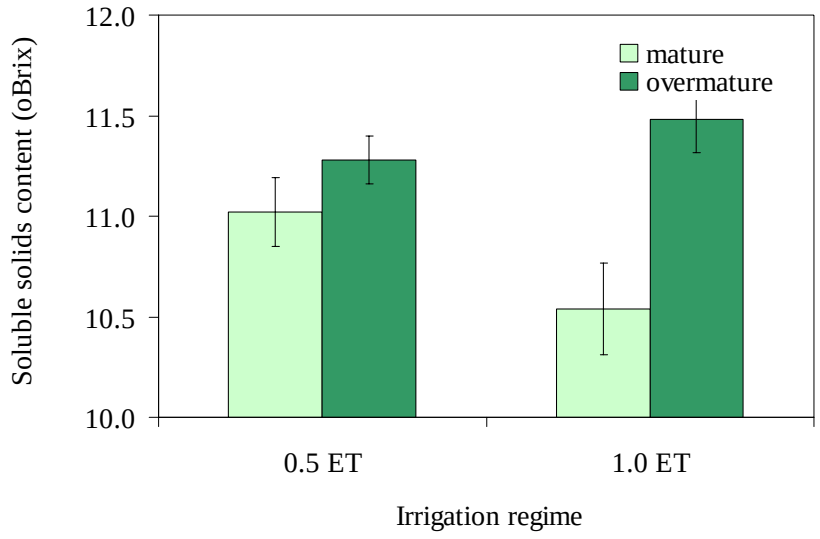

Fig. 5. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and maturity on soluble solids content during standard growing season. Bar represents standard error.

Carotenoids

Diploid and triploid red-fleshed watermelon cultivars had significantly higher carotenoid content than orange- and yellow-fleshed cultivars. The major carotenoid was lycopene (more than 65%), followed by prolycopene (20%) and β-carotene (7%) (Fig. 6). Tomes and Johnson (1965) reported the major carotenoid of orange watermelon appeared to be prolycopene and/or ζ-carotene. 'Early Moonbeam', a canary yellow watermelon cultivar, contained trace of carotenoids (Tadmor et al., 2004). This is similar to 'Amarillo'. It was also reported that predominant carotenoids were prolycopene in 'Orange Flesh Tendersweet' and β-carotene in 'NY162003'.

Total carotenoid content was not affected by irrigation regime during standard growing season (Table 8). Inconsistency of total carotenoid was shown between HPLC analysis and spectrophotometer measurement. It is recognized that the problem might be in the extraction protocol and procedure. Current methods may not be able to extract all type of carotenoids at one time. Moreover, standard carotenoids for the quantification were not available except for a few such as lycopene, β-carotene and lutein. Therefore, other carotenoids have been quantified equivalent to those standards.

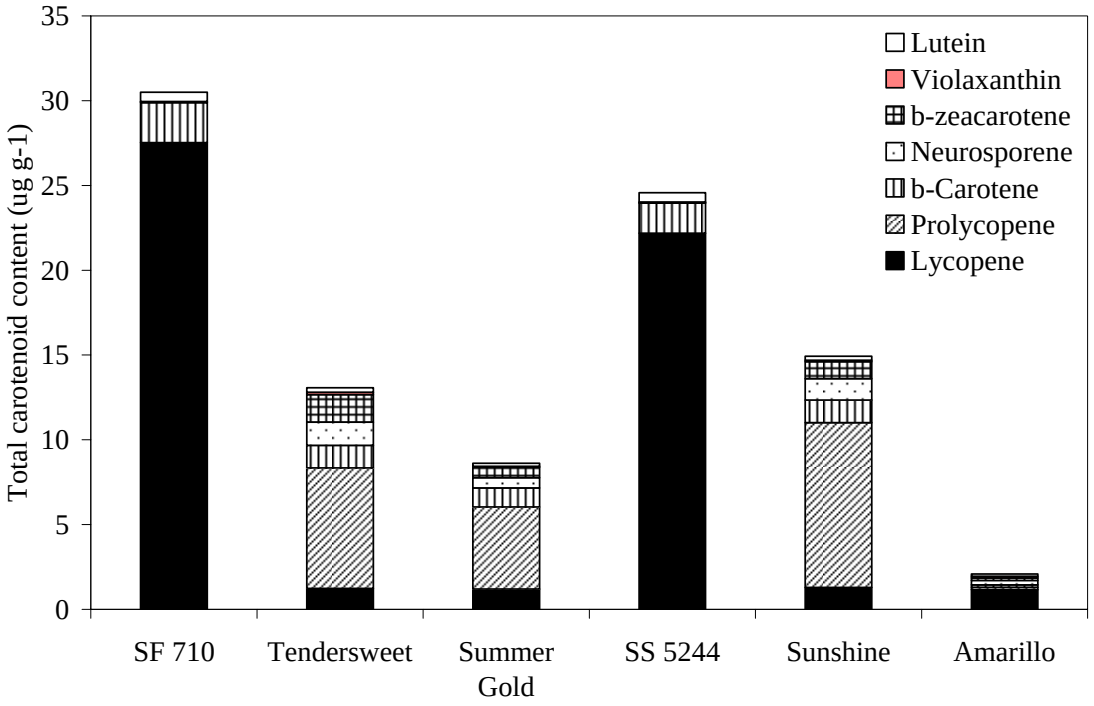

Fig. 6. Effect of deficit irrigation regime based on cultivar on total carotenoid profiles of diploid and triploid watermelons. Diploids were Summer Flavor 710 (red), Orange Flesh Tendersweet (orange), and Summer Gold (salmon yellow) and triploids were Summer Sweet 5244 (red), Sunshine (orange), and Amarillo (canary yellow).

Table 8. Effect of deficit irrigation regime based on evapotranspiration (ET) rate and cultivar on carotenoid content of diploid and triploid watermelons during standard growing season.

Treatment		Carotenoid content ($\mu g \cdot g^{-1}$)								
		Neurosporene	β-Zeacarotene	Prolycopene	Lycopene	β-Carotene	Violaxanthin	Lutein	HPLC total	SPEC
Irrigation regime										
0.5 ET		0.58±0.09	0.52±0.09	3.36±0.54	8.83±1.28	1.30±0.11	0.08±0.01	0.31±0.03	14.99±1.29	18.42±2.26
1.0 ET		0.61±0.10	0.66±0.10	4.11±0.60	9.22±1.32	1.39±0.10	0.08±0.01	0.32±0.03	16.40±1.29	19.28±2.26
Cultivar	***Ploidy***									
Summer Flavor 710	2*x*	0.00±0.00	0.04±0.02	0.00±0.00	27.53±2.06	2.35±0.22	0.03±0.01	0.54±0.05	30.49±2.12	51.68±2.09
OrangeTendersweet	2*x*	1.37±0.22	1.63±0.26	7.11±0.99	1.24±0.11	1.32±0.15	0.14±0.02	0.26±0.04	13.08±1.60	5.55±0.56
Summer Gold	2*x*	0.60±0.11	0.61±0.11	4.84±0.97	1.20±0.13	1.13±0.11	0.07±0.01	0.18±0.02	8.64±1.18	4.25±0.46
SummerSweet 5244	3*x*	0.00±0.00	0.03±0.02	0.00±0.00	22.18±1.62	1.78±0.17	0.04±0.01	0.55±0.05	24.59±1.65	45.54±2.00
Sunshine	3*x*	1.26±0.17	1.00±0.13	9.72±1.04	1.29±0.12	1.34±0.13	0.09±0.01	0.23±0.02	14.94±1.47	5.03±0.35
Amarillo	3*x*	0.27±0.07	0.15±0.07	0.14±0.04	1.16±0.53	0.14±0.02	0.09±0.02	0.13±0.03	2.07±0.58	1.76±0.74
Maturity										
Mature		0.65±0.10	0.69±0.10	4.46±0.63	9.43±1.32	1.28±0.10	0.06±0.01	0.33±0.03	16.91±1.26	19.04±2.28
Overmature		0.54±0.10	0.49±0.09	2.99±0.50	8.60±1.28	1.42±0.12	0.10±0.01	0.30±0.03	14.43±1.31	18.64±2.24

CHAPTER V

CLONING OF THE GENES PRODUCING SPECIFIC ENZYMES IN THE CAROTENOID BIOSYNTHETIC PATHWAY

Materials and Methods

Genes encoding specific enzymes in the carotenoid pathway were cloned using degenerate PCR and RACE (rapid amplification of cDNA ends) approaches.

RNA & DNA isolation and cDNA synthesis

Red watermelon flesh, ovary and young leaf tissue of 'Black Diamond' and 'Charleston Gray' were used as plant materials. To increase RNA yield and eliminate polysaccharides, excess water in flesh tissue was squeezed out using a sterilized cloth. For RNA isolation, 100 mg of flesh and ovary tissues were ground in liquid nitrogen. Then, total RNA was extracted using RNeasy Plant Mini Kit (Qiagen; Valencia, CA). RNase-free DNase (Qiagen) was used for additional digestion of remaining DNA during RNA purification. Absorbance was measured at 260 nm and A_{260}/A_{280} ratio was recorded with spectrophotometer to calculate RNA yield and check the purity. Pure RNA should have 1.9-2.1 for A_{260}/A_{280} ratio. RACE-ready first-strand cDNA was synthesized from 1 μg of RNA using SMART RACE cDNA amplification Kit (BD Biosciences; Palo Alto, CA). One hundred mg of young leaf tissue was ground in liquid nitrogen for genomic DNA isolation. DNA was extracted using a DNeasy Plant Mini

Kit (Qiagen).

Primer design

To design degenerate primers for degenerate PCR, the sequence of homologous genes in other species were collected from GenBank database (www.ncbi.nlm.nih.gov). Then, the highly conserved blocks among amino acid sequences were identified using Blockmaker software (www.blocks.fhcrc.org). Degenerate primers were designed based on conserved blocks using CODEHOP software (www.blocks.fhcrc.org/blockmkr-bin/codehop), as genes that have same function are highly homologous in different species in general, particularly within functional domains. Optimal primer sets were selected by criteria as follows: PCR product size 400-600 bp, approximately 50% of GC content, 32-128 degeneracy, and 60-70 ^{o}C of T_m. Degenerate primer sequences are shown in Table 9.

Table 9. Sequence of degenerate primers for gene cloning in the carotenoid biosynthetic pathway of watermelon.

Genes	Forward/Reverse	Degenerate primers (5'-3')
PSY	F	CTATTTGGGCAATTTATGTDTGGTGYAG
	R	AGGTGCRATKCCCATDAYHGG
PDS	F	GATGAAATTTCWGCDGAYCARAGYAAAGC
	R	TTGAAGCCAARTATTTYTGHTTBGTGTARTC
ZDS	F	GCTGGSCTTGCDGGGATGTCVAC
	R	ACRTGSAGKCCCATTTCRATRTGGTT
CRTISO	F	GCTCCWTCDTTTCTTTCHATTCAYATGG
	R	TTTGGAGGGAGWCCCTCCCARTCYTCWATG
LCYB	F	CTTAAAYTSATHTGGCCHAMAA
	R	GTTGGAHGAAAATGGCATNGCRTA
	R	AGTCCTTGCYACCATRTADCCNGT
CHYB	F	TGGAGTTTTGGGCNMGRTGGGCNCA
	R	ACGTTGGCDATGGGHCCNACWGGGAA
ZEP	F	GGWGGMAARATGCARTGGTAYGCATTT
	R	CCMCCTTGRCCCADATTWGGCTGCAT

Degenerate PCR and RACE

For degenerate PCR, PCR reaction mixture was prepared with 0.05 μg template, 5 μL 10× PCR buffer, 1 μL dNTP (10mM), 1 μL forward primer (10μM), 1 μL reverse primer (10 μM), and 1 μL Taq polymerase mix (50× Advantage 2 polymerase mix; BD Biosciences) in a total volume of 50 μL. Denaturation at 94 ^{o}C for 3 min was followed by 40 cycles of 94 ^{o}C for 30 s, 55-68 ^{o}C (depending on primer set) for 30 s, and 72 ^{o}C for 3 min, then a final extension at 72 ^{o}C for 20 min. The PCR products were separated on 1% agarose gel and then, bands were cut and DNA was purified with NucleoTrap Gel Extraction Kit (BD Biosciences). Partial sequences of a fragment obtained by degenerate PCR enable to design watermelon gene specific primers for RACE.

RACE was carried out to get the sequence of full-length cDNA. The RACE reaction mixture contained 2.5 μL of RACE-ready cDNA, 5 μL 10× PCR buffer, 1 μL dNTP (10mM), 1 μL gene specific primer (10μM; reverse for 5'-RACE, forward for 3'-RACE), 5 μL UPM (10× Universal primer A mix; BD Biosciences), and 1 μL Taq polymerase mix in a total volume of 50 μL. For PCR amplification, denaturation at 94 ^{o}C for 3 min was followed by 5 cycles of 94 ^{o}C for 5 s, 72 ^{o}C for 3 min, 5 cycles of 94 ^{o}C for 5 s, 72 ^{o}C for 10 s, and 72 ^{o}C for 3 min, 25 cycles of 94 ^{o}C for 5 s, 65-68 ^{o}C (depending on primer set) for 10 s, and 72 ^{o}C for 3 min, and 72 ^{o}C for 3 min, then a final extension at 72 ^{o}C for 20 min. The PCR products were separated on 1% agarose gel and then, bands were cut and DNA was purified with NucleoTrap Gel Extraction Kit (BD Biosciences). When a single band was produced, QIAquick PCR purification Kit (Qiagen) was used for purification.

Cloning and sequencing

TOPO TA Cloning Kit (Invitrogen; Carlsbad, CA) was used for cloning of PCR products using a mixture of 4 μL of purified PCR product, 1 μL of salt solution, and 1 μL of pCR®4-TOPO vector which was transformed into competent cell (TOP10 *E. coli* cell) provided with kit. Transformed cells were plated on a LB media and incubated overnight at 37 ^{o}C. To analyze positive clones among colonies, colonies were picked and cultured on LB medium containing 50 $\mu g \cdot mL^{-1}$ kanamycin for 12 hrs at 37 ^{o}C. Once transformed clones were cultured, plasmids were purified with QIAprep Spin Miniprep Kit (Qiagen). The sequences of purified plasmids were obtained using automated Big Dye DNA Cycle Sequencing (ABI Prism BigDye Terminator Cycle Sequencing Ready Kits; Applied Biosystems; Foster City, CA) and ABI 3100 capillary seqeuncer (ABI 3100 Genetic Analyzer; Applied Biosystems) by the Laboratory for Plant Genome Technology sequencing facility of Texas A&M University. The positive DNA and deduced amino acid sequences were identified by the BLAST search. Full-length cDNA sequences and deduced amino acid sequences were aligned by Clustal W (Chenna et al., 2003; Thompson et al., 1994). Neighbor-joining phylogenetic tree was obtained using ClustalX 1.81 and MEGA version 3.1 (Kumar et al., 2004; Thompson et al., 1997).

Results and Discussion

Phytoene synthase (PSY)

PSY is involved in the first committed step of the carotenoid biosynthetic pathway that converts two molecules of GGPP into phytoene. Two members of *PSY* family in watermelon were identified by degenerate PCR and RACE, which are similar to *PSY1* and *PSY2* in tomato (Bartley and Scolink, 1993; 1995; Fraser et al., 1999). Full-length cDNA of each was obtained and they were designated as *PSY-A* and *PSY-B,* respectively. The deduced amino acid sequence alignment of *PSY* with other species is shown in Fig. 7.

In general, the position of the splicing site is conserved in homologous genes. However, a noticeable feature of phytoene synthase structure was that both *PSY-A* and *PSY-B* had only 5 exons and 4 introns as compared to 6 exons and 5 introns in other known *PSY* genes of other species (Fig. 8). The fifth intron as in tomato *PSY1* was missing in watermelon *PSY-A,* and the first intron as in tomato *PSY2* was missing in watermelon *PSY-B.*

```
PSY-A                    --------------MSGVNANSLLSPKPRIR---------ISSKPFGSRRLSFFSDG--- 34
PSY-B                    -----------------MSSYICITPKPSIFIRECKGKLFPKRFTLIMSKSGVIAAP--- 40
TomatoPSY1               -MSVALLWVVSPCDVSNGTSFMESVREG--NRFFDSSR---HRNLVSNERINRGGGK--Q 52
TomatoPSY2               ------------------------------------------------------------
MaizePSY1                -MAIILVRAAS-PGLSAADSISHQGTLQ-CSTLLKTKRPAARRWMPCSL----LGLH--P 51
MaizePSY2                --------------MAAGSSAVWAAQHPACS--GGKFHHLSPSHSHCRPRRALQTPPALP 44
RicePSY1 (BAD62106)      MAAITLLRSASLPGLSDALARDAAAVQHVCSSYLPNNKEKKRRWILCSLKYACLGVDPAP 60
RicePSY2                 ---------------------------GAPR------------------------------ 4
RicePSY (AAK07735)       --------------MTGEGSPNQNCRGAPRGLLAGGFEGGPPPPRQVEQDSSHFSQGYNE 46
Tomato_GTom5 for PSY[z]  -MSVALLWVVSPCDVSNGTSFMESVREG--NRFFDSSR---HRNLVSNERINRGGGK--Q 52

PSY-A                    -----------VLASSAAVVNPSRSSEERVYEVVLKQAALVR--EPKRDIQRALDWEKTI 81
PSY-B                    -------KNPQRLKFPTLSKQGIPLADLNVDEIVERQSHANN-----------FSREESC 82
TomatoPSY1               TNNGRKFS-VRSAILATPSGERTMTSEQMVYDVVLRQAALVK---RQLRSTN-ELEVKP- 106
TomatoPSY2               ------------------------------------------------------DP- 2
MaizePSY1                WEAGRPSPAVYSSLAVNPAGEAVVSSEQKVYDVVLKQAALLK---RQLRTP--VLDARPQ 106
MaizePSY2                ARRSGASPPRASLAAAAPAVAVRTASEEAVYEVVLRQAALVEAATPQRRRTRQPRWAEEE 104
RicePSY1 (BAD62106)      GEIARTSP-VYSSLTVTPAGEAVISSEQKVYDVVLKQAALLK---RHLRPQPHTIPIVPK 116
RicePSY2                 --------------------------WAEED------------------------------ 9
RicePSY (AAK07735)       GEEAGCCRLVGEAAAGREPGGGHGAGGRGVEEDGGGEASRGRLGTRCGRPHRAARGRRGG 106
Tomato_GTom5             TNNGRKFS-VRSAILATPSGERTMTSEQMVYDVVLRQAALVK---RQLRSTN-ELEVKP- 106

PSY-A                    QNEGITDGNLLSEAYSRCGEVCAEYAKTFYLGTQLMTPERRRAVWAIYVWCRRTDELVDG 141
PSY-B                    KKKQQFHPSFLEEAYESCRKICAEYAKTFYLGTLLMTKERQRAIWAIYVWCRRTDELVDG 142
TomatoPSY1               DIPIPGNLGLLSEAYDRCGEVCAEYAKTFNLGTMLMTPERRRAIWAIYVWCRRTDELVDG 166
TomatoPSY2               DIVLPGNLGLLSEAYDRCGEVCAEYAKTFYLGTMLMTPDRRRAIWAIYVWCRRTDELVDG 62
MaizePSY1                DMDMPRNG--LKEAYDRCGEICEEYAKTFYLGTMLMTEERRRAIWAIYVWCRRTDELVDG 164
MaizePSY2                EEERVLGWGLLGDAYDRCGEVCAEYAKTFYLGTQLMTPERRKAVWAIYVWCRRTDELVDG 164
RicePSY1 (BAD62106)      DLDLPRNG--LKQAYHRCGEICEEYAKTFYLGTMLMTEDRRRAIWAIYVWCRRTDELVDG 174
RicePSY2                 ---AVDWGLLLGDAYHRCGEVCAEYAKTFYLGTQLMTPERRKAVWAIYVWCRRTDELVDG 66
RicePSY (AAK07735)       RGGRVDWGLLLGDAYHRCGEVCAEYAKTFYLGTQLMTPERRKAVWAIYVWCRRTDELVDG 166
Tomato_GTom5             DIPIPGNLGLLSEAYDRCGEVCAEYAKTFNLGTMLMTPERRRAIWAIYVWCRRTDELVDG 166
                                   * :**  * ::* ****** *** *** :*::*:****************

PSY-A                    PNASHITPKALERWEKRLTDLFEGRPYDMYDAALSDTVSKYPVDIQPFKDMIEGMRLDLR 201
PSY-B                    PNAVYMNPKVLDRWEERLEDIFEGCPYDLLDAALSHTVSRFPIDMKPFKDMIEGMRMDTK 202
TomatoPSY1               PNASYITPAALDRWENRLEDVFNGRPFDMLDGALSDTVSNFPVDIQPFRDMIEGMRMDLR 226
TomatoPSY2               PNASHITPQALDRWEARLEDIFNGRPFDMLDAALSDTVSRFPVDIQPFRDMVEGMRMDLW 122
MaizePSY1                PNANYITPTALDRWEKRLEDLFTGRPYDMLDAALSDTISRFPIDIQPFRDMIEGMRSDLR 224
MaizePSY2                PNASYITPTALDRWEKRLEDLFEGRPYDMYDAALSDTVSKFPVDIQPFKDMVQGMRLDLW 224
RicePSY1 (BAD62106)      PNASHITPSALDRWEKRLDDLFTGRPYDMLDAALSDTISKFPIDIQPFRDMIEGMRSDLR 234
RicePSY2                 PNSSYITPKALDRWEKRLEDLFEGRPYDMYDAALSDTVSKFPVDIQPFKDMIEGMRLDLW 126
RicePSY (AAK07735)       PNSSYITPKALDRWEKRLEDLFEGRPYDMYDAALSDTVSKFPVDIQPFKDMIEGMRLDLW 226
Tomato_GTom5             PNASYITPAALDRWENRLEDVFNGRPFDMLDGALSDTVSNFPVDIQPFRDMIEGMRMDLR 226
                         **: ::.* .*:*** ** *:* * *:*: *.***.*:*.:*:*::**:**::*** *
```

Fig. 7. Alignment of the deduced amino acid sequences of watermelon *PSY-A* and *PSY-B* with *PSY1* and *PSY2* genes of other species. '*': amino acids in the column are identical, ':': conserved substitution, '.': semi-conserved substitution.

[z]Tomato GTom5 gene for PSY (CAA42969).

```
PSY-A                   KSRYENFDELYLYCYYVAGTVGLMSVPVMGLAPESKASVESVYNAALALGLANQLTNILR 261
PSY-B                   KCRYENFEELYLYCYYVAGTVGLMSVPVMGIAPDSSLPTQTIYSAALHLGIGNQLTNILR 262
TomatoPSY1              KSRYKNFDELYLYCYYVAGTVGLMSVPIMGIAPESKATTESVYNAALALGIANQLTNILR 286
TomatoPSY2              KSRYNNFDELYLYCYYVAGTVGLMSVPIMGIAPESKATTESVYNAALALGIANQLTNILR 182
MaizePSY1               KTRYNNFDELYMYCYYVAGTVGLMSVPVMGIATESKATTESVYSAALALGIANQLTNILR 284
MaizePSY2               KSRYMTFDELYLYCYYVAGTVGLMTVPVMGIAPDSKASTESVYNAALALGIANQLTNILR 284
RicePSY1 (BAD62106)     KTRYKNFDELYMYCYYVAGTVGLMSVPVMGIAPESKATTESVYSAALALGIANQLTNILR 294
RicePSY2                KSRYRSFDELYLYCYYVAGTVGLMTVPVMGIAPDSKASTESVYNAALALGIANQLTNILR 186
RicePSY (AAK07735)      KSRYRSFDELYLYCYYVAGTVGLMTVPVMGIAPDSKASTESVYNAALALGIANQLTNILR 286
Tomato_GTom5            KSRYKNFDELYLYCYYVAGTVGLMSVPIMGIAPESKATTESVYNAALALGIANQLTNILR 286
                        * ** .*:***:************:**:**:*.:*. ..::::*.*** **:.********

PSY-A                   DVGEDARRGRVYLPQDELAQAGLCDDDIFRGKVTDKWRFFMKGQIKRARRFFDEAEKGVA 321
PSY-B                   DVGEDAIRGRIYLPQDELAQFGLCDDDILAMRVTEKWREFMKEQIKRAKFYFKLAEKGAS 322
TomatoPSY1              DVGEDARRGRVYLPQDELAQAGLSDEDIFAGRVTDKWRIFMKKQIHRARKFFDEAEKGVT 346
TomatoPSY2              DVGEDARRGRVYLPQDELAQAGLSDEDIFAGKVTDKWRIFMKKQIQRARKFFDEAEKGVT 242
MaizePSY1               DVGEDARRGRIYLPQDELAQAGLSDEDIFKGVVTNRWRNFMKRQIKRARMFFEEAERGVT 344
MaizePSY2               DVGEDARRGRIYLPLDELAQAGLTEEDIFRGKVTGKWRRFMKGQIQRARLFFDEAEKGVT 344
RicePSY1 (BAD62106)     DVGEDARRGRIYLPQDELAEAGLSDEDIFNGVVTNKWRSFMKRQIKRARMFFEEAERGVT 354
RicePSY2                DVGEDSRRGRIYLPLDELAEAGLTEEDIFRGKVTDKWRKFMKGQILRARLFFDEAEKGVA 246
RicePSY (AAK07735)      DVGEDSRRGRIYLPLDELAEAGLTEEDIFRGKVTDKWRKFMKGQILRARLFFDEAEKGVA 346
Tomato_GTom5            DVGEDARRGRVYLPQDELAQAGLSDEDIFAGRVTDKWRIFMKKQIHRARKFFDEAEKGVT 346
                        *****: ***:*** ****: ** ::**:   ** :** *** ** **: :*. **:*.:

PSY-A                   ELSAASRWPVWASLMLYKQILDSIEANDYDNFTKRAYVGKAKKLLSLPIAFGRAMVGPSS 381
PSY-B                   QLDKASRWPVWSSLMLYRKILEAIEENDYNNFTKRAYVRRSKKLLTLPLAYTKAISAPSL 382
TomatoPSY1              ELSSASRFPVWASLVLYRKILDEIEANDYNNFTKRAYVSKSKKLIALPIAYAKSLVPPTK 406
TomatoPSY2              ELSSASRWPVLASLLLYRKILDEIEANDYNNFTRRAYVSKPKKLLTLPIAYARSLVPPKS 302
MaizePSY1               ELSQASRWPVWASLLLYRQILDEIEANDYNNFTKRAYVGKGKKLLALPVAYGKSLLLPCS 404
MaizePSY2               HLDSASRWPVLASLWLYRQILDAIEANDYNNFTKRAYVGKAKKLLSLPLAYARAAVAP-- 402
RicePSY1 (BAD62106)     ELSQASRWPVWASLLLYRQILDEIEANDYNNFTKRAYVGKAKKLLALPVAYGRSLLMPYS 414
RicePSY2                HLDSASRWPVLASLWLYRQILDAIEANDYNNFTKRAYVNKAKKLLSLPVAYARAAVAS-- 304
RicePSY (AAK07735)      HLDSASRWPVLASLWLYRQILDAIEANDYNNFTKRAYVNKAKKLLSLPVAYARAAVAS-- 404
Tomato_GTom5            ELSSASRFPVWASLVLYRKILDEIEANDYNNFTKRAYVSKSKQVDCITYCICKISCASYK 406
                        .*. ***:** :** **::**: ** ***:***:**** : *::  :. .  :    .

PSY-A                   FKDLVTR- 388
PSY-B                   VFH----- 385
TomatoPSY1              TAS-LQR- 412
TomatoPSY2              TSCPLAKT 310
MaizePSY1               LRN-GQT- 410
MaizePSY2               --------
RicePSY1 (BAD62106)     LRN-SQK- 420
RicePSY2                --------
RicePSY (AAK07735)      --------
Tomato_GTom5            TAS-LQR- 412
```

Fig. 7. Continued.

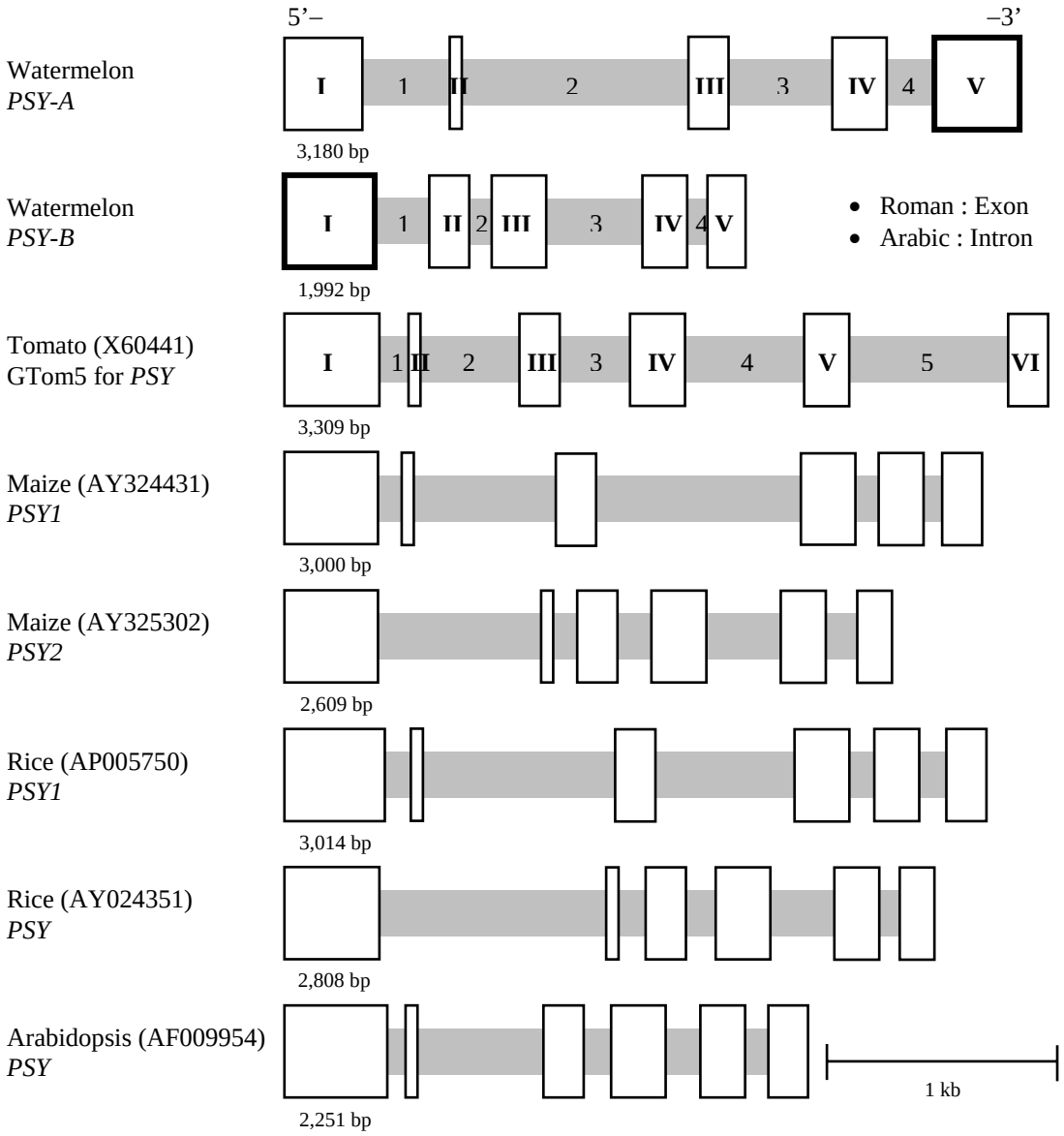

Fig. 8. Comparison of gene structures of genomic sequences of *PSY-A* and *PSY-B* with *PSY* of other species. White and gray box represent an exon and an intron. The numbers in the brackets are GenBank accession numbers.

Phylogenetic analysis indicated that watermelon *PSY-A* clustered with carrot and showed closer relationship than *PSY-B* or melon *PSY* (Fig. 9 and Table 10). Watermelon *PSY-B* gene was most closely related to rice *PSY*. The result from phylogenetic analysis suggests that *PSY-B* has a different transcriptional behavior from *PSY-A*, similar to *PSY2* which is not involved in fruit ripening (Batley and Scolnik, 1993; Fraser et al., 1999). Phylogenetic analysis of grass family by Gallegher et al. (2004) showed that *PSY1*-like genes and *PSY2*-like genes were closely related respectively, so that they were clustered together indicating common ancestry in grass (monocot). Fig. 9 shows that in dicots, they were not clustered as *PSY1*-like genes and *PSY2*-like genes, but instead they diverged different.

The orthologous genes of *PSY-A* or *PSY-B* were identified in tomato, maize, and rice. Watermelon *PSY-A* and tomato *PSY1* showed 65% identity which is higher compared to *PSY-B* (Table 10). However, there was only 54% identity of deduced amino acid sequences between *PSY-A* and *PSY-B*, which is relatively low. This result was similar to the report by Gallergher et al. (2004). Overall, the identity of *PSY1*-like or *PSY2*-like genes between species was higher than the identity between *PSY1* and *PSY*2 within a species. The amino acid sequence identity was lower between *PSY1*-like genes or *PSY2*-like genes among dicots, when compared to higher identity in monocot (data not shown). Rice *PSY1* and maize *PSY1* shared 80%, and rice *PSY2* and maize *PSY2* shared 92%.

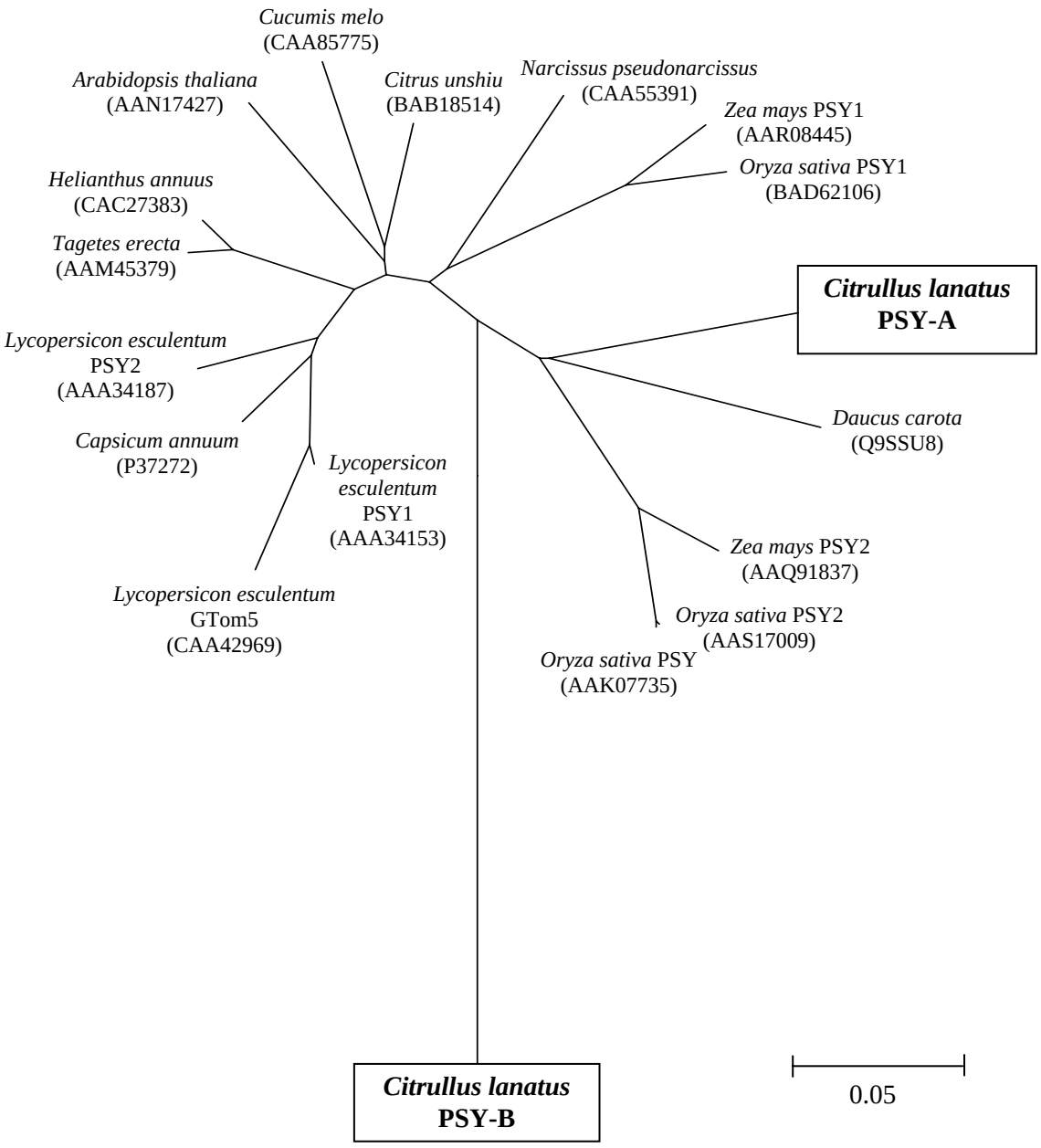

Fig. 9. Phylogenetic analysis of *PSY-A* and *PSY-B* based on amino acid sequences. The numbers in the brackets are GenBank accession numbers.

Table 10. Identity comparison of the deduced amino acid sequences of structural genes encoding enzymes in the carotenoid biosynthetic pathway of watermelon with other species.

Gene	Species	Amino acid identity (%)	Gene	Species	Amino acid identity (%)
PSY-A	*PSY-B*	54	*PDS*	*Tagetes erecta*	85
	Daucus carota	71		*Citrus unshiu*	83
	Tagetes erecta	67		*Zea mays*	74
	Helianthus annuus	67		*Narcissus pseudonarcissus*	75
	Citrus unshiu	67		*Capsicum annuum*	78
	Arabidopsis thaliana	68		*Lycopersicon esculentum*	78
	Capsicum annuum	67		*Glycine max*	80
	Oryza sativa			*Arabidopsis thaliana*	79
	PSY1 (BAD62106)	64			
	PSY2 (AAS17009)	82	*ZDS*	*Citrus unshiu*	84
	PSY (AAK07735)	63		*Lycopersicon esculentum*	81
	Lycopersicon esculentum			*Capsicum annuum*	81
	PSY1	65		*Narcissus pseudonarcissus*	81
	PSY2	78		*Helianthus annuus*	82
	GTom5 for *PSY*	63		*Arabidopsis thaliana*	82
	Zea mays PSY1	63		*Zea mays*	74
	Zea mays PSY2	70			
	Cucumis melo	66	*CRTISO*	*Lycopersicon esculentum*	76
	Narcissus pseudonarcissus	65		*Arabidopsis thaliana*	79
PSY-B	*Daucus carota*	52	*LCYB*	*Arabidopsis thaliana*	76
	Tagetes erecta	56		*Sandersonia aurantiaca*	73
	Helianthus annuus	54		*Adonis palaestina*	79
	Citrus unshiu	54		*Tagetes erecta*	77
	Arabidopsis thaliana	52		*Lycopersicon esculentum*	80
	Capsicum annuum	52		*Narcissus pseudonarcissus*	74
	Oryza sativa			*Capsicum annuum*	80
	PSY1 (BAD62106)	54		*Citrus sinensis*	83
	PSY2 (AAS17009)	66		*Nicotiana tabacum*	80
	PSY (AAK07735)	52			
	Lycopersicon esculentum		*CHYB*	*Brassica rapa*	72
	PSY1	53		*Citrus unshiu*	71
	PSY2	66		*Arabidopsis thaliana*	70
	GTom5 for *PSY*	52		*Lycopersicon esculentum*	66
	Zea mays PSY1	54		*Vitis vinifera*	65
	Zea mays PSY2	53		*Capsicum annuum*	61
	Cucumis melo	57		*Tagetes erecta*	61
	Narcissus pseudonarcissus	53		*Narcissus pseudonarcissus*	60
			ZEP	*Prunus armeniaca*	73
				Citrus unshiu	72
				Nicotiana tabacum	69
				Lycopersicon esculentum	68
				Arabidopsis thaliana	67
				Oryza sativa	65

Additional homologous *PSY* genes have been identified in tomato and rice. Four homologous *PSY* genes were cloned in tomato (GenBank Accession No. (protein) *PSY1*, AAA34153; *PSY2*, AAA34187; *GTom5 for PSY*, CAA42969; *PSY* pseudogene X60440) and three were in rice (GenBank Accession No. (protein) *PSY1*, BAD62106; *PSY2*, AAS17009; *PSY*, AAK07735). The amino acid sequence identity ranged from 57% to 97% (data not shown) among them. This implies that other homologous genes possibly exist in other species such as watermelon, Arabidopsis, and maize, but may not have been identified so far.

It was proposed that gene duplication might precede evolution of the grass family (Poaceae) by Gallergher et al. (2004). It was also suggested the gene duplication may be common in monocots based on the result that gene duplication was identified in 12 species of Poaceae. Therefore, gene duplication was considered to be unusual in dicot by Gallergher et al. (2004) because tomato and tobacco were the only example examined in dicots. However, our watermelon gene cloning results with the study of tomato and pepper (Romer et al., 1993; Bartley and Scolnik, 1993) will provide additional evidence that gene duplication of *PSY* may be common in dicots. Moreover, they are likely to be duplicated evolutionarily a long time ago, possibly even prior to evolution of monocot and dicot division. Consequently, these two orthologous *PSY* genes may be under different regulatory mechanisms.

Phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS)

Plants require two types of desaturases, *PDS* and *ZDS* whereas bacteria have one desaturase, *crtI* (Bartley et al., 1999) that catalyzes multiple stpes.

PDS catalyzes desaturation of phytoene (van den Berg et al., 2000). Degenerate PCR was used to obtain and sequence a fragment of the *PDS* gene in watermelon. Alignment of the deduced amino acid sequence of *PDS* between species is shown in Fig. 10. The overall percentage of the identity comparisons of *PDS* with other species was higher than with other genes such as *PSY* (Table 10). Pecker et al. (1992) concluded that the *PDS*-encoding *crtI* gene in bacteria and fungi was highly conserved in deduced amino acid sequences.

ZDS is involved in a subsequent desaturation step in the carotenoid biosynthetic pathway. The full-length cDNA of *ZDS* gene from watermelon was 2,062 bp including 181 bp 5'-untranslated region (UTR), 1,758 bp open reading frame (ORF), and 153 bp 3'UTR (Fig. 11). Relatively high identity of deduced amino acid sequences were observed across species, indicating that *ZDS* is highly conserved compared to other genes in the carotenoid biosynthetic pathway.

Based on identity comparison of both *PDS* and *ZDS*, the desaturases playing a role in the desaturation step of the carotenoid biosynthesis are highly conserved among species and are probably stable throughout the evolution.

```
Watermelon     MSLCGSV--SALNLRWEKGIPKATS------RCCSPLSCEKSNALAFWGSEIVGDGLKVS 52
Citrus         MSLCFSVSESAFNLRYG----------------------------FRDSEPMGQSLKIR 31
Soybean        MAACGYI--SAANFNYLVGA-----------RNISKFASSDATIS-FSFGGSDSMGLTLR 46
Arabidopsis    MVVFGNV--SAANLPYQNG------------FLEALSSGGCELMGHSFRVPTSQALKTR 45
Pepper         MPQIGLV--SAVNLRVQGNSAYLWSSRSS-LGTDSQDGCSQRNSLCFGGSDSMSHRLKIR 57
Tomato         MPQIGLV--SAVNLRVQGSSAYLWSSRSSSLGTESRDGCLQRNSLCFAGSESMGHKLKIR 58
Maize          -MDTGCL--SSMNITGASQT--------------RSFAGQLPPQRCFASSHYTSFAVKKL 43
Daffodil       MSIVGLV--SVVCPSGGIKK--------------RYFSKGLDNFQGFRSSECLGIQLQVP 44
                     :  *                                    .     .  :

Watermelon     G-RHVSRKLSKGNVPLKVVCVDYPRPQIDDTVNFIEAASLSASFRASARPSKPLKIVIAG 111
Citrus         ----VKTGTRKGFCPSKVVCVDYPRPDIDNTSNFLEAAYLSSSFRTSPRPSKPLKVVIAG 87
Soybean        P-APIRAPKRNHFSPLRVVCVDYPRPELENTVNFVEAAYLSSTFRASPRPLKPLNIVIAG 105
Arabidopsis    ------TRRRSTAGPLQVVCVDIPRPELENTVNFLEAASLSASFRSAPRPAKPLKVVIAG 99
Pepper         NPHSITRRLAKDFRPLKVVCIDYPRPELDNTVNYLEAAFLSSSFRSSPRPTKPLEIVIAG 117
Tomato         TPHATTRRLVKDLGPLKVVCIDYPRPELDNTVNYLEAAFLSSTFRASPRPTKPLEIVIAG 118
Maize          VSRNKGRRSHRRHPALQVVCKDFPRPPLESTINYLEAGQLSSFFRNSERPSKPLQVVVAG 103
Daffodil       VPFYSGIRQSPRATSLQVVCKDCPRPELEGAVNFLEAAQLSASFRSSPRPEKGLEVVVVG 104
                           . :*** * *** ::.: *::**. **: ** : ** * *::*:.*

Watermelon     AGLAGLSTAKYLADAGHKPVLLEARDVLGGKVAAWKDNDGDWYETGLHIFFGAYPNVQNL 171
Citrus         AGLAGLSTAKYLADAGHKPLLLEARDVLGGKVAAWKDGDGNWYETGLHIFFGAYPNIQNL 147
Soybean        AGLAGLSTAKYLADAGHKPILLEARDVLGGKVAAWKDKDGDWYETGLHIFFGAYPYVQNL 165
Arabidopsis    AGLAGLSTAKYLADAGHKPLLLEARDVLGGKIAAWKDEDGDWYETGLHIFFGAYPNVQNL 159
Pepper         AGLGGLSTAKYLADAGHKPILLEARDVLGGKVAAWKDDDGDWYETGLHIFFGAYPNMQNL 177
Tomato         AGLGGLSTAKYLADAGHKPILLEARDVLGGKVAAWKDDDGDWYETGLHIFFGAYPNIQNL 178
Maize          AGLAGLSTAKYLADAGHKPILLEARDVLGGKVAAWKDEDGDWYETGLHIFFGAYPNIQNL 163
Daffodil       AGLAGLSTAKYLADAGHKPILLESRDVLGGKIAAWKDKDGDWYETGLHIFFGAYPNVQNL 164
               ***.***************:***:*******:***** **:************** :***

Watermelon     FGELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEKLPAPVNGIWAILRNNEMLTWPEKI 231
Citrus         FGELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEVLPAPLNGILAILRNNEMLTWPEKV 207
Soybean        FGELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEVLPSPLNGIWAILRNNEMLTWPEKV 225
Arabidopsis    FGELGINDRLQWKEHSMIFAMPSKPGEFSRFDFPDVLPAPLNGIWAILRNNEMLTWPEKI 219
Pepper         FGELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEALPAPLNGILAILKNNEMLTWPEKV 237
Tomato         FGELGINDRLQWKEHSMIFAMPSKPGEFSRFDFSEALPAPLNGILAILKNNEMLTWPEKV 238
Maize          FGELRIEDRLQWKEHSMIFAMPNKPGEFSRFDFPETLPAPINGIWAILRNNEMLTWPEKV 223
Daffodil       FGELGINDRLQWKEHSMIFAMPNKPGEFSRFDFPEVLPAPLNGIWAILRNNEMLTWPEKV 224
               **** *:***************.**********.: **:*:*** ***:**********:

Watermelon     KFAIGLLPAMLGGQSYVEAQDNLTVQEWMRSRGVPDRVTTEVFIAMSKALNFINPDELSM 291
Citrus         KFAIGLLPAIIGGQAYVEAQDGLTVQEWMRKQGVPDRVTTEVFIAMSKALNFINPDELSM 267
Soybean        KFAIGLLPAMLGGQPYVEAQDGLSVQEWMKKQGVPERVADEVFIAMSKALNFINPDELSM 285
Arabidopsis    KFAIGLLPAMVGGQAYVEAQDGLSVKEWMEKQGVPERVTDEVFIAMSKALNFINPDELSM 279
Pepper         KFAIGLLPAMLGGQSYVEAQDGISVKDWMRKQGVPDRVTDEVFIAMSKALNFINPDELSM 297
Tomato         KFAIGLLPAMLGGQSYVEAQDGISVKDWMRKQGVPDRVTDEVFIAMSKALNFINPDELSM 298
Maize          KFAIGLLPAMVGGQPYVEAQDGLTVSEWMKKQGVPDRVNDEVFIAMSKALNFINPDELSM 283
Daffodil       RFAIGLLPAMVGGQAYVEAQDGLTVTEWMRRQGVPDRVNDEVFIAMSKALNFINPDELSM 284
               :********::***.******.::* :**. :***:**  ********************
```

Fig. 10. Alignment of the deduced amino acid sequences of watermelon *PDS* with *PDS* genes of other species. ‘*’: amino acids in the column are identical, ‘:’: conserved substitution, ‘.’: semi-conserved substitution.

```
Watermelon     QCILIALNRFLQEKHGSKMAFLDGNPPERLCEPIVEHIQSLGGEVRFNSRIQKIELNNDG 351
Citrus         QCILIALNRFLQEKHGSKMAFLDGNPPERLCLPIVEHIQSLGGEVRLNSRVQKIELNDDG 327
Soybean        QCILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVDYIQSLGGEVHLNSRIQKIELNDDG 345
Arabidopsis    QCILIALNRFLQEKHGSKMAFLDGNPPERLCMPVVDHIRSLGGEVQLNSRIKKIELNDDG 339
Pepper         QCILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVEHIESKGGQVRLNSRIKKIELNEDG 357
Tomato         QCILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVEHIESKGGQVRLNSRIKKIELNEDG 358
Maize          QCILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVDHIRSRGGEVRLNSRIKKIELNPDG 343
Daffodil       QCILIALNRFLQEKHGSKMAFLDGNPPERLCMPIVDHIQSLGGRAQLNSRLQKIELNPDG 344
               ****************************** *:*::*.* **..::***::***** **

Watermelon     TVKRFLLNDGNVIEGDAYVFATPVDILKLLLPNDWKAIPYFKKLEKLVGVPVINVHIWFD 411
Citrus         TVKNFLLTNGNVIDGDAYVFATPVDILKLQLPENWKEMAYFKRLEKLVGVPVINIHIWFD 387
Soybean        TVKSFLLNNGKVMEGDAYVFATPVDILKLLLPDNWKGIPYFQRLDKLVGVPVINVHIWFD 405
Arabidopsis    TVKSFLLTNGSTVEGDAYVFAAPVDILKLLLPDPWKEIPYFKKLDKLVGVPVINVHIWFD 399
Pepper         SVKCFILNDGSTIEGDAFVFATPVDIFKLLLPEDWKEIPYFQKLEKLVGVPVINVHIWFD 417
Tomato         SVKSFILSDGSAIEGDAFVFAAPVDIFKLLLPEDWKEIPYFQKLEKLVGVPVINVHIWFD 418
Maize          TVKHFALSDGTQITGDAYVCATPVDIFKLLVPQEWSEITYFKKLEKLVGVPVINVHIWFD 403
Daffodil       TVKHFVLGNGNIITGDAYVVAAPVDILKLLLPQEWREIPYFQKLDKLVGVPVINVHIWFD 404
               :** * * :*. : ***:* *:****:** :*: *  :.**::*:*********:*****

Watermelon     RKLKNTYDHLLFSRSPLLSVYADMSVTCKEYYNPNQSMLELVFAPAEEWISRSDSEIIDA 471
Citrus         RKLKNTYDHLLFSRSSLLSVYADMSLTCKEYYNPNQSMLELVFAPAEEWISCSDSEIIDA 447
Soybean        RKLKNTYDHLLFSRSPLLSVYADMSVTCKEYYSPNQSMLELVFAPAEEWISRSDDDIIQA 465
Arabidopsis    RKLKNTYDHLLFSRSNLLSVYADMSLTCKEYYDPNRSMLELVFAPAEEWISRTDSDIIDA 459
Pepper         RKLKNTSDNLLFSRSPLLSVYADMSVTCKEYYDPNKSMLELVFAPAEEWVSRSDSEIIDA 477
Tomato         RKLKNTYDHLLFSRSSLLSVYADMSVTCKEYYNPNQSMLELVFAPAEEWISRSDSEIIDA 478
Maize          RKLNNTYDHLLFSRSSLLSVYADMSVTCKEYYDPNRSMLELVFAPADEWIGRSDTEIIDA 463
Daffodil       RKLKNTYDHLLFTRSPLLSVYADMSVTCKEYYDPNRSMLELVFAPAEEWISRSDSEIIER 464
               ***:** *:***:** *********:******.**:**********:**:. :* :**:

Watermelon     TMVELAKLFPDEISADQSKAKIVKYHVVKTPRSVYKTVPDCEPCRPLQRSPIEGFYLAGD 531
Citrus         TMKELAKLFPDEISADQSKAKIVKYHVVKTPRSVYKTIPNCEPCRPLQRSPVEGFYLAGD 507
Soybean        TMTELAKLFPDEISADQSKAKILKYHVVKTPRSVYKTVPNCEPCRPIQRSPIEGFYLAGD 525
Arabidopsis    TMKELEKLFPDEISADQSKAKILKYHVVKTPRSVYKTIPNCEPCRPLQRSPIEGFYLAGD 519
Pepper         TMKELAKLFPDEISADQSKAKILKYHVVKTPRSVYKTVPGCEPCRLLQRSPVEGFYLAGD 537
Tomato         TMKELATLFPDEISADQSKAKILKYHVVKTPRSVYKTVPGCEPCRPLQRSPIEGFYLAGD 538
Maize          TMEELAKLFPDEIAADQSKAKILKYHIVKTPRSVYKTVPNCEPCRPLQRSPIEGFYLAGD 523
Daffodil       TMKELAKLFPDEIAADQSKAKILKYHVVKTPRSVYKTIPDCEPCRPLQRSPIEGFYLAGD 524
               ** ** .******:********:***:**********:*.***** :****:********

Watermelon     YTKQKYLASMEGAVLSGKLCAQAIVKDYEVLVAREQRRVAEAGIRGQELLR 582
Citrus         YTKQKYLASMEGAVLSGKLCAQAIVQDYVLLAARGKGRLAEASMCP----- 553
Soybean        YTKQKYLASMEGAVLSGKLCAQAIVQDSELLATRGQKRMAKASVV------ 570
Arabidopsis    YTKQKYLASMEGAVLSGKFCSQSIVQDYELLAASGPRKLSEATVSSS---- 566
Pepper         YTKQKYLASMEGAVLSGKLCAQAIVQDYELLVGRSQRKLAETSVV------ 582
Tomato         YTKQKYLASMEGAVLSGKLCAQAIVQDYELLVGRSQKKLSEASVV------ 583
Maize          YTKQKYLASMEGAVLSGKLCAQSIVQDYSRLALRSQKSLQSGEVPVPS--- 571
Daffodil       YTNQKYLASMEGAVLSGKLCAQSIVQDYELLVRRSKK-ASTAEMTVV---- 570
               **:***************:*:*:**:*   *.          :
```

Fig. 10. Continued.

```
Watermelon     MASGI------LFPPVSFTGKHGNCRN-----FRIPARNSVVLLKGQKFLVRSSLDKDVS 49
Arabidopsis    MASSV------VFAAT-------------------GSLSVPPLKSRRFYVNSSLDSDVS 34
Citrus         MGSSV------LFPATSITG---------------VSWSRVQEKCPRFCVRASLDANVS 38
Daffodil       MASST------CLIHSSSFGVGGK-----------KVKMNTMIRSKLFSIRSALDTKVS 42
Tomato         MATSS---AYLSCPATSATGKKHVFPNGSPGFLVFGGTRLSNRLVTRKSVIRADLDSMVS 57
Pepper         MATCS---AYLCCPATSASLKKRVFPDGSAGFLFFGGRRLSNRLVTPKSVIRADLNSMVS 57
Sunflower      MATSSSSTASLCFPATSAAGTRSSFHTTTSTLLRCRRSRQLTRLKVRKAVIRSDLDRDVS 60
Maize          MASVA-------ATTTLAPALAPR-----------RARPGTGLVPPRRASAVAARSTVTS 42
               *.:                                                 :  .  .*

Watermelon     DMSVSAPKGLFPPEPERYRGPKLKVAIIGAGLAGMSTAVELLDQGHEVDIYESRTFIGGK 109
Arabidopsis    DMSVNAPKGLFPPEPVPYKGPKLKVAIIGAGLAGMSTAVELLDQGHEVDIYDSRTFIGGK 94
Citrus         DMSVNAPKGLFPPEPEHYRGPKLKVAIIGAGLAGMSTAVELLDQGHEVDIYESRSFIGGK 98
Daffodil       DMSVNAPKGLFPPEPEHYRGPKLKVAIIGAGLAGMSTAVELLDQGHEVDIYESRQFIGGK 102
Tomato         DMSTNAPKGLFPPEPEHYRGPKLKVAIIGAGLAGMSTAVELLDQGHEVDIYESRTFIGGK 117
Pepper         DMSTNAPKGLFPPEPEHYRGPKLKVAIIGAGLAGMSTAVELLDQGHEVDIYESRTFIGGK 117
Sunflower      DMRTNAPKGLFPPEPEHYRGPKLKVAIIGAGLAGMSTAVELLNQGHEVDIYESRTFIGGK 120
Maize          PTWRQRSQRLFPPEPEHYRGPKLKVAIIGAGLAGMSTAVELLDQGHEVDLYESRPFIGGK 102
                   . .: ******  *:**********************:******:*:** *****

Watermelon     VGSFVDKRGNHIEMGLHVFFGCYNNLFRLMKKVGAEKNLLVKDHTHTFVNKGGEIGELDF 169
Arabidopsis    VGSFVDRRGNHIEMGLHVFFGCYNNLFRLMKKVGAEKNLLVKDHTHTFINKDGTIGELDF 154
Citrus         VGSFVDKRGNHIEMGLHVFFGCYNNLFRLMKKVGADKNLLVKDHTHTFVNQGGEIGELDF 158
Daffodil       VGSFVDKRGNHIEMGLHVFFGCYNNLFRLMKKVGADENLLVKDHTHTFVNRGGEIGELDF 162
Tomato         VGSFVDRRGNHIEMGLHVFFGCYNNLFRLLKKVGAEKNLLVKEHTHTFVNKGGEIGELDF 177
Pepper         VGSFVDKRGNHIEMGLHVFFGCYNNLFRLMKKVGAEKNLLVKEHTHTFVNKGGEIGELDF 177
Sunflower      VGSFVDKQGNHIEMGLHVFFGCYNNLFRLLKKVGAEKNLLVKDHTHTFVNKGGELGELDF 180
Maize          VGSFVDRQGNHIEMGLHVFFGCYSNLFRLMKKVGADNNLLVKEHTHTFVNKGGTIGELDF 162
               ******::****************.*****:*****::*****:*****:*:.* :***** 

Watermelon     RFPIGAPIHGIRAFLATNQLGTYDKARNALALALSPVVKALVDPDAAMKDIRNLDSISFS 229
Arabidopsis    RFPVGAPIHGIRAFLVTNQLKPYDKLRNSLALALSPVVKALVDPDGAMRDIRNLDSISFS 214
Citrus         RFPIGAPLHGIRAFLSTNQLKTYDKARNALALALSPVVKALVDPDGALKDIRDLDSISFS 218
Daffodil       RLPMGAPLHGIRAFLTTNQLKPYDKARNAVALALSPVVRALIDPNGAMQDIRNLDNISFS 222
Tomato         RFPVGAPLHGINAFLSTNQLKIYDKARNAVALALSPVVRALVDPDGALQQIRDLDNVSFS 237
Pepper         RFPVGAPLHGINAFLSTNQLKTYDKARNAVALALSPVVRALVDPDGALQQIRDLDSVSFS 237
Sunflower      RFPVGAPLHGINAFLTTNHLKTYDKARNAVALALSPVVRALVDPDGAMTQIRNLDNISFS 240
Maize          RFPVGAPLHGIQAFLRTNQLKVYDKARNAVALALSPVVRALVDPDGALQQVRDLDDISFS 222
               *:*:***:***.*** **:*  *** **::********:**:**:.*: ::*:**.:***

Watermelon     EWFLSKGGTRASIQRMWDPVAYALGFIDCDNISARCMLTIFSLFATKTEASLLRMLKGSP 289
Arabidopsis    DWFLSKGGTRASIQRMWDPVAYALGFIDCDNMSARCMLTIFSLFATKTEASLLRMLKGSP 274
Citrus         DWFLSKGGTRTSIQRMWDPVAYALGFIDCDNISARCMLTIFALFATKTEASLLRMLKGSP 278
Daffodil       DWFLSKGGTRMSIQRMWDPVAYALGFIDCDNISARCMLTIFSLFATKTEASLLRMLKGSP 282
Tomato         EWFLSKGGTRASIQRMWDPVAYALGFIDCDNMSARCMLTIFALFATKTEASLLRMLKGSP 297
Pepper         DWFMSKGGTRASIQRMWDPVAYALGFIDCDNISARCMLTIFALFATKTEASLLRMLKGSP 297
Sunflower      EWFMSKGGTRTSIQRMWDPVAYALGFIDCDNISARCMLTIFSLFATKTEASLLRMLKGSP 300
Maize          DWFMSKGGTRESITRMWDPVRYALGFIDCDNISARCMLTIFTLFATKTEASLLRMLKGSP 282
               :**:****** ** ****** **********:*********:*****************
```

Fig. 11. Alignment of the deduced amino acid sequences of watermelon *ZDS* with *ZDS* genes of other species. ‘*’: amino acids in the column are identical, ‘:’: conserved substitution, ‘.’: semi-conserved substitution.

```
Watermelon     DVFLSGPIRKYITDRGGRFHLRWGCREVLYDKFADGETYIAGLAMSKATNKKIVKADAYV 349
Arabidopsis    DVYLSGPIKQYITDRGGRIHLRWGCREILYDKSADGETYVTGLAISKATNKKIVKADVYV 334
Citrus         DVYLSGPIRKYITDKGGRFHLRWGCREILYDKAANGETYVKGLAMSKATDKKVVQADAYV 338
Daffodil       DVYLSGPIRKYITDKGGRFHLRWGCREILYDELSNGDTYITGIAMSKATNKKLVKADVYV 342
Tomato         DVYLSGPIKKYIMDKGGRFHLRWGCREVLYETSSDGSMYVSGLAMSKATQKKIVKADAYV 357
Pepper         DVYLSGPIKKYIIDKGGRFHLRWGCREVLYETSSDGSMYVSGLAMSKATQKKIVKADAYV 357
Sunflower      DVYLSGPIRDYIIEKGGRFHLRWGCREILYEKSANGDTYVTGLAMSKATQKKIVKADAYI 360
Maize          DVYLSGPIKKYITDRGGRFHLRWGCREVLYEKSPDGETYVKGLLLTKATSREIIKADAYV 342
               **:*****:.** ::***:********:**:  .:*. *: *: ::***.:::::**.*:

Watermelon     AACDVPGIKRLIPSQWREWEFFDNIYKLIGVPVVTVQLRYNGWVTELQDLERSRQLREAV 409
Arabidopsis    AACDVPGIKRLLPKEWRESRFFNDIYELEGVPVVTVQLRYNGWVTELQDIELARQLKRAV 394
Citrus         AACDVPGIKRLLPSSWREMKFFNNIYALVGVPVVTVQLRYNGWVTELQDLERSRQLRRAL 398
Daffodil       AACDVPGIKRLIPSEWREWDLFDNIYKLVGVPVVTVQLRYNGWVTEMQDLEKSRQLRAAV 402
Tomato         AACDVPGIKRLVPQKWRELEFFDNIYKLVGVPVVTVQLRYNGWVTELQDLERSRQLKRAA 417
Pepper         AACVVPGIKRLVPQKWRELEFFGNIYKLIGVPVVTVQLRYNGWVTELQDLERSRQSKRAT 417
Sunflower      AACDVPGIKRLLPSNWREWEFFDNIYKLVGVPVVTVQLRYNGWVTELQDLERSRQLRQAA 420
Maize          AACDVPGIKRLLPSEWREWEMFDNIYKLDGVPVVTVQLRYNGWVTELQDLEKSRQLQRAV 402
               *** *******:*..***  :*.:** * *****************:**:* :** : *

Watermelon     GLDNLLYTPDADFSCFADLALTSPEDYYIEGQGSLLQCVLTPGDPYMPLLNDEIIARVAK 469
Arabidopsis    GLDNLLYTPDADFSCFADLALASPADYYIEGQGTLLQCVLTPGDPYMRMPNDKIIEKVAM 454
Citrus         GLDNLLYTPDADFSCFADLALTSPEDYYREGQGSLLQCVLTPGDPYMPLPNDEIIRRVAK 458
Daffodil       GLDNLLYTPDADFSCFSDLALSSPEDYYIEGQGSLIQAVLTPGDPYMPLPNDAIIERVRK 462
Tomato         GLDNLLYTPDADFSCFADLALASPDDYYIEGQGSLLQCVLTPGDPYMPLSNDEIIKRVTK 477
Pepper         GLDNLLYTPDADFSCFADLALASPEDYYIEGQGSLLQCVLTPGDPYMPLPNEEIIRRVSK 477
Sunflower      GLDNLLYTPDADFSCFADLALASPEDYYIEGQGSLLQCVLTPGDPYMPLPNEEIISRVSK 480
Maize          GLDNLLYTADADFSCFSDLALSSPADYYIEGQGSLIQAVLTPGDPYMPLPNEEIISKVQK 462
               ********.*******:****:** *** ****:*:*.********* : *: ** :*

Watermelon     QVLDLFPSSQGLEVTWSSVVKIGQSLYREAPGKDPFRPDQKTPIKNFFLAGSYTKQDYID 529
Arabidopsis    QVTELFPSSRGLEVTWSSVVKIAQSLYREAPGKDPFRPDQKTPIKNFFLAGSYTKQDYID 514
Citrus         QVLALFPSSQGLEVIWSSVVKIGQSLYREGPGKDPFRPDQKTPVKNFFLAGSYTKQDYID 518
Daffodil       QVLDLFPSSQGLEVLWSSVVKIGQSLYREGPGKDPFRPDQKTPVKNFFLAGSYTKQDYID 522
Tomato         QVLALFPSSQGLEVTWSSVLKIGQSLYREGPGKDPFRPDQKTPVENFFLAGSYTKQDYID 537
Pepper         QVLALFPSSQGLEVTWSSVVKIGQSLYREGPGKDPFRPDQKTPVENFFLAGSYTKQDYID 537
Sunflower      QVLALFPSSQGLEVTWSSVVKIGQSLYREGPGKDPFRPDQKTPVKNFFLAGSYTKQDYID 540
Maize          QVVELFPSSRGLEVTWSSVVKIGQSLYREAPGNDPFRPDQKTPVKNFFLSGSYTKQDYID 522
               **  *****:**** ****:**.******.**:**********::****:**********

Watermelon     SMEGATLSGRQASAYICDSGEELMMLREKIAGID-----SETAKLSDELSLV 576
Arabidopsis    SMEGATLSGRQASSYICDAGEELAELNKKLS--------SSATAVPDELSLV 558
Citrus         SMEGATLSGRQASAYICNAGEELVALRKQLAAFESQEQMEAPTTTNDELSLV 570
Daffodil       SMEGATLSGRQAAAYICSAGEDLAALRKKIAADHPEQLINKDSNVSDELSLV 574
Tomato         SMEGATLSGRQASAYICNVGEQLMALRKKITAAELN-DISKGVSLSDELSLV 588
Pepper         SMEGATLSGRQASAYICDAGEQLLALRKKIAAAELN-EISKGVSLSDELSLV 588
Sunflower      SMEGATLSGRQASAFICDAGEELAALRKVLAAIQSI-DNVG----VDELSLV 587
Maize          SMEGATLSGRRTSAYICGAGEELLALRKKLLIDDGE----KALGNVQVLQAS 570
               **********:::::**. **:*  *.: :               : *.
```

Fig. 11. Continued

Carotenoid isomerase (CRTISO)

CRTISO catalyzes the isomerization in conjunction with desaturation from phytoene to lycopene in the carotenoid biosynthetic pathway of plants (Isaacson et al., 2002; 2004). Full-length cDNA of *CRTISO* had previously been identified only in tomato and *Arabidopsis*. Less information of homologous genes in other species made it difficult in cloning *CRTISO* of watermelon. Full-length cDNA sequence was isolated and characterized including 125 bp 5'UTR, 1,998 bp ORF, and 253 bp 3'UTR (Fig. 12). Tomato *CRTISO* contained 1,845 bp of ORF with 67.5 kD of molecular mass (Isaacson et al., 2002). Over 5 kb of genomic sequence of watermelon *CRTISO* contains 12 introns in the coding region like tomato and *Arabidopsis*. It showed 76% identity with tomato and 79% with *Arabidopsis* in comparison of sequence identity of deduced amino acids (Table 10).

In a tomato, Isaacson (2002) reported that deletions in the promoter region and coding region of *CRTISO* resulted in two different color mutants of *tangerine*, which has prolycopene and ζ-carotene in carotenoid composition. This strongly suggests that watermelon *CRTISO* mutations might also cause salmon yellow or orange mutant which accumulates prolycopene and ζ-carotene as major carotenoids in fruit (data not shown).

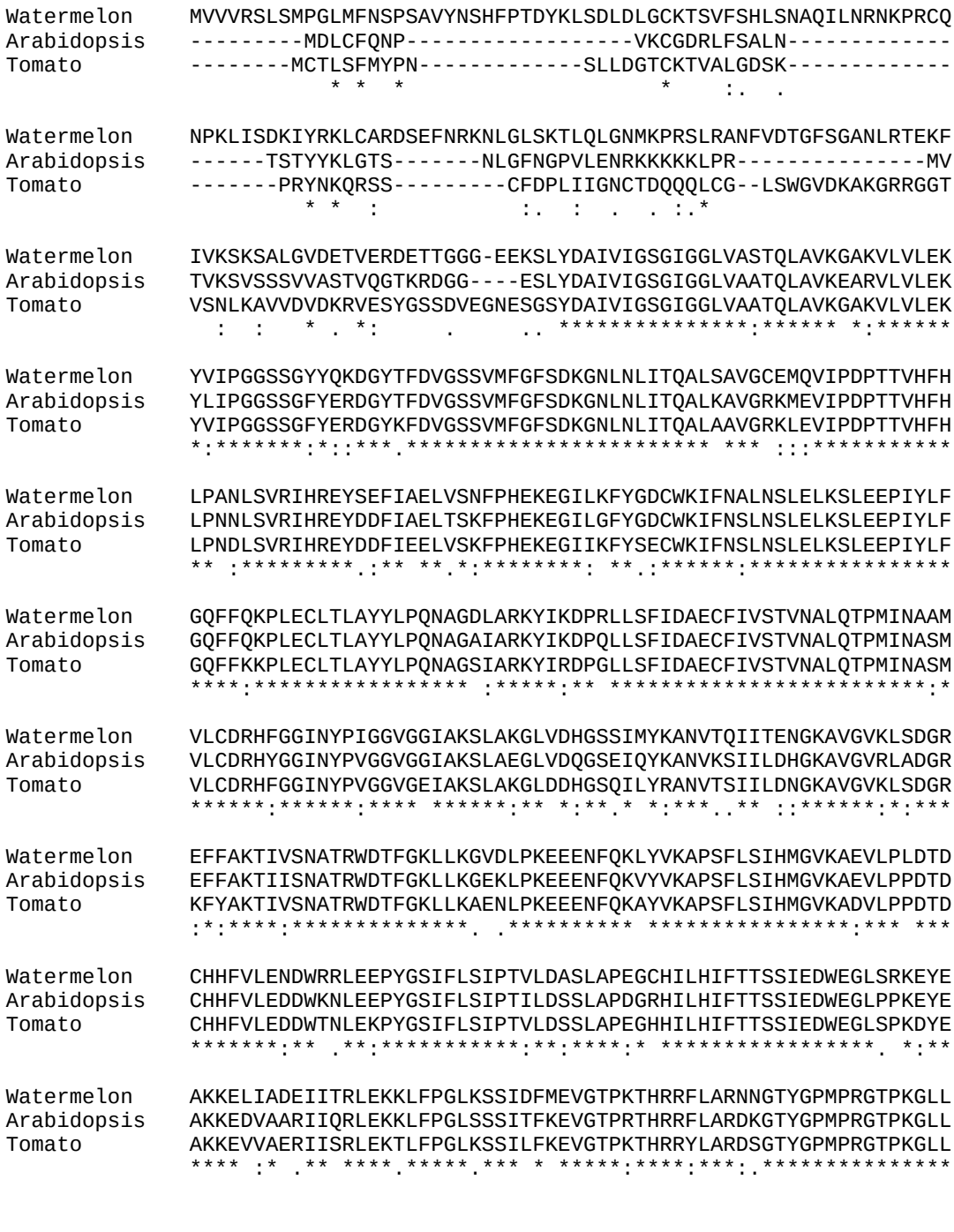

Fig. 12. Alignment of the deduced amino acid sequences of watermelon *CRTISO* with *CRTISO* genes of other species. '*': amino acids in the column are identical, ':': conserved substitution, '.': semi-conserved substitution.

```
Watermelon     GMPFNTTGIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRVAADIGLEKKSPILDAALLRLL 659
Arabidopsis    GMPFNTTAIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRVAADIGLEKKSRVLDVGLLGLL 588
Tomato         GMPFNTTAIDGLYCVGDSCFPGQGVIAVAFSGVMCAHRVAADLGFEKKSDVLDSALLRLL 608
               *******.**********************************:*:**** :** .** **

Watermelon     GWLRTLA 666
Arabidopsis    GWLRTLA 595
Tomato         GWLRTLA 615
               *******
```

Fig. 12. Continued.

Lycopene cyclase (LCY)

Two types of lycopene cyclases are known; *LCYB* catalyzes two β-ring formations of β-carotene by cyclization of lycopene and *LCYE* catalyzes a α-ring formation. It has been reported that two homologous genes, *LCYB* (*or Crt-L*) and chromoplast-specific lycopene β-cyclase (*CYCB*) were identified in tomato (Ronen et al., 2000; Hirschberg, 2001). Tomato *CYCB* shares approximately 50% amino acid sequence identity with tomato *LCYB*, but it shows 85% identity with pepper capsanthin-capsorbin synthase (*CCS*). In comparison of identity with other species, watermelon *LCYB* had 80% identity with tomato, pepper, and tobacco (Fig. 13 and Table 10).

In watermelon, only the *LCYB* gene was isolated through degenerate PCR and RACE. The *CYCB* homolog was not detected in watermelon. If the *CYCB* homolog exists in watermelon, it should have been detected by degenerate PCR since they share identity.

```
Watermelon     MDTLLKINNKYGFLQPLHGVSEKVS---GVRSTKFQSQEFGFGHRKGRLKWR-KGGCLNV 56
Citrus         MDTLLKTHNKLEFLPQVHGALEKSS---SLSSLKIQNQELKFGLKKSRQKRN-RSCFIKA 56
Tomato         MDTLLKTPNNLEFLNPHHGFAVKAS---TFRSEKHHN----FGSRKFCETLG-RSVCVKG 52
Pepper         MDTLLRTPNNLEFL---HGFGVKVS---AFSSVKSQK----FGAKKFCEGLGSRSVCVKA 50
Tobacco        MDTLLKTPNKLEFLHPVHGFSVKAS---SFNSVKPHK----FGSRKICENWG-KGVCVKA 52
Adonis         MDTLLRTHNKLELLPTLHGFAEKQH---LVSTSKLQNQVFRIASRNIHPCRN---GTVKA 54
Marigold       MDTFLRTYNSFEFVHPSNKFAGNLNNLNQLNQSKSQFQDFRFGPKKSQFKLG-QKYCVKA 59
Arabidopsis    MDTLLKTPNKLDFFIPQFHGFERLC------SNNPYHSRVRLGVKKRAIKIV---SSVVS 51
Sandersonia    MDTLLKTHSRLELLHLQSQRHILTS------TAKPSS---LISAKKPHLMRC----SVVA 47
Daffodil       MDTLLRTHNRLELLYPLHELAKRHFLS---PSPNPQNPNFKFFSRKPYQKKC-RNGYIGV 56
               ***:*:  .   :.                   :       :  ::           :

Watermelon     RSSSLLELVPETKKENLEVELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCAI 116
Citrus         SSSALLELVPETKKENLEFELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCSI 116
Tomato         SSSALLELVPETKKENLDFELPMYDPSKGVVVDLAVVGGGPAGLAVAQQVSEAGLSVCSI 112
Pepper         SSSALLELVPETKKENLDFELPMYDPSKGVVVDLAVVGGGPAGLAVAQQVSEAGLSVCSI 110
Tobacco        KSSALLELVPETKKENLDFELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVVSI 112
Adonis         RGSALLELVPETKKENLEFDLPAYDPSRGIVVDLAVVGGGPAGLAIAQQVSEAGLLVCSI 114
Marigold       SSSALLELVPEIKKENLDFDLPMYDPSRNVVVDLVVVGGGPSGLAVAQQVSEAGLTVCSI 119
Arabidopsis    GSAALLDLVPETKKENLDFELPLYDTSKSQVVDLAIVGGGPAGLAVAQQVSEAGLSVCSI 111
Sandersonia    KSSALLELVPEVKKENLDMELPLYDPSKSLTVDLAVVGGGPAGLAVAQQVSQSGLSVCSI 107
Daffodil       SSNQLLDLVPEIKKEHLEFDLPLYDPSKALTLDLAVVGGGPLARSCSTSLG-GGLSVVSI 115
                .  **:**** ***:*:.:** **.*:  .:**.:***** . : : .:. .** * :*

Watermelon     DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVFTNEQSTKDLARPYARVNRKQLK 176
Citrus         DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVHIDDNTKKDLDRPYGRVNRKLLK 176
Tomato         DPNPKLIWPNNYGVWVDEFEAMDLLDCLDATWSGAAVYIDDNTAKDLHRPYGRVNRKQLK 172
Pepper         DPNPKLIWPNNYGVWVDEFEAMDLLDCLDATWSGAAVYIDDKTTKDLNRPYGRVNRKQLK 170
Tobacco        DPSPKLIWPNNYGVWVDEFEAMDLLDCLDATWSGTVVYIDDNTTKDLDRPYGRVNRKQLK 172
Adonis         DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVYTDDNSKKYLDRPYGRVNRKQLK 174
Marigold       DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSSAVVYIDEKSTKSLNRPYARVNRKQLK 179
Arabidopsis    DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVYVDEGVKKDLSRPYGRVNRKQLK 171
Sandersonia    DPSPKLIWPNNYGVWVDEFEAMDLLDCLDASWPGAVVYLDESTKKLLDRPYARVNRKQLK 167
Daffodil       DPNPKLIWPNNYGVWVDEFEDMDLLDCLDATWSGAIVYVDDRSTKNLSRPYARVNRKNLK 175
               **.***************** ********::*..: *. ::   * * ***.***** **

Watermelon     SKMLQKCISNGVKFHEAKVIKVIHEEFKSLLICNDGVTIQAAIVLDATGVSRCLVQYDKP 236
Citrus         SKMLQKCITNGVKFHQAKVIKVIHEESKSLLICNDGVTIQAAVVLDATGFSRCLVQYDKP 236
Tomato         SKMMQKCIMNGVKFHQAKVIKVIHEESKSMLICNDGITIQATVVLDATGFSRSLVQYDKP 232
Pepper         SKMMQKCILNGVKFHQAKVIKVIHEESKSMLICNDGITIQATVVLDATGFSRSLVQYDKP 230
Tobacco        SKMMQKCILNGVKFHHAKVIKVIHEEAKSMLICNDGVTIQATVVLDATGFSRCLVQYDKP 232
Adonis         SKMLQKCVTNGVKFHQAKVIKVIHEESKSLLICNDGITINATVVLDATGFSRCLVQYDKP 234
Marigold       TKMLQKCIANGVKFHQAKVIKVIHEELKSLLICNDGVTIQATLVLDATGFSRSLVQYDKP 239
Arabidopsis    SKMLQKCITNGVKFHQSKVTNVVHEEANSTVVCSDGVKIQASVVLDATGFSRCLVQYDKP 231
Sandersonia    SKMMHKCVANGVRFHQAKVVKVIHEEAKSNIICNDGVTIQARVVLDATGFSRCLVQYDKP 227
Daffodil       SKMMKKCVSNGVRFHQATVVKAMHEEEKSYLICSDGVTIDARVVLDATGFSRCLVQYDKP 235
               :**::**: ***:**.::* :.:*** :* ::*.**:.*:* :******.**.*******
```

Fig. 13. Alignment of the deduced amino acid sequences of watermelon *LCYB* with *LCYB* genes of other species. '*': amino acids in the column are identical, ':': conserved substitution, '.': semi-conserved substitution.

```
Watermelon     YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLNNNMILKERNSKIPTFLYAMPFSSN 296
Citrus         YNPGYQVAYGILAEVEEHPFDLDKMVFMDWRDSHLNNNSELKEANSKIPTFLYAMPFSSN 296
Tomato         YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLKNNTDLKERNSRIPTFLYAMPFSSN 292
Pepper         YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLKNNVELKERNSRIPTFLYAMPFSSN 290
Tobacco        YKPGYQVAYGILAEVEEHPFDTSKMVLMDWRDSHLGNNMELKERNRKVPTFLYAMPFSSN 292
Adonis         YNPGYQVAYGIMAEVEEHPFDLDKMLFMDWRDSHLNEKLELKDKNRKIPTFLYAMPFSST 294
Marigold       YNPGYQVAYGILAEVEEHPFDVDKMLFMDWRDSHLDQNLEIKARNSRIPTFLYAMPFSST 299
Arabidopsis    YNPGYQVAYGIVAEVDGHPFDVDKMVFMDWRDKHLDSYPELKERNSKIPTFLYAMPFSSN 291
Sandersonia    YNPGYQVAYGILAQVEEHPFDLDKMVFMDWRDMHLRDGKDMKDRNRRIPTFLYAMPFSSE 287
Daffodil       YNPGYQVAYGILAEVEEHPFDVDKMVFMDWRDSHLNGKAELNERNAKIPTFLYAMPFSSN 295
               *:**********:*:*: **** .**::***** **     ::  * ::***********

Watermelon     RIFLEETSLVARPGLQMSDIQERMEVRLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVV 356
Citrus         RIFLEETSLVARPGVPMKDIQERMVARLKHLGIKVRSIEEDEHCVIPMGGPLPVLPQRVV 356
Tomato         RIFLEETSLVARPGLRIDDIQERMVARLNHLGIKVKSIEEDEHCLIPMGGPLPVLPQRVV 352
Pepper         RIFLEETSLVARPGLGMDDIQERMVARLSHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVV 350
Tobacco        KIFLEETSLVARPGLRMDDIQERMVARLNHLGIKVKSIEEDEHCVIPMGGSLPVIPQRVV 352
Adonis         KIFLEETSLVARPGLRFEDIQERMVARLKHLGIKVKSIEEDERCVIPMGGPLPVLPQRVV 354
Marigold       RIFLEETSLVARPGLKMEDIQERMAYRLKHLGIKVKSIEEDERCVIPMGGPLPVLPQRVL 359
Arabidopsis    RIFLEETSLVARPGLRMEDIQERMAARLKHLGINVKRIEEDERCVIPMGGPLPVLPQRVV 351
Sandersonia    RIFLEETSLVARPGLAMEDIQERMVARLRHLGIRVKSIXXDERCIIPMGGPLPVLPQRVV 347
Daffodil       RIFLEETSLVARPGLKMEDIQERMVARLNHLGIRIKSIEEDERCVIPMGGPLPVIPQRVV 355
               :*************: :.******  ** ****.:: *  **:*:*****.***:****:

Watermelon     GIGGTAGMVHPSTGYMVARTLAAAPIVASAIVRCLGSDG----RFRGDAISSEVWKDLWP 412
Citrus         GIGGTAGMVHPSTGYMVARTLAAAPIVANAIVRSLSSDR----SISGHKLSAEVWKDLWP 412
Tomato         GIGGTAGMVHPSTGYMVARTLAAAPVVANAIIQYLGSER----SHSGNELSTAVWKDLWP 408
Pepper         GIGGTAGMVHPSTGYMVARTLAAAPVVANAIIQYLSSER----SHSGDELSAAVWKDLWP 406
Tobacco        GTGGTAGLVHPSTGYMVARTLAAAPVVANAIIHYLGSEK----DLLGNELSAAVWKDLWP 408
Adonis         GIGGTAGMVHPSTGYMVARTLAAAPVVAKSIVQYLGSDR----SLSGNELSAEVWKDLWP 410
Marigold       GIGGTAGMVHPSTGYMVARTLAAAPIVAKSIIRYLNNEKSMVADVTGDDLAAGIWRELWP 419
Arabidopsis    GIGGTAGMVHPSTGYMVARTLAAAPIVANAIVRYLGSPSS--NSLRGDQLSAEVWRDLWP 409
Sandersonia    GIGGTAGMVHPSTGYMVARTLAAAPIVAGSIVRYLSSNR----GISGDGISARVWKDLWP 403
Daffodil       GIGGTAGMVHPSTGYMVARTLAAAPIVANSIVQYLVSDS----GLSGNDLSADVWKDLWP 411
               * *****:*****************:** :*:: * .         *. ::: :*::***

Watermelon     IERRRQREFFCFGMDILLKLDLKGTRRFFDAFFDLEPRYWHGFLSSRLFLPELLLFGLSL 472
Citrus         IERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLFLPELLVFGLSL 472
Tomato         IERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLFLPELIVFGLSL 468
Pepper         IERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLFLPELIVFGLSL 466
Tobacco        IERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLYLPELIFFGLSL 468
Adonis         IERRRQREFFCFGMDILLKLDLQGTRRFFDAFFDLEPHYWHGFLSSRLFLPELLFFGLSL 470
Marigold       IERRRQREFFCFGMDILLKLDLEGTRRFFDAFFDLEPRYWHGFLSSRLFLPELVTFGLSL 479
Arabidopsis    IERRRQREFFCFGMDILLKLDLDATRRFFDAFFDLQPHYWHGFLSSRLFLPELLVFGLSL 469
Sandersonia    IERRRQREFFCFGMDILLKLDLQGTRRFFDAFFDLEPHYWHGFLSSRLFLPELVFFGLSL 463
Daffodil       IERRRQREFFCFGMDILLKLDLEGTRRFFDAFFDLEPRYWHGFLSSRLFLPELVPFGLSL 471
               ********************** .***********:*:**********:****: *****
```

Fig. 13. Continued.

```
Watermelon     FSHASNASRLEIMAKGTPSLVNMIGNLVKDRD 504
Citrus         FSHASNTSRLEIMAKGTLPLVNMINNLVQDTD 504
Tomato         FSHASNTSRFEIMTKGTVPLVNMINNLLQDKE 500
Pepper         FSHASNTSRLEIMTKGTLPLVHMINNLLQDKE 498
Tobacco        FSRASNTSRIEIMTKGTLPLVNMINNLLQDTE 500
Adonis         FSHASNASRIEIMAKGTVPLVNMMNNLIQDTD 502
Marigold       FGHASNTCRVEIMAKGTLPLATMIGNLVRDRE 511
Arabidopsis    FSHASNTSRLEIMTKGTVPLAKMINNLVQDRD 501
Sandersonia    FGHASNTCRLEIMAKGSLPLVHMVNNLLQDRD 495
Daffodil       FSHASNTCKLEIMAKGTLPLVNMINNLVQDRD 503
               *.:***:.:.***:**: .*. *:.**::* :
```

Fig. 13. Continued.

Like other species, intron interference in the coding region was not detected. While cloning of *LCYB*, 229 bp of leader intron was identified in 541 bp 5'UTR and unspliced mRNA with leader intron existed dominantly when concentrations of unspliced and spliced mRNA were compared (Fig 14). Although it is unknown so far if the leader intron affects the regulation, this is the first reported leader intron in *LCYB* genes cloned so far.

Studies by Ronen et al. (1999; 2000) reported that color mutants of *Delta*, *Beta*, and *og* in tomato resulted from mutations of *CYCB* gene. This suggests that color determination between canary yellow and red watermelon mutants may also be due to a mutation of *LCYB* gene. Phylogenetic relationship with *LCYB* genes in other species indicates that watermelon *LCYB* gene is closely related to *LCYB* genes in tomato, pepper, and citrus rather than *CYCB* gene which causes mutation in tomato (Fig. 15).

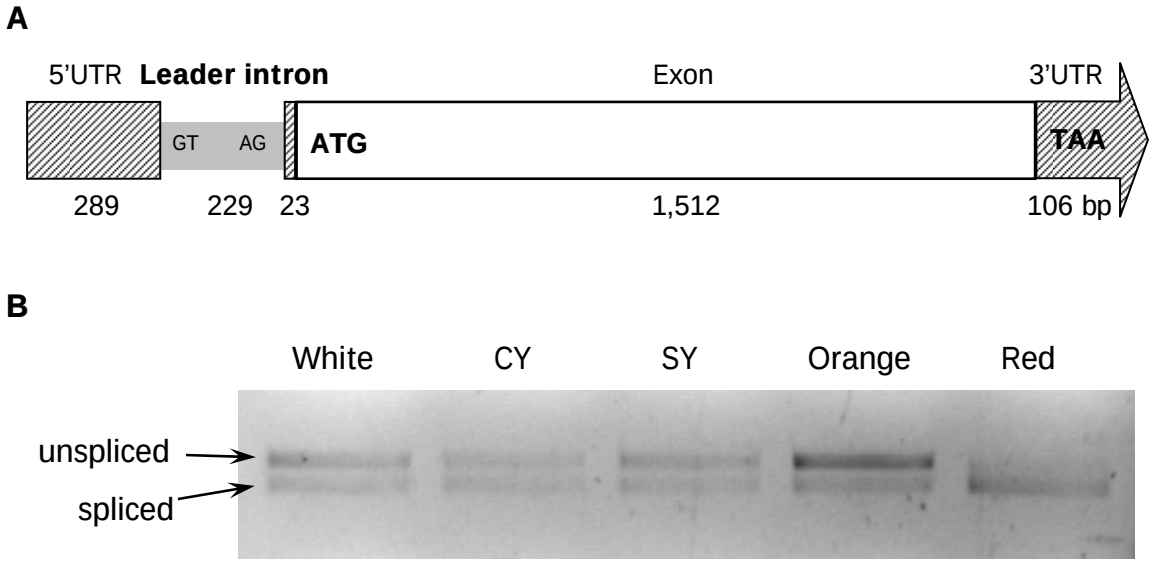

Fig. 14. Gene structure of *LCYB* gene in watermelon. A. A gray box represents leader intron located in 5'-untranslated region (UTR). Shaded and white box represent a UTR and exon. B. Unspliced and spliced leader introns were amplified by RT-PCR across different colored fleshes. CY; canary yellow, SY; salmon yellow.

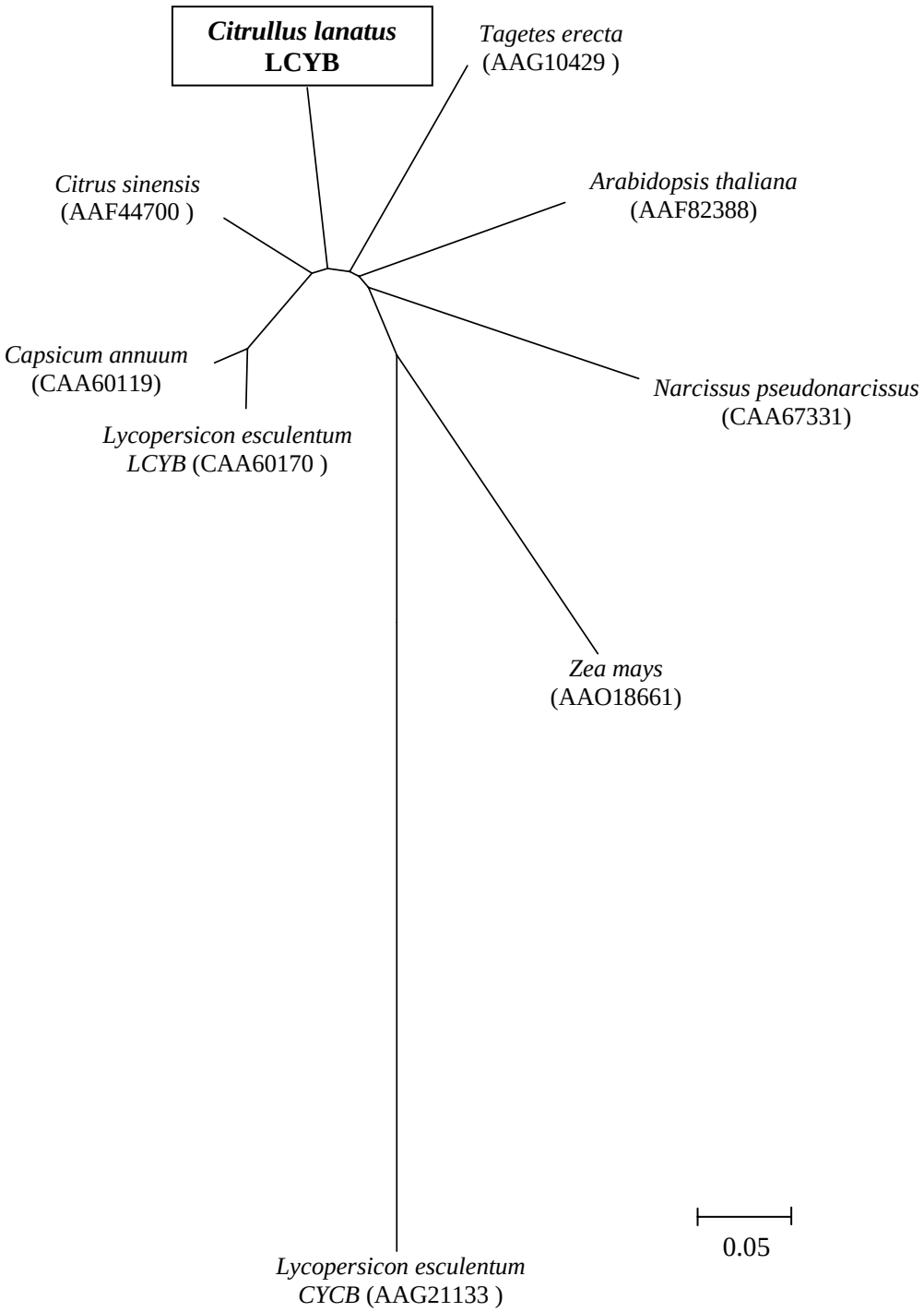

Fig. 15. Phylogenetic analysis of *LCYB* based on amino acid sequences. The numbers in the brackets are GenBank accession numbers.

β-carotene hydroxylase (CHYB) and Zeaxanthin epoxidase (ZEP)

CHYB is involved in the hydroxylation of β-carotene. The size of full-length cDNA of *CHYB* from watermelon was 1,139 bp including 178 bp 5'UTR, 948 bp ORF, and 13 bp 3'UTR (Fig. 16). The protein consisted of 316 amino acids. The identity comparison shows over 70% identity with citrus and *Arabidopsis* and 66% with tomato (Table 10).

ZEP converts zeaxanthin to violaxanthin through antheraxanthin. The *ZEP* clone from watermelon consists of 2,394 bp including 107 bp 5'UTR, 1,995 bp ORF, and 292 bp 3'UTR showing 60-70% identity with other species (Fig. 17 and Table 10).

```
Watermelon     --MAAGLSAALVPKPLHLFLT--SSHLSPKPRTPFLFPPPVFRNSRFQWKMRRKT-LFTV 55
Brassica       --MAAGLSTTVTFNPLHRSFS--SSSS-VRLHHPRSLTG--LPSS---LRFRG----FSV 46
Citrus         --MAVGLLAAIVPKPFCLLTTKLQPSSLLTTKPAPLYAPLGTHHGFFNGKNRRKLNSFTV 58
Arabidopsis    --MAAGLSTAVTFKPLHRSFS--SSSTDFRLRLPKSLSG--FSPS---LRFKR----FSV 47
Tomato         MAAAARISASSTSRTFYFRHSPFLGPKPTSTTSHVSPISPFSLNLGPILRSRRKP-SFTV 59
Grape          --MATGISASLNSMSCRLGRNSFTATGPSSVISLSSFLTPVTHLKGNIFPLQRRR-SLKV 57
Pepper         --MAAEISISASSRAICLQRNPFPAPKYFATAPPLLFFSPLTCNLDAILRSRRKP-RLAA 57
Marigold       --MAAAIAVPCSSRPFGLGRMRLLGHKPTTITCHFPFSFSIKS-FTPIVRGRR----CTV 53
Daffodil       --MAVWISAAPPALAIS------SAPRIRRVILFSPLHS--RQIGWPPIRNRRKRSKSTV 50
                  *. :  .    .                                       :       .

Watermelon     CVLVEDQNSSGEVEN-LSDE---GSPIV----IPQIPSPHVSERLARKKSERFTYLVAAV 107
Brassica       CYVVEEQRQSSPVDNDERPE---RTNVI----DPELLALRLAEKLERKKSERFTYLIAAV 99
Citrus         CFVLEEKKQSTQIETFTEEE---EEESG----TQISTAARVAEKLARKRSERFTYLVAAV 111
Arabidopsis    CYVVEERRQNSPIENDERPESTSSTNAI----DAEYLALRLAEKLERKKSERSTYLIAAM 103
Tomato         CFVLEDEKLKPQFDDEAEDFEKK--------IEEQILATRLAEKLARKKSERFTYLVAAI 111
Grape          CLVLEKEIEDGIEIEDDSPE----------------SSNRASERLARKKAERYTYLVAAM 101
Pepper         CFVLKDDKLYTAQSGKQSDTEAIGDEIEVETNEEKSLAVRLAEKFARKKSERFTYLVAAV 117
Marigold       CFVAGGDSNSNSNNNSDSNSNNPGLDLN---PAVMNRNRLVEEKMERKKSERFTYLVAAI 110
Daffodil       FFASDVDVGKSNGGDGIVDKIERLKKQE----QLMISKSRTTERMERKRSERTTYLIAAM 106
                                                  *:: **::** ***:**:
```

Fig. 16. Alignment of the deduced amino acid sequences of watermelon *CHYB* with *CHYB* genes of other species. '*': amino acids in the column are identical, ':': conserved substitution, '.': semi-conserved substitution.

```
Watermelon     MSSFGITSMAVMAVYYRFYWQMEGGEIPFSEMFGTFSLSVGAAVGMEFWARWAHRALWHS 167
Brassica       MSSFGITSMAVMAVYYRFSWQMEGGVIPMSEMFGTFALSVGAAVGMEFWARWAHRALWHA 159
Citrus         MSSFGITSMAVMAVYYRFWWQMEGGEVPLAEMFGTFALSVGAAVGMEFWARWAHKALWHA 171
Arabidopsis    LSSFGITSMAVMAVYYRFSWQMEGGEISMLEMFGTFALSVGAAVGMEFWARWAHRALWHA 163
Tomato         MSSFGITSMAVMAVYYRFSWQMEGGEVPVTEMLGTFALSVGAAVGMEFWARWAHKALWHA 171
Grape          MSSLGITSMAIVAVYYRLSWQMEGGEIPVLEMLGTFALSVGAAVGMEFWARWAHKALWHA 161
Pepper         MSSLGITSMAVISVYYRFSWQMEGGEMPFSEMFCTFALAFGAAIGMEYWARWAHRALWHA 177
Marigold       MSTFGITSMAVMAVYYRFSWQMEGGEIPYVEMFGTFALSVGAAVGMEYWARWAHEALWHA 170
Daffodil       MSSLGITSMAIVSVYYRFAWQMEEGEIPVTEMLGTFALSVGAAVGMEFWARWAHRALWHA 166
               :*::******::::****: **** * :.  **: **:*:.***:***:******.****:

Watermelon     SLWHMHESHHKPREGPFELNDVFAIVNAVPAIALLSYGFFHKGLVPGLCFGAGLGITVFG 227
Brassica       SLWNMHESHHKPREGPFELNDVFAIINAVPAIGLLSYGFFNKGLVPGLCFGAGLGITVFG 219
Citrus         SLWHMHESHHRPREGPFELNDVFAIINAVPAIALLSVGFFHKGLVPGLCFGAGLGITVFG 231
Arabidopsis    SLWNMHESHHKPREGPFELNDVFAIVNAGPAIGLLSYGFFNKGLVPGLCFGAGLGITVFG 223
Tomato         SLWHMHESHHKPREGPFELNDVFAITNAVPAIALLNYGFFHKGLIAGLCFGAGLGITVFG 231
Grape          SLWHMHESHHRPREGPFELNDVFAIINAVPAISLLSYGLFNKGLVPGLCFGAGLGITVFG 221
Pepper         SLWHMHESHHRPREGPFELNDIFAIINAVPAIAFFSFGFNHKGLIPGICFGAGLGITVFG 237
Marigold       SLWHMHESHHKPREGPFELNDVFAITNAVPAIALLSYGFFHKGIIPGLCFGAGLGITVFG 230
Daffodil       SLWHMHESHHKPRDGPFELNDVFAVINAVPAISLLYYGFFNRGLVPGLCFGAGLGITLYG 226
               ***:******:**:*******:**: ** ***.::  *: ::*::.*:*********::*

Watermelon     MAYMFVHDGLVHKRFPVGPIANVPYFRKVAAAHQLHHSDKFNGVPYGLFLGPKELEEVGG 287
Brassica       IAYMFVHDGLVHKRFPVGPIADVPYLRKVAAAHQLHHTDKFDGVPYGLFLGPKELEEVGG 279
Citrus         MAYMFVHDGLVHKRFPVGPIAGVPYFRRVAAAHQLHHSDKFHGVPYGLFLGPKELEEVEG 291
Arabidopsis    IAYMFVHDGLVHKRFPVGPIADVPYLRKVAAAHQLHHTDKFNGVPYGLFLGPKELEEVGG 283
Tomato         MAYMFVHDGLVHKRFPVGPVANVPYLRKVAAAHSLHHSEKFNGVPYGLFFGPKELEEVGG 291
Grape          MAYMFVHDGLVHRRFPVGPIANVPYLRKVASAHQLHHSDKFNGVPYGLFLGPMELEEVGG 281
Pepper         MAYMFVHDGLVHKRFPVGPIAKVPYFQRVAAAHQLHHSDKFDGVPYGLFLGPKELEEVGV 297
Marigold       MAYMFVHDGLVHRRFQVGPIANVPYLRRVAAAHQLHHTEKFNGVPYGLFLGPKELEEVGG 290
Daffodil       MAYMFVHDGLVHRRFPVGPIADVPYFRRVAAAHRIHHTEKFNGVPYGLFLGPKELEEVGG 286
               :***********:** ***:* ***:::**:** :**::**.*******:** *****

Watermelon     LEELEKEINRRIKLTAPKSNHGSSSTNIM 316
Brassica       DEELDKEISRRIKLYKKSSSS-------- 300
Citrus         LEELEKEISKRIKSYNRVPK--------- 311
Arabidopsis    NEELDKEISRRIKSYKKASGSGSSSSS-- 310
Tomato         TEELEKEVIRRTRLSKGS----------- 309
Grape          MEELEKEISRRIKSSDSS----------- 299
Pepper         IEELEKEVNRRIKSLKRL----------- 315
Marigold       TEELDKEIQRRIKLYNNTK---------- 309
Daffodil       EEELEKLIKRRIEINSRSLDVK------- 308
                ***:* : :* .
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Fig. 16. Continued.

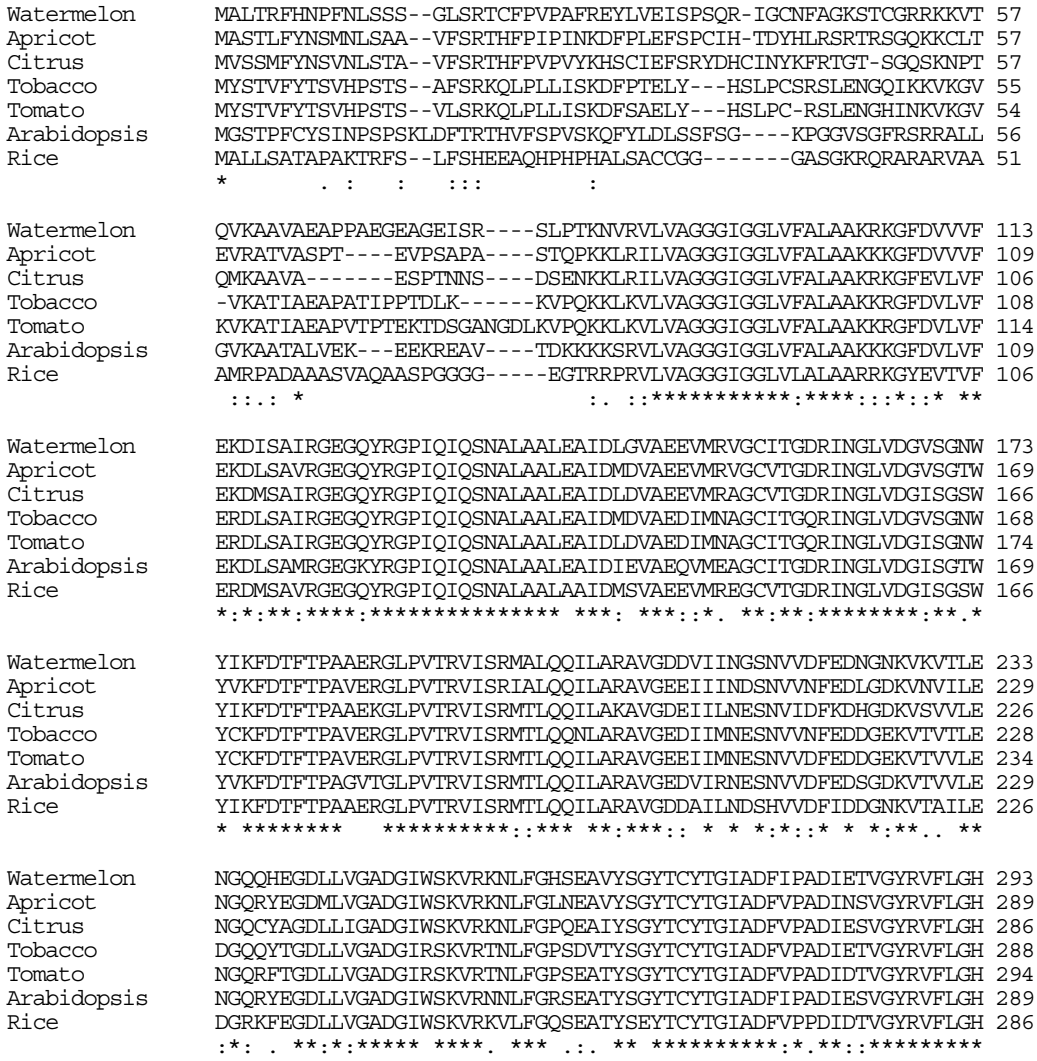

Fig. 17. Alignment of the deduced amino acid sequences of watermelon *ZEP* with *ZEP* genes of other species. '*': amino acids in the column are identical, ':': conserved substitution, '.': semi-conserved substitution.

```
Watermelon      KQYFVSSDVGAGKMQWYAFHKEPPGGTDPPNSKKERLFKIFEGWCDNVIDLIHATDEDSV 353
Apricot         KQYFVSSDVGGGKMQWYAFHKESPGGVDSPNGKKERLLKIFEGWCDNVIDLLLATEEDAI 349
Citrus          KQYFVSSDVGAGKMQWYAFHKEPAGGVDDPEGKKERLLKIFEGWCDNVVDLILATDEEAI 346
Tobacco         KQYFVSSDVGGGKMQWYAFHNEPAGGVDDPNGKKARLLKIFEGWCDNVIDLLVATDEDAI 348
Tomato          KQYFVSSDVGGGKMQWYAFYNEPAGGADAPNGKKERLLKIFGGWCDNVIDLLVATDEDAI 354
Arabidopsis     KQYFVSSDVGGGKMQWYAFHEEPAGGADAPNGMKKRLFEIFDGWCDNVLDLLHATEEEAI 349
Rice            KQYFVSSDVGAGKMQWYAFHKEPAGGTDPENGKNKRLLEIFNGWCDNVVDLINATDEEAI 346
                **********.********::*..**.*  :. : **::** ******:**: **:*:::

Watermelon      LRRDIYDRTPIFTWSLGRVTLLGDSVHAMQPNMGQGGCMAIEDGYQLALELDKAWNKSVV 413
Apricot         LRRDIYDRTPILTWGKGHVTLLGDSVHAMQPNMGQGGCMAIEDGYQLALELDKAWKKSSE 409
Citrus          LRRDIYDRTPIFTWGRGRVTLLGDSVHAMQPNLGQGGCMAIEDGYQLAVELEKACKKSNE 406
Tobacco         LRRDIYDRPPTFSWGKGRVTLLGDSVHAMQPNLGQGGCMAIEDSYQLALELDKALSRSAE 408
Tomato          LRRDIYDRPPTFSWGRGRVTLLGDSVHAMQPNLGQGGCMAIEDSYQLALELEKACSRSAE 414
Arabidopsis     LRRDIYDRSPGFTWGKGRVTLLGDSIHAMQPNMGQGGCMAIEDSFQLALELDEAWKQSVE 409
Rice            LRRDIYDRPPTFNWGKGRVTLLGDSVHAMQPNLGQGGCMAIEDGYQLAVELEKSWQESAK 406
                ********.* :.*. *:*******:******:**********.:***:**::: ..*

Watermelon      SGSPIDIVSSLKSYESSRRIRVAVIHGMARMAALMASTYKAYLGVGLGPLSFLTQFRIPH 473
Apricot         TGTPVDVASSLRSYENSRRLRVAIIHGMARMAALMASTYKAYLGVGLGPLSFLTKFRIPH 469
Citrus          SKTPIDIVSALKSYERARRLRVAVIHGLARSAAVMASTYKAYLGVGLGPLSFLTKFRIPH 466
Tobacco         SGTPVDIISSLRSYESSRKLRVGVIHGLARMAAIMASTYKAYLGVGLGPLSFLTKFRIPH 468
Tomato          FGSPVDIISSLRSYESARKLRVGVIHGLARMAAIMASTYKAYLGVGLGPLSFLTQYRIPH 474
Arabidopsis     TTTPVDVVSSLKRYEESRRLRVAIIHAMARMAAIMASTYKAYLGVGLGPLSFLTKFRVPH 469
Rice            SGTPMDIVSSLRRYEKERILRVSVIHGLARMAAIMATTYRPYLGVGLGPLSFLTKLRIPH 466
                  :*:*: *:*: **  * :**.:**.:** **:**:**:.*************: *:**

Watermelon      PGTFGGRFFIDLAMPLMLNWVLGGNSSKLEGRPPACRLSDKANDQLRQWFEDDDALERAI 533
Apricot         PGRVGGRVFIDKAMPLMLSWVLGGNSSKLEGRSPSCRLSDKASDQLRNWFEDDDALERAI 529
Citrus          PGRVGGRFFIDLAMPLMLSWVLGGNSSKLEGRSPCCKLSDKASDNLRTWFRDDDALERAM 526
Tobacco         PGRVGGRFFIDLGMPLMLSWVLGGNGEKLEGRIQHCRLSEKANDQLRNWFEDDDALERAT 528
Tomato          PGRVGGRVFIDLGMPLMLSWVLGGNGDKLEGRIKHCRLSEKANDQLRKWFEDDDALERAT 534
Arabidopsis     PGRVGGRFFVDIAMPSMLDWVLGGNSEKLQGRPPSCRLTDKADDRLREWFEDDDALERTI 529
Rice            PGRVGGRFFIKYGMPLMLSWVLGGNSTKLEGRPLSCRLSDKANDQLRRWFEDDDALEQAM 526
                ** .***.*:. .** **.******. **:**   *:*::**.*.** **.******::

Watermelon      NGDWFLLPQGGEASVSHPICLP-RDENQPCLIGSVEQEVDSGLSIAIPLPQVSEKHARIH 592
Apricot         DGEWYLIPCGQDNDASQLICLN-RDEKNPCIIGSAPHGDVSGISIAIPKPQVSEMHARIS 588
Citrus          NGEWFLVPSGSENVVSQPIYLSGSHENEPYLIGSESHEDFPRTSIVIPSAQVSKMHARIS 586
Tobacco         DAEWLLLPAGNSNAALETLVLS-RDENMPCNIGSVSHANIPGKSVVIPLPQVSEMHARIS 587
Tomato          DAEWLLLPAGNGSSGLEAIVLS-RDEDVPCTVGSISHTNIPGKSIVLPLPQVSEMHARIS 593
Arabidopsis     KGEWYLIPHGDDCCVSETLCLT-KDEDQPCIVGSEPDQDFPGMRIVIPSSQVSKMHARVI 588
Rice            GGEWYLLPTSSGD--SQPIRLI-RDEKKSLSIGSRSDPSNSTASLALPLPQISENHATIT 583
                 .:* *:* .      . : *   .*. .  :**  .   .   :.:* .*:*: ** :

Watermelon      YKDGAFFLTDLRSEHGTWLSDHEGRRYRVPPNFPVHFHQFNIIELGSDKKAAFRVKVIRS 652
Apricot         YKDGAFYLTDLRSEHGTWIADIEGKRYRVPPNFPARFRPSDAIEIGS-QKVAFRVKVMKS 647
Citrus          YKDGAFYLIDLQSEHGTYVTDNEGRRYRVSSNFPARFRPSDTIEFGSDKKAIFRVKVIGT 646
Tobacco         YKGGAFFVTDLRSEHGTWITDNEGRRYRASPNFPTRFHPSDIIEFGSDKKAAFRVKVMKF 647
Tomato          CKDGAFFVTDLRSEHGTWVTDNEGRRYRTSPNFPTRFHPSDVIEFGSDK-AAFRVKAMKF 652
Arabidopsis     YKDGAFFLMDLRSEHGTYVTDNEGRRYRATPNFPARFRSSDIIEFGSDKKAAFRVKVIRK 648
Rice            CKNKAFYVTDNGSEHGTWITDNEGRRYRRTSELPCPFPS-----LGCH------------ 626
                 *. **:: *  *****:::* **:*** ..::*  *       :*.
```

Fig. 17. Continued.

```
Watermelon      SVEYDREKVKMNS------ 665
Apricot         SPG--SVEKEG--ILQAA- 661
Citrus          PPNNNSERKEAGEILQ--- 662
Tobacco         PPKTAAK-EERQAVGAA-- 663
Tomato          PLKTSERKEEREAVEAA-- 669
Arabidopsis     TPKSTRKNESNNDKLLQTA 667
Rice            ------------------
```

Fig. 17. Continued.

CHAPTER VI

DIFFERENTIAL EXPRESSION OF CLONED GENES IN THE CAROTENOID BIOSYNTHETIC PATHWAY

Materials and Methods

Plant materials

Different colored fruit samples were collected from materials used in the inheritance study: white (PI 595203), canary yellow (PI 165002), salmon yellow (‘Luscious Golden’), orange (‘Orange Flesh Tendersweet’), and red (‘Charleston Gray’). These watermelons were selected based on vivid and distinct color. These were used to examine differential expression of genes encoding enzymes in the carotenoid biosynthetic pathway across different colored fleshes at the transcriptional level. Ovary, petal, leaf, and root tissues of ‘Black Diamond’ were used to examine differential expression of genes encoding enzymes in the carotenoid biosynthetic pathway across different tissues at the transcriptional level. The 18S rRNA gene was used as a control for cDNA synthesis.

RNA isolation and cDNA synthesis

To increase RNA yield and eliminate polysaccharides, water from all different colored fleshes was squeezed out using a sterilized cloth. For RNA isolation, 100 mg of flesh and ovary tissues were ground in liquid nitrogen. Then, total RNA was extracted

using RNeasy Plant Mini Kit (Qiagen; Valencia, CA). RNase-free DNase (Qiagen) was used for additional digestion of DNA during RNA purification. Absorbance was measured at 260 nm and A_{260}/A_{280} ratio was recorded with spectrophotometer to calculate RNA yield and check the purity. Pure RNA should have 1.9-2.1 for A_{260}/A_{280} ratio. cDNA was synthesized from 1 μg of RNA of each flesh color and each tissue using a RT-PCR Kit (Advantage RT-for-PCR Kit; BD Biosciences; Palo Alto, CA).

Reverse transcription-PCR (RT-PCR)

After full-length cDNA sequences were obtained during cloning, watermelon specific primers for RT-PCR were designed to have a T_m above 70 ^oC and to flank at least one intron to see if the PCR product was amplified from the remaining genomic DNA, except for *LCYB* because it does not have any intron interference.

Each a PCR reaction mixture was prepared with 1-3 μL template, 5 μL 10× PCR buffer, 1 μL dNTP (10mM), 1 μL forward gene specific primer (10μM), 1 μL reverse gene specific primer (10 μM), and 1 μL Taq polymerase mix (50× Advantage 2 polymerase mix) in a total volume of 50 μL. Denaturation at 94 ^oC for 3 min was followed by 35 cycles at 94 ^oC for 30 s, 68 ^oC for 30 s, and 72 ^oC for 3 min, then a final extension at 72 ^oC for 10 min. PCR products were separated on a 1% agarose gel.

Results and Discussion

Extensive studies of gene expression of carotenoid biosynthesis have been

investigated in tomato color mutants. Several watermelon color variants have been reported that may be similar to the tomato mutants. It has been proposed that 'Early Moonbeam' (canary yellow) corresponds to the *r* mutant of tomato, 'Malali' and 'NY162003' corresponds to the *B*, 'Yellow Crimson', 'Orangeglo', and 'Orange Flesh Tendersweet' correspond to the *t*, 'Moon and Stars' corresponds to the *og* and 'Crimson Sweet' corresponds to the *Delta* (Lewinsohn et al., 2005; Tadmor et al., 2004). However, they were categorized based on HPLC profile pattern, not based on the pattern of orthologous gene expression. Therefore, categorizing mutant types is not consistent, as in the case of 'Moon and Stars'. It was compared to the *og* mutant in the report of Tadmor et al. (2004), but later it was compared to wild type of tomato by Lewinsohn et al. (2005). Supporting data of gene expression for each color mutant seem to be required for reliable comparison.

Differential expression of cloned genes was examined at the transcriptional level using RT-PCR. *PSY-A* did not show differential expression across flesh colors (Fig. 18). It was expressed in various colored fleshes, whereas *PSY1* of tomato was not expressed in the *r* (yellow fruit) mutant of tomato, but was expressed in wild type and other mutants (Bartley et al., 1992; Fray and Grierson, 1993). Similarly, *PSY1* expression was not detected in the white endosperm mutant of maize (Gallagher et al., 2004). In Fig. 19, *PSY-A* transcript was detected in ovary, petal, leaf, and root tissues in addition to the different colored fleshes.

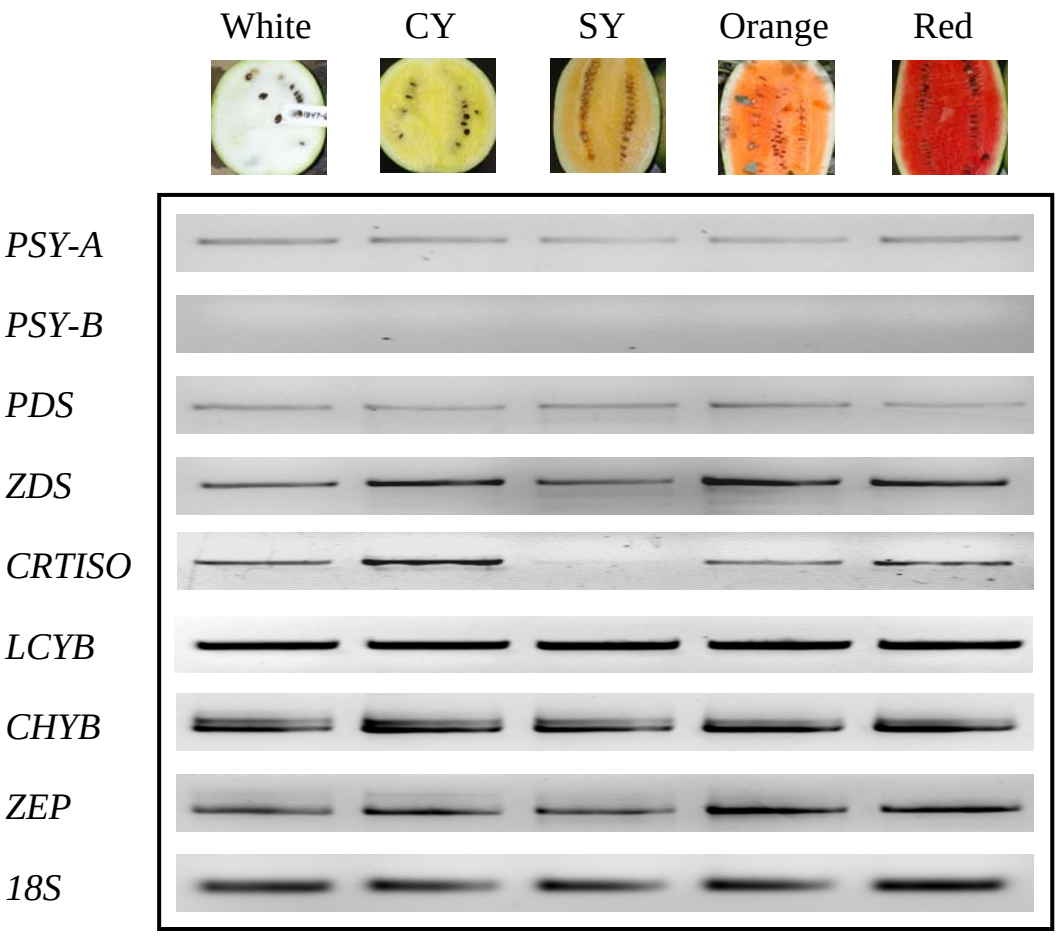

Fig. 18. RT-PCR of structural genes among different colored watermelon in the carotenoid biosynthetic pathway. 18S gene was used as a positive control for cDNA synthesis. CY, canary yellow; SY, salmon yellow.

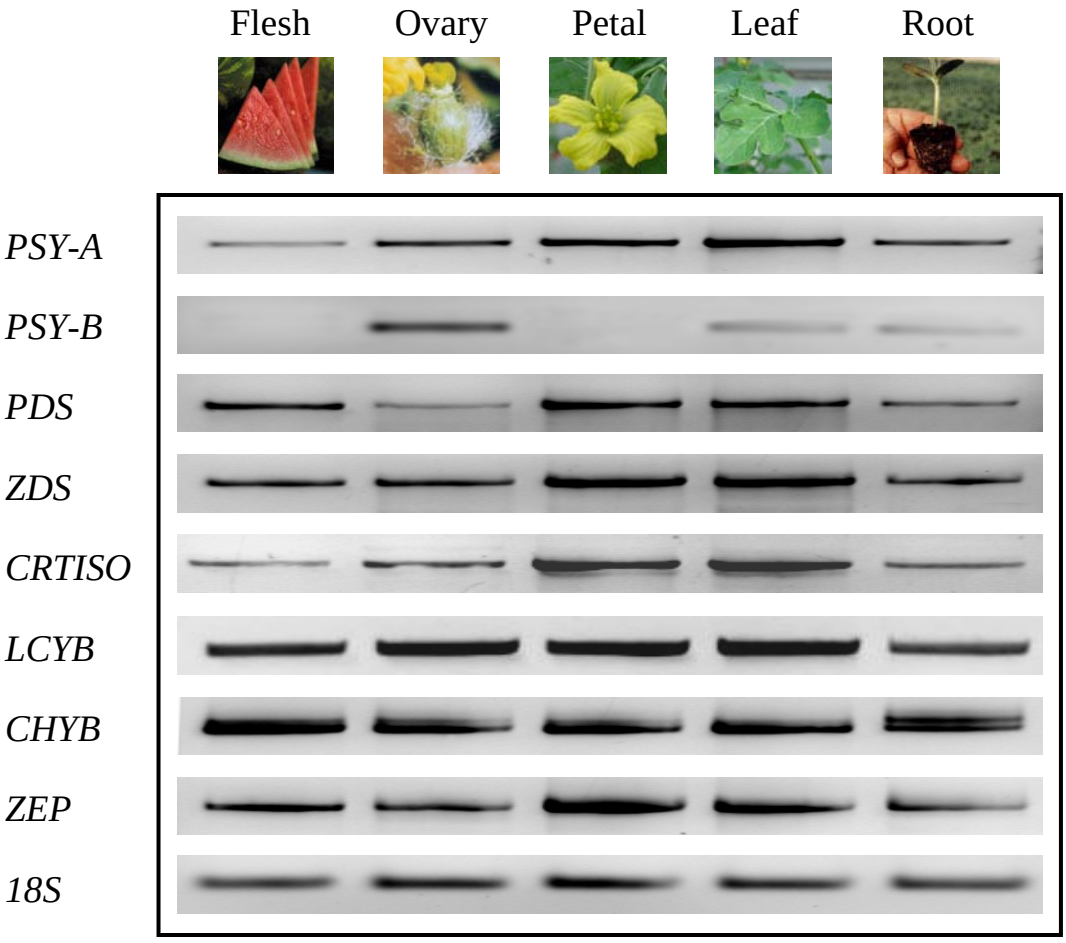

Fig. 19. RT-PCR of structural genes among different watermelon tissues in the carotenoid biosynthetic pathway. 18S gene was used as a positive control for cDNA synthesis. CY, canary yellow; SY, salmon yellow.

PSY-B was not expressed in any flesh color, but its expression was detected in ovary, leaf, and root tissues. This result indicates that *PSY-B* might be involved in carotenogenesis of chloroplast-containing tissues, similar to *PSY2* of tomato. It also seemed to be consistent with a report that *PSY-2* in tomato, which was down-regulated

during fruit development and is known to be involved in carotenoid biosynthesis of chloroplast (Fraser et al., 1999).

In tomato, *PSY* expression during fruit ripening was comprehensively studied (Bartley and Scolnik, 1993; Fray and Grierson, 1993; Gallagher et al., 2004 ; Guiliano et al., 1993; Ikoma et al., 2001 ; Karvouni et al., 1995; Romer et al., 1993; Salvini et al., 2005). Tomato *PSY1* and *PSY2* transcripts were detected in leaf, green, orange and red fruit during fruit development (Bartley and Scolnik, 1993), whereas *PSY-B* transcript was not detected in mature watermelon fruit (Fig. 18). Salvini (2005) reported that high expression of *HaPSY* was detected in cotyledon and leaf, but low in stem and root. *MEL5*, is a homolog of *PSY* that is induced by fruit ripening similar to tomato and citrus (Guiliano et al., 1993; Ikoma et al., 2001; Karvouni et al., 1995). *MEL5* transcript was extremely low in leaf and root in melon. In pepper, *PSY* expression was undetectable in fruit at the green and mature green stage, unlike tomato (Romer et al, 1993) or watermelon (Fig. 18).

RT-PCR of *PDS* transcript indicated that expression of *PDS* was not affected in color mutants. However, *PDS* expression appeared to be slightly lower in ovary than in red flesh. Real time-PCR would be necessary to determine whether it is induced by fruit ripening. In tomato, decreased *PDS* expression was observed at pink and red stage in the *Beta* mutant (Ronen et al., 2000). Tomato *PDS* mRNA transcript significantly increased with *PSY* during fruit ripening (Bartley and Scolnik, 1993; Guiliano et al., 1993; Pecker et al., 1992). *PDS* expression was detected in leaf, petal and root tissues of watermelon. In contrast, it was not detected in mature green fruit or very low in leaf and root of

tomato (Guiliano et al., 1993; Pecker et al., 1992).

Expression of *ZDS* was detected in all different colored fruits and all type of tissues. In sunflower, *HaZDS* mRNA transcript was very low in stem and root (Fambrini et al., 2004) compared to high expression in cotyledon and leaf tissues.

Any differential expression of other genes except *CRTISO* was not detected across any colored flesh, nor different tissues (Fig. 18 and 19). The expression of *CRTSIO* gene in salmon yellow was not detected. It is still possible that *CRTISO* expressed at a level undetectable by RT-PCR. Real-time PCR could provide more accurate difference of gene expression level. However, reduced or unexpressed *CRTISO* transcript indicated that color determination of salmon yellow might be controlled at the transcriptional level. *CRTISO* expression in orange appeared to be slightly reduced, but it was not significant. These salmon yellow and orange mutantsof watermelon may be equivalent to *tangerine* mutants of tomato, since similar results of *CRTISO* expression were observed in tomato. No mRNA transcript of *CRTISO* resulted from the deletion in promoter region of *tangerine*^{3183} containing almost equal amounts of prolycopene and ζ-carotene (Isaacson et al., 2002). In *tangerine*^{mic}, the deletion in the ORF resulted in the mutant having more ζ-carotene. Nonetheless, its expression level was not reduced, which suggested that regulation is at the post-transcriptional level. Therefore, salmon yellow can be compared to *tangerine*^{3183} since mRNA transcript was not detected in either mutant. The orange mutant of watermelon might be comparable to *tangerine*^{mic} since the expression level was not reduced. However, the sequence analyses of promoter and coding region of *CRTSIO* in watermelon and tomato color mutants, including

carotenoid profiles, will be necessary to make any determinations.

The genes affecting color pigmentation of tomato have been studied by Ronen et al. (1999; 2000). The orange color mutants, *Delta* and *Beta*, were affected by lycopene cyclase. The *Delta* mutant resulted from upregulation of *LCYE,* resulting in the accumulation of δ-carotene. The *Beta* orange color mutant results from the upregulation of *CYCB,* so that mutants accumulate β-carotene. An interesting feature was observed that *PDS* expression decreased at the pink stage of *Beta* mutants, whereas it increased at the pink stage of wild type (Ronen et al., 2000). *Old-gold,* a recessive mutation which accumulates lycopene is based on a frame-shift in *CYCB* gene. Bramley (2002) suggested that feedback inhibition by an end-product may regulate tomato carotenogenesis. Lack of end-product, such as β-carotene in *old-gold,* may increase enzyme activity in earlier steps resulting in an accumulation of lycopene.

Based on these reports, gene expression involved in carotenogenesis in tomato seems to be regulated at both the transcriptional and post-transcriptional levels. In watermelon, RT-PCR results of *PSY-A, PSY-B, and CRTISO* indicated that they may be regulated at the transcriptional level like tomato. However, regulatory mechanisms of other genes in the carotenoid biosynthetic pathway of watermelon need to be established.

CHAPTER VII

INHERITANCE STUDY OF CAROTENOIDS ASSOCIATED WITH SPECIFIC FLESH COLOR

Materials and Methods

Forty open pollinated varieties and ninety plant introductions (PI) were grown and screened in the greenhouse for an inheritance study in 2003. Visual color ratings and HPLC profiles were used to categorize watermelons into different color groups. To determine the number of color related loci and interactions of loci, crosses were made between various colored watermelon groups differing in their carotenoid profiles.

Crossing blocks were made between canary yellow (PI165002) and red (PI593380), salmon yellow ('Yellow Flesh Black Diamond') and red ('Sugar Baby') in the greenhouse at College Station. F_1s were also selfed and backcrossed in the greenhouse to create F_2 and backcross population. F_2 and backcross populations were seeded out on 23 Feb. 2004, including parental lines for inheritance study. Five-week old seedlings were transplanted in the greenhouse at College Station and in the field of Texas A&M Agricultural Research and Extension Center at Uvalde to analyze flesh colors in the progeny. Additional F_2 and backcross populations were seeded out 4 Apr. 2005 and grown in the greenhouse and field at College Station. The soil at Uvalde is a Uvalde silty clay loam. Fertilizer (30-17-38) was applied through the drip system weekly for 5 weeks using urea, KNO_3 and H_3PO_4 as sources of N, P, and K, respectively. Plants

for each experimental plot were grown on a single raised bed on 2.03 m centers with one row per bed and 0.9 m within row spacing and each plot was separated by a 2-m blank row. A subsurface drip system (20 cm depth) and black plastic mulch were used.

Fruits were harvested when the majority of them were overmature and color was visually scored. The number of genes involved in color determination for each cross was estimated by the chi-square goodness-of-fit test.

Results and Discussion

Color is a very important trait in watermelon, but its inheritance mechanism is complex. Several loci have been shown to be involved in color inheritance of watermelon (Poole, 1944; Henderson et al., 1998). The *C* locus and *I* locus were identified by Poole (1944) and by Henderson et al. (1998), respectively, conditioning color difference between canary yellow and red. Canary yellow (*C*) was dominant to red (*c*) and the *I* locus was known as an inhibitor of the *C* locus. Since Porter (1937) has reported a single gene determining color difference between red and salmon yellow, Henderson (1989) confirmed this previous result.

However, the identities of color-conditioning genes are still unknown. In contrast, comprehensive tomato color inheritance studies have been identified a few genes that determine tomato fruit color. For example, impaired *PSY* resulted in the *r* tomato mutant (pale yellow) and a deletion in promoter region or coding region of *CRTISO* gene caused the *tangerine*^{mic} and *tangerine*^{3183} mutants (MacKinney and Jenkins, 1949; Jenkins and

MacKinney, 1953; Fray and Grierson, 1993; Issacson et al., 2002). Lycopene β-cyclase (*CYCB*) was postulated to encode the *B* gene of which function was considered to be related to β-carotene production (Hirschberg, 2001; Lincoln and Porter; 1950; Ronen et al., 2000).

In our population, the segregation derived from a cross between canary yellow and red is shown in Fig. 20. Chi-square goodness-of-fit test of the segregation ratio of F_2 population and backcross was not in accordance with the result of Henderson et al. (1998) (Table 11). Observed data from our population did not fit to 9:7 ratio, but significantly fit to 3:1 in the F_2 population. Chi-square goodness-of-fit test result of segregation ratio indicated that only a single gene determined fruit flesh color in our population, not the two genes as compared to be *C* and *I* locus.

A distinct fruit color pattern was also observed in which canary yellow and red were mixed in the flesh, as was reported by Navot et al. (1990). This may be due to a modifier gene or incomplete dominance (Henderson et al., 1998). However, it might be due to environmental effects in our population because the mixed patterns were observed in watermelon which had obvious developmental problems during growth. Our population was scored based on portion of canary yellow and red. If it had more than 50% of red portion in the flesh, it was classified as red and vise versa.

The segregation and phenotypes of the cross between red and salmon yellow is depicted in Fig. 21. The expected ratios were 3:1 in the F_2 population and 1:1 in each backcross, and the chi-square goodness-of-fit test of the segregation ratio fit to the expected ratio (Table 12). The *Y* locus was proposed by Poole (1944), but identification

of the *Y* locus has not been studied.

Identification of genes determining flesh color in watermelon is necessary to fully understand genetic mechanisms and to accelerate breeding for specific colors with health promoting compounds. This inheritance study provides important background information to identify color determining genes. If molecular markers for allelic selections in color mutants based on color determining gene are developed, they will be very useful in watermelon breeding programs. Therefore, we investigated the identification of color determining genes as a next step using a candidate gene approach.

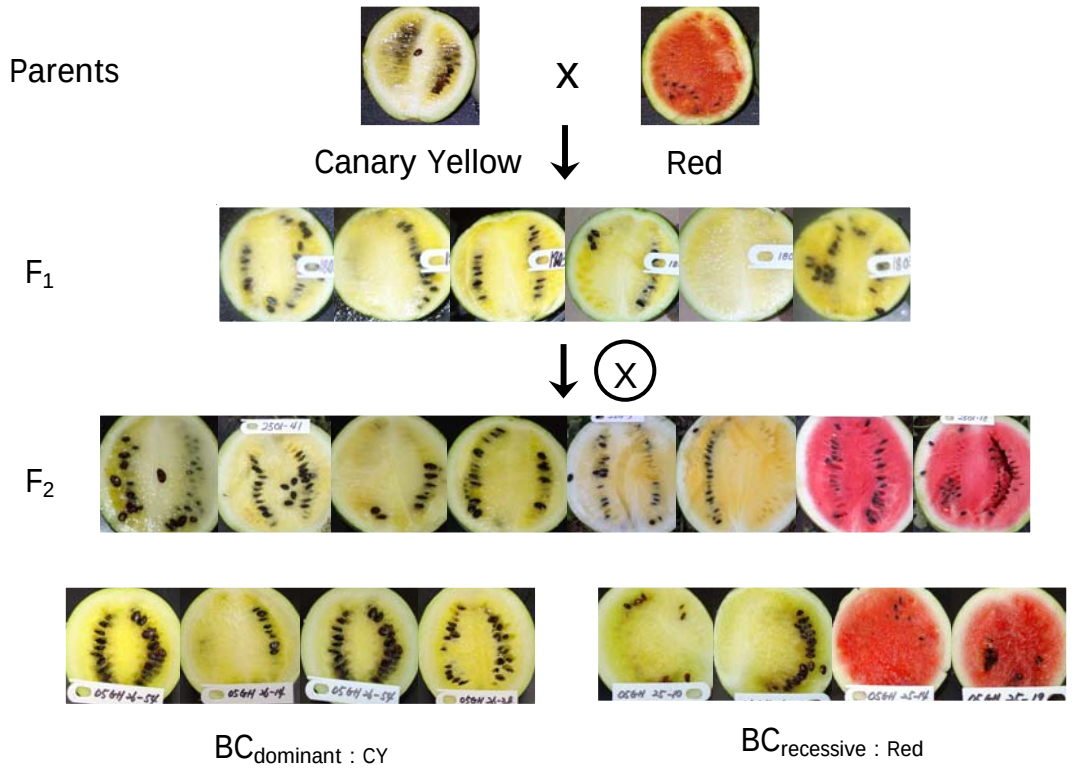

Fig. 20. The segregation and phenotypes of a F_2 population originating from a cross between canary yellow (PI 165002) and red (PI 593380). CY; canary yellow.

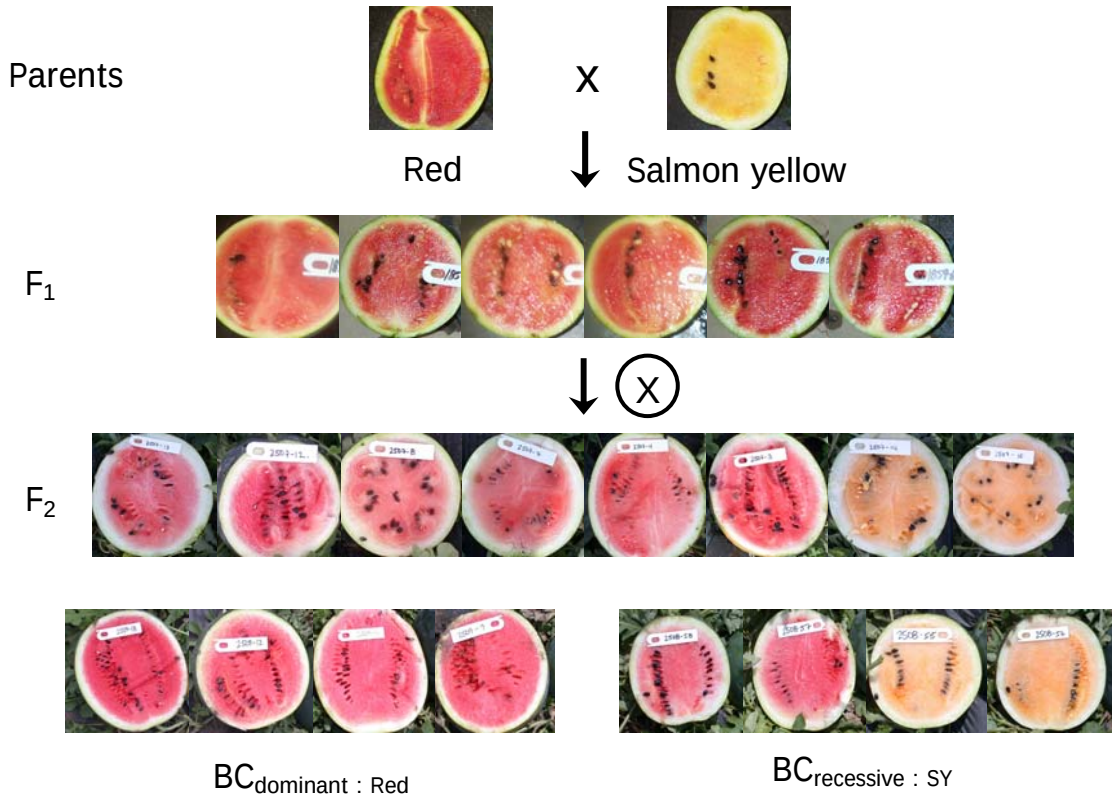

Fig. 21. The segregation and phenotypes of a F_2 population originating from a cross between red (‘Sugar Baby’) and salmon yellow (‘Yellow Flesh Black Diamond’). SY; salmon yellow

Table 11. Chi-square test of the segregation ratio of colors in the F_2 population originating from the cross between canary yellow and red lines.

Population	Genotype	Observed (CY[z] : Red)	Expected (CY : Red)	χ^2	*P*
F_1	*Cc*	27 : 0	1 : 0	0	1
F_2	Segregating	61 : 24	3 : 1	0.47	0.49
			9 : 7	8.31	<0.01
$BC_{dominant(CY)}$	*C_*	69 : 0	1 : 0	0	1
$BC_{recessive(Red)}$	Segregating	17 : 15	1 : 1	0.13	0.72
			1 : 3	13.5	<0.01

[z] CY = canary yellow

Table 12. Chi-square test of the segregation ratio of colors in the F_2 population originating from the cross between red and salmon yellow lines.

Population	Genotype	Observed (Red : SY[z])	Expected (Red : SY)	χ^2	*P*
F_1	*Yy*	9 : 0	1 : 0	0	1
F_2	Segregating	32 : 17	3 : 1	2.46	0.12
$BC_{dominant(Red)}$	*Y_*	25 : 0	1 : 0	0	1
$BC_{recessive(SY)}$	Segregating	39 : 30	1 : 1	1.17	0.28

[z] SY = salmon yellow

CHAPTER VIII

DEVELOPMENT OF MOLECULAR MARKERS FOR ALLELIC SELECTION FOR CANARY YELLOW AND RED

Materials and Methods

Plant materials

A cross between canary yellow (PI 165002) and red (PI 593380) was made to produce F_2 and BC populations as described in Chapter VII. Leaf tissues from individuals of parents, F_1, F_2, and backcross populations were collected for genotyping and stored at -20 ^{o}C until analysis. A bulk of 5-10 individual leaf samples were ground for bulk segregant analysis to search for polymorphism between canary yellow and red flesh color in the F_2 and backcross populations. Samples were selected based on the evaluation of individual plants from the F_2 and backcross populations. Commercial varieties and plant introductions were grown in the greenhouse and leaf samples were collected at the seedling stage. They were genotyped using a molecular marker, and grown until maturity unless they are genetically fixed in color. Seven canary yellow and six red breeding lines were provided by Syngenta for additional marker test. Tissue from leaves (100 mg) was ground in liquid nitrogen for genomic DNA isolation. Genomic DNA was isolated using a DNeasy Plant Mini Kit (Qiagen).

Isolation of LCYB gene and sequencing

The *LCYB* gene was expected to encode enzymes governing the color determinant between canary yellow and red watermelons, since it was indicated that a single gene was involved in color difference between two colors from the inheritance study (Chapter VII). Therefore, sequencing of the *LCYB* gene of canary yellow and red was carried out to search for a polymorphism.

PCR reaction mixture was prepared with 1 μL template, 5 μL 10× PCR buffer, 1 μL dNTP (10mM), 1 μL forward *LCYB* primer (10μM), 1 μL reverse LCYB primer (10 μM), and 1 μL Taq polymerase mix (50× Advantage 2 polymerase mix) in a total volume of 50 μL. Denaturation at 94 ^{o}C for 3 min was followed by 40 cycles of 94 ^{o}C for 30 s, 68 ^{o}C for 30 s, and 72 ^{o}C for 3 min, then a final extension at 72 ^{o}C for 10 min. PCR products were run on a 1% agarose gel. As a single band was produced for each color, PCR products were purified with PCR purification kit (QIAquick PCR purification Kit; Qiagen) for direct sequencing.

The sequences were obtained using automated Big Dye DNA Cycle Sequencing (ABI Prism BigDye Terminator Cycle Sequencing Ready Kits; Applied Biosystems) and ABI 3100 capillary seqeuncer (ABI 3100 Genetic Analyzer; Applied Biosystems) by the Laboratory for Plant Genome Technology sequencing facility of Texas A&M University.

Development of CAPS marker

Differential restriction sites between the two alleles were identified using NEBcutter V2.0 (Vincze et al., 2003) based on genomic DNA sequences of *LCYB* genes

from canary yellow and red. PCR products were digested with restriction enzyme *BsaH*I. The reaction mixtures contained 4 μL of PCR product, 2 μL 10× buffer, 0.2 μL BSA, 1.5 μL *BsaH*I in a total volume of 20 μL and incubated at 37 ^{o}C for 4 hrs. Digested products were separated on a 1% agarose gel for genotyping of individuals.

Results and Discussion

The *C* and *I* loci were proposed to be involved in color inheritance of canary yellow and red colors (Henderson et al., 1998). As in other plants and tissues, color of watermelon flesh is associated with specific phytochemicals. Major colorants in watermelon and tomato are carotenoids (Lincoln and Porter, 1950; Tomes and Johnson, 1965; Mackinney and Jenkins, 1949).

Since canary yellow was dominant to red (Poole, 1944), it seemed likely that early enzymatic steps from *PSY* up to *ZDS* in both canary yellow and red watermelon might be active to accumulate lycopene which is the major carotenoid in red types. Therefore, a possible candidate gene for the color determinant would be the gene downstream of the pathway. In tomato, increased lycopene accumulation of tomato *og* mutant was resulted from frame shift mutation of . The function *B* gene is known to accumulate β-carotene (Ronen et al., 2000). In our inheritance study, the mutation of *LCYB* in watermelon seemed to account for lycopene accumulation and significant β-carotene reduction of red watermelon. Therefore, we targeted structural genes in the carotenoid biosynthetic pathway as candidate genes for color determination. In this study,

we hypothesized that *LCYB* might be responsible for color differentiation between canary yellow and red in watermelons. However, significant differential expression was not detected at the transcriptional level according to analysis by RT-PCR. This result led us to search for sequence polymorphism in the respective genomic DNA. Sequence comparison of genomic DNAs from canary yellow and red watermelon revealed a single nucleotide polymorphism (SNP) in the coding region of the *LCYB* gene (Fig. 22).

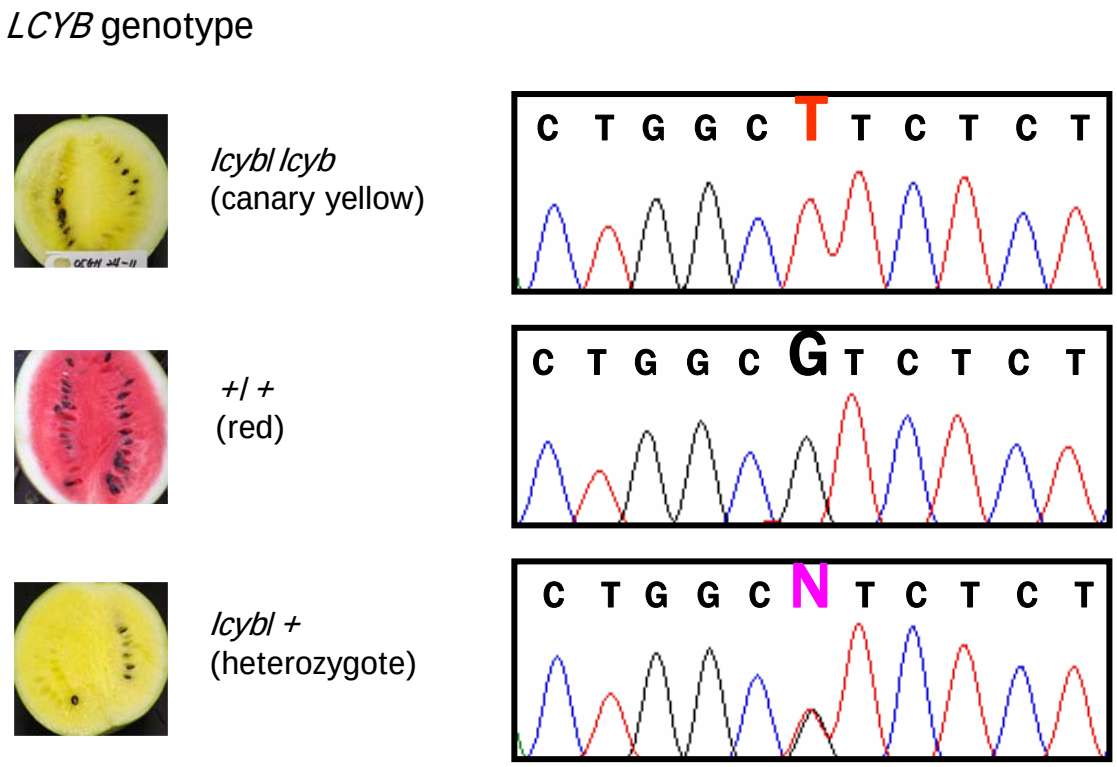

Fig. 22. SNP marker utilized for genotyping of *LCYB* alleles. Heterozygous individuals can be identified using SNP, where both chromatographic peaks are present at the polymorphic sequence region.

A nucleotide thymine peak was detected in homozygous canary yellow, whereas a guanine peak was detected in homozygous red, and both thymine and guanine peaks in heterozygous canary yellow in the chromatography of the sequences. Full-sequence comparison of genomic DNAs enabled to detect an additional SNP which is adenine in red and guanine in canary yellow (Fig. 23). The first SNP was positioned at the 12^{th} nucleotide and second was at the 676^{th} nucleotide from the putative transcription start site.

To investigate co-segregation of both SNPs with color phenotypes, genotyping of the F_2 population derived from the cross between canary yellow and red was carried out. Color phenotypes of individuals from the F_2 and backcross populations showed perfectly co-segregated with the SNP markers (data not shown). The findings indicating that a single gene determines color differ from the report that *I* gene epistatically inhibits the *C* locus when *I* is homozygous recessive (*ii*) and the two genes were involved in their color determination by Henderson et al. (1998). It is conceivable that this discrepancy would arise if *I* were fixed as a homozygous dominant (*II*) in both parents of the population that we used for inheritance study. In our population, the perfect co-segregation indicated that a single gene determined flesh colors between canary yellow and red. To obtain supporting data of no inhibitory effect on color determination of canary yellow, it was examined whether the SNP marker co-segregates with color phenotypes in other populations such as commercial varieties and PIs. A total of 170 different individuals from 31 sources were checked, and test results showed perfect co-segregation (Fig. 24). This suggests that there is no effect of *ii* and a single gene conditioning in color

determination between canary yellow and red.

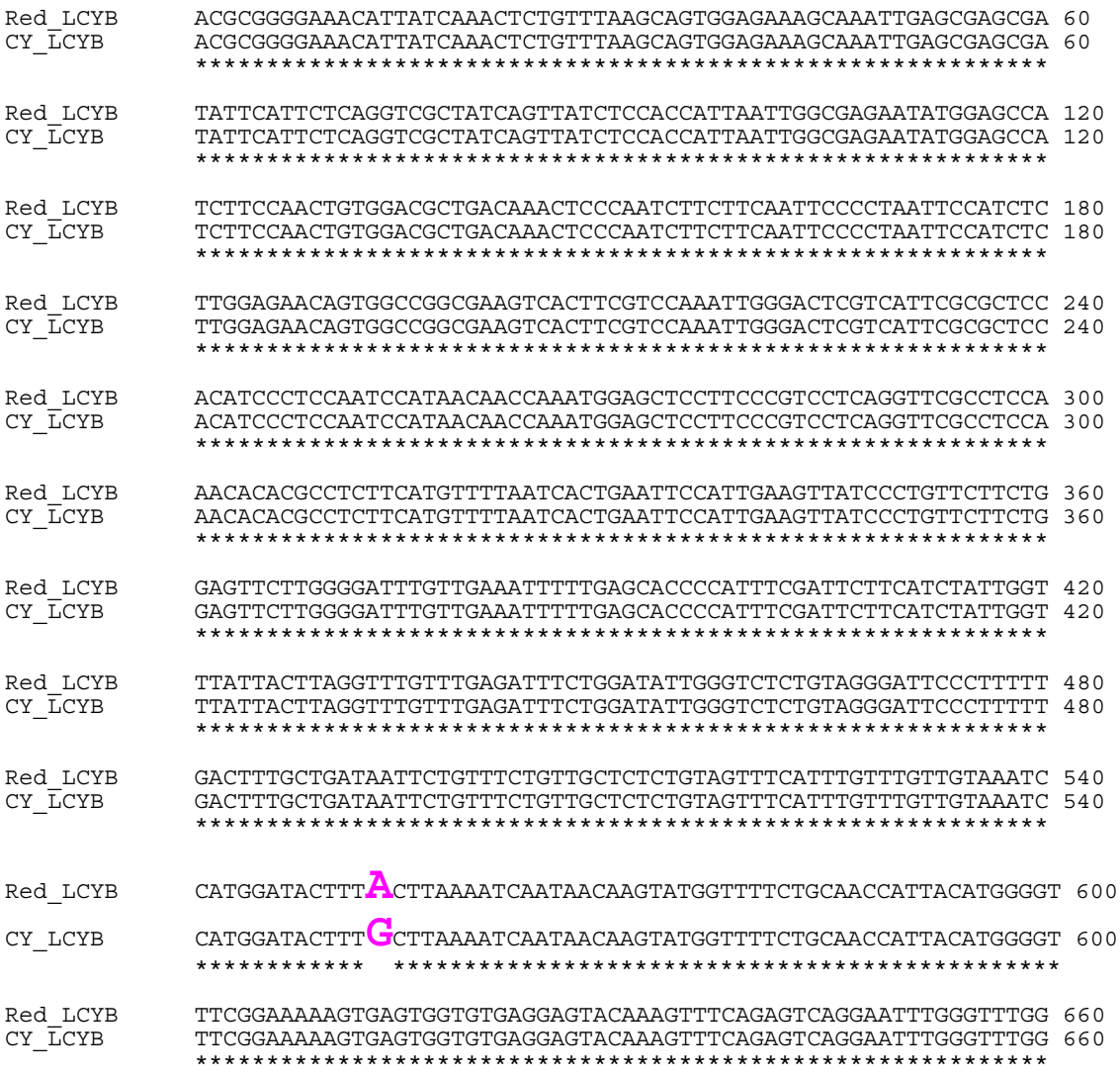

Fig. 23. Alignment of nucleotide sequences of *LCYB* gene in canary yellow and red watermelon. Bold letters are start codon (ATG) and stop codon (TAA). '*': amino acids in the column are identical.

```
Red_LCYB      TCATAGGAAGGGTCGTCTGAAATGGAGGAAAGGGGGTTGTCTTAATGTGAGAAGTAGTTC 720
CY_LCYB       TCATAGGAAGGGTCGTCTGAAATGGAGGAAAGGGGGTTGTCTTAATGTGAGAAGTAGTTC 720
              ************************************************************

Red_LCYB      TCTTTTGGAGCTTGTTCCTGAAACCAAGAAGGAGAATCTTGAGGTTGAACTTCCCATGTA 780
CY_LCYB       TCTTTTGGAGCTTGTTCCTGAAACCAAGAAGGAGAATCTTGAGGTTGAACTTCCCATGTA 780
              ************************************************************

Red_LCYB      TGATCCTTCGAAGGGCCTTGTTGTCGATCTTGCGGTCGTGGGAGGCGGCCCAGCAGGGCT 840
CY_LCYB       TGATCCTTCGAAGGGCCTTGTTGTCGATCTTGCGGTCGTGGGAGGCGGCCCAGCAGGGCT 840
              ************************************************************

Red_LCYB      TGCTGTTGCGCAACAGGTTTCAGAGGCAGGGCTTTCAGTTTGTGCAATTGACCCATCTCC 900
CY_LCYB       TGCTGTTGCGCAACAGGTTTCAGAGGCAGGGCTTTCAGTTTGTGCAATTGACCCATCTCC 900
              ************************************************************

Red_LCYB      CAAGTTGATTTGGCCCAACAATTATGGGGTTTGGGTGGATGAATTTGAGGCAATGGATTT 960
CY_LCYB       CAAGTTGATTTGGCCCAACAATTATGGGGTTTGGGTGGATGAATTTGAGGCAATGGATTT 960
              ************************************************************

Red_LCYB      GCTAGATTGTCTCGACACGACTTGGTCTGGTGCTGTCGTGTTCACCAATGAGCAATCAAC 1020
CY_LCYB       GCTAGATTGTCTCGACACGACTTGGTCTGGTGCTGTCGTGTTCACCAATGAGCAATCAAC 1020
              ************************************************************

Red_LCYB      AAAAGATCTTGCTCGACCTTATGCGAGGGTTAATAGAAAGCAACTCAAGTCAAAAATGTT 1080
CY_LCYB       AAAAGATCTTGCTCGACCTTATGCGAGGGTTAATAGAAAGCAACTCAAGTCAAAAATGTT 1080
              ************************************************************

Red_LCYB      GCAGAAATGCATTTCCAATGGTGTTAAGTTTCATGAAGCTAAAGTTATTAAAGTTATACA 1140
CY_LCYB       GCAGAAATGCATTTCCAATGGTGTTAAGTTTCATGAAGCTAAAGTTATTAAAGTTATACA 1140
              ************************************************************

Red_LCYB      TGAGGAGTTCAAATCCTTGTTAATTTGCAATGATGGTGTGACCATTCAAGCTGCCATTGT 1200
CY_LCYB       TGAGGAGTTCAAATCCTTGTTAATTTGCAATGATGGTGTGACCATTCAAGCTGCCATTGT 1200
              ************************************************************

Red_LCYB      TCTTGATGCCACTGGCGTCTCTCGATGCCTTGTCCAATATGATAAGCCTTACAATCCAGG 1260

CY_LCYB       TCTTGATGCCACTGGCTTCTCTCGATGCCTTGTCCAATATGATAAGCCTTACAATCCAGG 1260
              ****************  ****************************************

Red_LCYB      CTACCAGGTAGCTTATGGGATTTTAGCTGAGGTGGAGGAACATCCATTTGATGTTAACAA 1320
CY_LCYB       CTACCAGGTAGCTTATGGGATTTTAGCTGAGGTGGAGGAACATCCATTTGATGTTAACAA 1320
              ************************************************************

Red_LCYB      GATGGTGTTTATGGACTGGAGAGATTCACATCTGAATAACAATATGATTTTGAAGGAGAG 1380
CY_LCYB       GATGGTGTTTATGGACTGGAGAGATTCACATCTGAATAACAATATGATTTTGAAGGAGAG 1380
              ************************************************************

Red_LCYB      AAATAGCAAAATTCCTACATTTCTCTATGCAATGCCCTTTTCATCAAATCGGATATTTCT 1440
CY_LCYB       AAATAGCAAAATTCCTACATTTCTCTATGCAATGCCCTTTTCATCAAATCGGATATTTCT 1440
              ************************************************************
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Fig. 23. Continued.

```
Red_LCYB      GGAGGAAACTTCTTTGGTAGCTCGACCTGGGTTACAAATGAGCGATATCCAGGAAAGAAT 1500
CY_LCYB       GGAGGAAACTTCTTTGGTAGCTCGACCTGGGTTACAAATGAGCGATATCCAGGAAAGAAT 1500
              ************************************************************

Red_LCYB      GGAGGTAAGATTGAAGCACTTGGGAATAAAAGTGAAGAGCATTGAAGAGGATGAGCATTG 1560
CY_LCYB       GGAGGTAAGATTGAAGCACTTGGGAATAAAAGTGAAGAGCATTGAAGAGGATGAGCATTG 1560
              ************************************************************

Red_LCYB      TGTCATTCCAATGGGTGGACCGCTGCCAGTTCTTCCTCAAAGAGTTGTTGGAATTGGTGG 1620
CY_LCYB       TGTCATTCCAATGGGTGGACCGCTGCCAGTTCTTCCTCAAAGAGTTGTTGGAATTGGTGG 1620
              ************************************************************

Red_LCYB      AACAGCAGGGATGGTGCACCCTTCAACTGGATATATGGTAGCAAGAACTCTAGCAGCGGC 1680
CY_LCYB       AACAGCAGGGATGGTGCACCCTTCAACTGGATATATGGTAGCAAGAACTCTAGCAGCGGC 1680
              ************************************************************
Red_LCYB      ACCTATTGTTGCTAGTGCAATAGTCCGGTGCCTTGGTTCAGATGGACGTTTCAGGGGTGA 1740
CY_LCYB       ACCTATTGTTGCTAGTGCAATAGTCCGGTGCCTTGGTTCAGATGGACGTTTCAGGGGTGA 1740
              ************************************************************

Red_LCYB      TGCGATATCCTCTGAAGTTTGGAAAGATCTATGGCCCATCGAAAGGAGGAGGCAGAGAGA 1800
CY_LCYB       TGCGATATCCTCTGAAGTTTGGAAAGATCTATGGCCCATCGAAAGGAGGAGGCAGAGAGA 1800
              ************************************************************

Red_LCYB      ATTTTTCTGTTTTGGGATGGATATTTTATTGAAGCTGGATCTAAAGGGTACAAGAAGGTT 1860
CY_LCYB       ATTTTTCTGTTTTGGGATGGATATTTTATTGAAGCTGGATCTAAAGGGTACAAGAAGGTT 1860
              ************************************************************

Red_LCYB      TTTTGATGCATTTTTTGATCTTGAACCTCGTTATTGGCATGGATTCTTGTCATCACGACT 1920
CY_LCYB       TTTTGATGCATTTTTTGATCTTGAACCTCGTTATTGGCATGGATTCTTGTCATCACGACT 1920
              ************************************************************

Red_LCYB      ATTCCTTCCTGAGCTGTTACTCTTTGGGCTTTCCTTATTCTCTCACGCATCTAATGCCTC 1980
CY_LCYB       ATTCCTTCCTGAGCTGTTACTCTTTGGGCTTTCCTTATTCTCTCACGCATCTAATGCCTC 1980
              ************************************************************

Red_LCYB      CAGGCTTGAAATCATGGCAAAGGGAACTCCATCTTTGGTAAACATGATCGGCAATCTGGT 2040
CY_LCYB       CAGGCTTGAAATCATGGCAAAGGGAACTCCATCTTTGGTAAACATGATCGGCAATCTGGT 2040
              ************************************************************

Red_LCYB      AAAGGATAGAGATTAAGATGAATATAGAGTTACTGTGTTGTAAGCTAATCACCATACTGA 2100
CY_LCYB       AAAGGATAGAGATTAAGATGAATATAGAGTTACTGTGTTGTAAGCTAATCACCATACTGA 2100
              ************************************************************

Red_LCYB      TGCACTTGCATCATCACATTTACTTCTGCAGATGATTGTTCATAAGATTATGAGTTAGCA 2160
CY_LCYB       TGCACTTGCATCATCACATTTACTTCTGCAGATGATTGTTCATAAGATTATGAGTTAGCA 2160
              ************************************************************

Red_LCYB      AAAAAAAAAAAAAAAAAAAA 2181
CY_LCYB       AAAAAAAAAAAAAAAAAAAA 2181
              ********************
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Fig. 23. Continued.

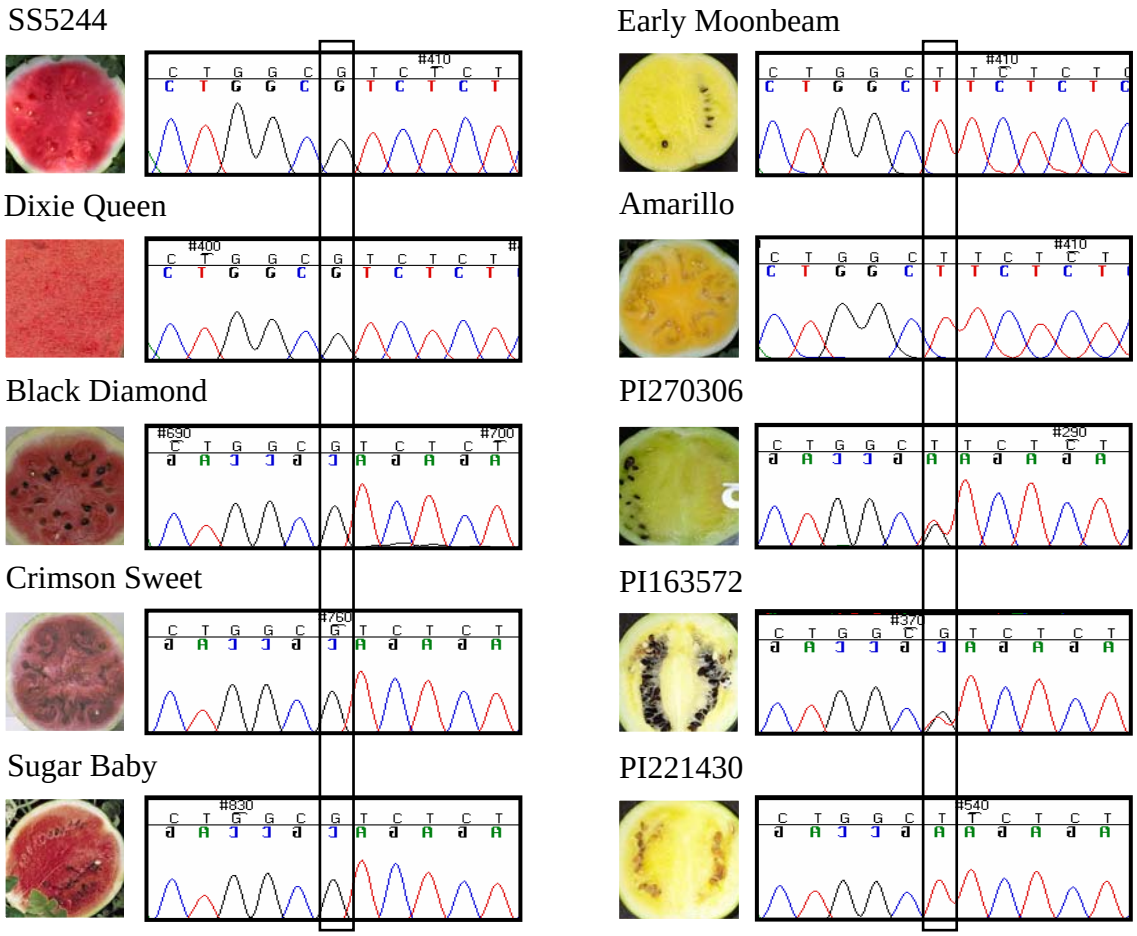

Fig. 24. Co-segregation of the SNP marker and color phenotype of commercial cultivars and PIs in watermelon. The red allele of *LCYB* has guanine peak and canary yellow allele of *LCYB* has thymine peak in a homozygote or both thymine and guanine in a heterozyte.

With regard to mixed colored (bicolor) fruit in F_2 and backcross populations, they were also tested with a SNP marker. The genotype did not match with the phenotype in bicolor fruit. Environmental condition or other factors could have had a larger impact on

phenotype than genetic composition.

Since a SNP marker testing requires expensive equipment and procedures, a cleaved amplified polymorphic sequence (CAPS) marker was developed from SNPs based on the restriction map (Fig. 25). The red allele of *LCYB* had a *BsaH*I restriction site around the second SNP (676^{th} nucleotide from the putative transcription start site), whereas no restriction site occurred in the canary yellow allele of *LCYB*. Therefore, *BsaH*I digestion of the PCR product produced 1182 bp and 412 bp fragments of the red allele and one 1594 bp fragment of the canary yellow allele (Fig. 26. A). This difference will allow detecting heterozygous canary yellow as the marker is co-dominant (Fig. 26. B). Additionally, a CAPS marker can be applied for *LCYB* allelic selection at seedling stages to predict flesh color, which will facilitate watermelon breeding.

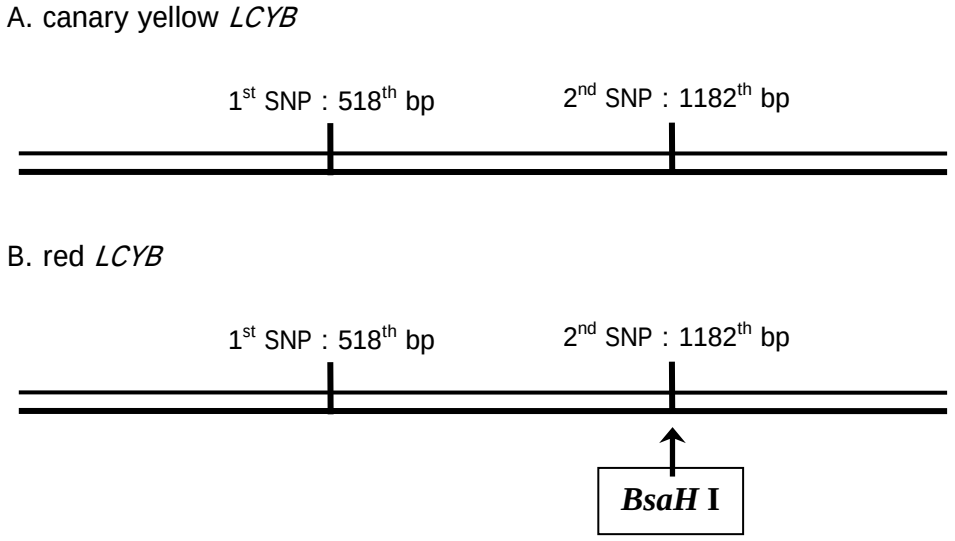

Fig. 25. Restriction map of the *LCYB* gene of canary yellow and red watermelon. The positions of SNPs were marked based on a primer pair used for PCR.

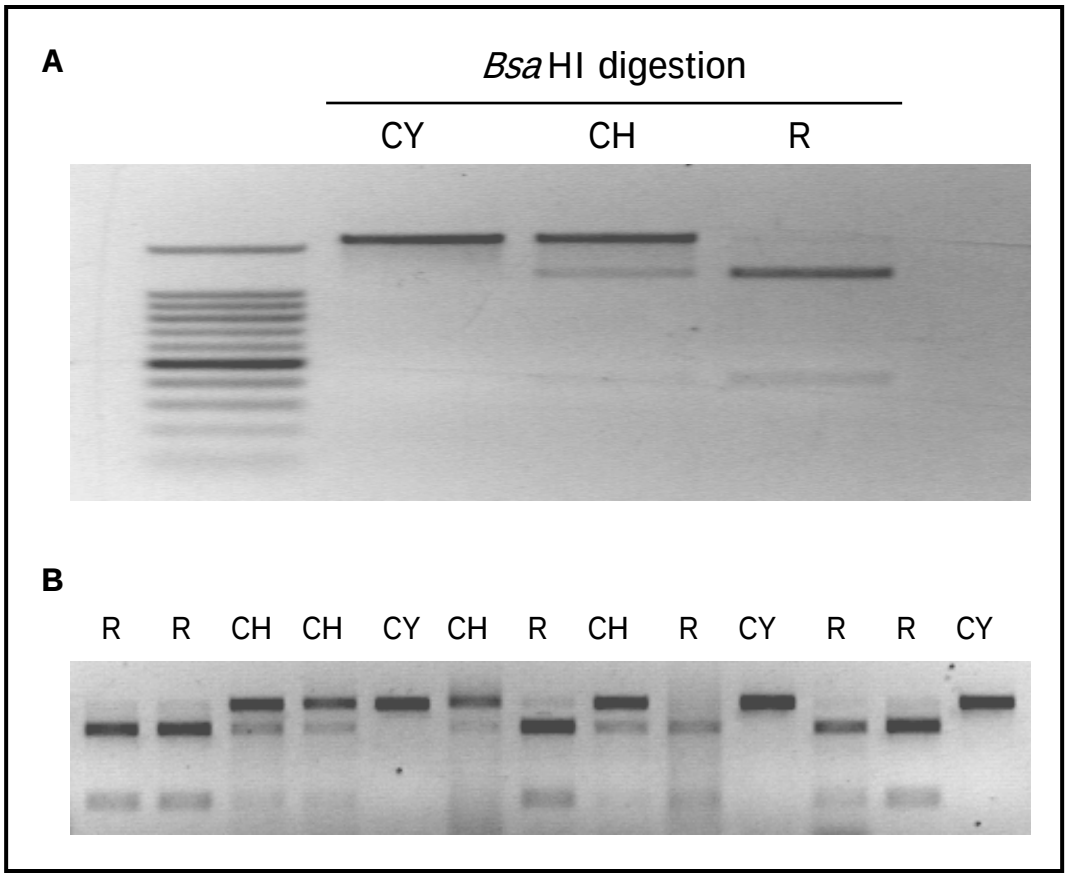

Fig. 26. Co-segregation of a CAPS marker and color phenotype in F_2 populations originating from the cross between a canary yellow line and a red line. A. Development of a CAPS marker from SNP of *LCYB* gene. The *LCYB* allele of red has *Bsa*HI restriction site. B. The genotypes of F_2 watermelons were identified by a CAPS marker. CY, homozygous canary yellow; CH, heterozygous canary yellow; R, homozygous red.

Together with the chi-square test results of the inheritance study (Table 11, Chapter VII), the genotyping results of the F_2 and backcross populations using the CAPS

marker strongly suggests that a single gene conditions the difference in color between canary yellow and red. Co-segregation of phenotypes and the *LCYB* allele in the segregating populations also indicates that the identity of the *C* locus (Henderson et al., 1998) might be the LCYB gene (Table 13).

Table 13. Co-segregation of *LCYB* allele and color phenotype in F_2 populations originating from the cross between a canary yellow line and a red line.

Parameter	Parent		F_1	F_2		
	CY[z]	Red		CY	Heterozygote	Red
Phenotype	CY	Red	CY	CY	CY	Red
Exprected *C* allele	*C*/*C*	*c*/*c*	*C*/*c*	*C*/*C*	*C*/*c*	*c*/*c*
LCYB allele	*lcyb*/*lcyb*	+ / +	*lcyb*/ +	*lcyb*/*lcyb*	*lcyb*/ +	+ / +

[z] CY = canary yellow

The first SNP (12th nucleotide from the putative transcription start site) did not change the amino acid sequence, but the second SNP resulted in an amino acid substitution from phenylalanine in canary yellow to valine in red watermelon (Fig. 27). Thus, the latter likely accounts for the modified phenotype, i.e. critical mutation. An interesting result of the amino acid sequence comparison with other species showed that amino acid in only red watermelon was substituted with valine whereas it was conserved as phenylalanine in other species (Fig. 28). There was no cross-over type identified based on the marker test. It is unlikely that a more critical mutation could be identified in

the promoter region, because the transcription of *LCYB* gene was normal. Therefore, this indicates that color determination may be due to a critical mutation by amino acid change significantly reducing the activity of *LCYB* in red watermelon. Complementation of red fruit would provide definite evidence whether *LCYB* gene is conditioning color difference.

```
Red_LCYB      MDTLLKINNKYGFLQPLHGVSEKVSGVRSTKFQSQEFGFGHRKGRLKWRKGGCLNVRSSS 60
CY_LCYB       MDTLLKINNKYGFLQPLHGVSEKVSGVRSTKFQSQEFGFGHRKGRLKWRKGGCLNVRSSS 60
              ************************************************************

Red_LCYB      LLELVPETKKENLEVELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCAIDPSP 120
CY_LCYB       LLELVPETKKENLEVELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCAIDPSP 120
              ************************************************************

Red_LCYB      KLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVFTNEQSTKDLARPYARVNRKQLKSKML 180
CY_LCYB       KLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVFTNEQSTKDLARPYARVNRKQLKSKML 180
              ************************************************************

Red_LCYB      QKCISNGVKFHEAKVIKVIHEEFKSLLICNDGVTIQAAIVLDATGVSRCLVQYDKPYNPG 240

CY_LCYB       QKCISNGVKFHEAKVIKVIHEEFKSLLICNDGVTIQAAIVLDATGFSRCLVQYDKPYNPG 240
              ******************************************.*********************

Red_LCYB      YQVAYGILAEVEEHPFDVNKMVFMDWRDSHLNNNMILKERNSKIPTFLYAMPFSSNRIFL 300
CY_LCYB       YQVAYGILAEVEEHPFDVNKMVFMDWRDSHLNNNMILKERNSKIPTFLYAMPFSSNRIFL 300
              ************************************************************

Red_LCYB      EETSLVARPGLQMSDIQERMEVRLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVVGIGG 360
CY_LCYB       EETSLVARPGLQMSDIQERMEVRLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVVGIGG 360
              ************************************************************

Red_LCYB      TAGMVHPSTGYMVARTLAAAPIVASAIVRCLGSDGRFRGDAISSEVWKDLWPIERRRQRE 420
CY_LCYB       TAGMVHPSTGYMVARTLAAAPIVASAIVRCLGSDGRFRGDAISSEVWKDLWPIERRRQRE 420
              ************************************************************

Red_LCYB      FFCFGMDILLKLDLKGTRRFFDAFFDLEPRYWHGFLSSRLFLPELLLFGLSLFSHASNAS 480
CY_LCYB       FFCFGMDILLKLDLKGTRRFFDAFFDLEPRYWHGFLSSRLFLPELLLFGLSLFSHASNAS 480
              ************************************************************

Red_LCYB      RLEIMAKGTPSLVNMIGNLVKDRD 504
CY_LCYB       RLEIMAKGTPSLVNMIGNLVKDRD 504
              ************************
```

Fig. 27. Alignment of the deduced amino acids sequences of *LCYB* gene in canary yellow and red watermelon. '*': amino acids in the column are identical.

```
Red_LCYB      MDTLLKINNKYGFLQPLHGVSEKVSGVRST---KFQSQEFGFGHRKGRLKWR-KGGCLNV 56
CY_LCYB       MDTLLKINNKYGFLQPLHGVSEKVSGVRST---KFQSQEFGFGHRKGRLKWR-KGGCLNV 56
Citrus        MDTVLKTHNKLEFLPQVHGALEKSSSLSSL---KIQNQELRFGLKKSRQKRN-MSCFIKA 56
Tomato        MDTLLKTPNNLEFLNPHHGFAVKASTFRSE---KHHN----FGSRKFCETLG-RSVCVKG 52
Pepper        MDTLLRTPNNLEFL---HGFGVKVSAFSSV---KSQK----FGAKKFCEGLGSRSVCVKA 50
Tobacco       MDTLLKTPNKLEFLHPVHGFSVKASSFNSV---KPHK----FGSRKICENWG-KGVCVKA 52
Marigold      MDTFLRTYNSFEFVHPSNKFAGNLNNLNQLNQSKSQFQDFRFGPKKSQFKLG-QKYCVKA 59
Arabidopsis   MDTLLKTPNKLDFFIPQFHGFERLCSNNPY---HSRVR---LGVKKRAIKIV---SSVVS 51
Daffodil      MDTLLRTHNRLELLYPLHELAKRHFLSPSP---NPQNPNFKFFSRKPYQKKC-RNGYIGV 56
Maize         ------MATTALLLRTHHHPCKPPAPRAS------------VLCRATAGMAG------PA 36
                     .   :.                           .   :

Red_LCYB      RSSSLLELVPETKKENLEVELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCAI 116
CY_LCYB       RSSSLLELVPETKKENLEVELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCAI 116
Citrus        SSSALLELVPETKKENLEFELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVCSI 116
Tomato        SSSALLELVPETKKENLDFELPMYDPSKGVVVDLAVVGGGPAGLAVAQQVSEAGLSVCSI 112
Pepper        SSSALLELVPETKKENLDFELPMYDPSKGVVVDLAVVGGGPAGLAVAQQVSEAGLSVCSI 110
Tobacco       KSSALLELVPETKKENLDFELPMYDPSKGLVVDLAVVGGGPAGLAVAQQVSEAGLSVVSI 112
Marigold      SSSALLELVPEIKKENLDFDLPMYDPSRNVVVDLVVVGGGPSGLAVAQQVSEAGLTVCSI 119
Arabidopsis   GSAALLDLVPETKKENLDFELPLYDTSKSQVVDLAIVGGGPAGLAVAQQVSEAGLSVCSI 111
Daffodil      SSNQLLDLVPEIKKEHLEFDLPLYDPSKALTLDLAVVGGGPLARSCSTSLG-GGLSVVSI 115
Maize         SAAALRSLAPPTRPELLSLDLPRYDPAPARPVDLAVVGGGPAGLAVAQRVAEAGLSVCAI 96
               :  * .*.*  : * *..:** **.:    :**.:***** . : :  :. .**:* :*

Red_LCYB      DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVFTNEQSTKDLARPYARVNRKQLK 176
CY_LCYB       DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVFTNEQSTKDLARPYARVNRKQLK 176
Citrus        DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVHIDDDTKKDLDRPYGRVNRKLLK 176
Tomato        DPNPKLIWPNNYGVWVDEFEAMDLLDCLDATWSGAAVYIDDNTAKDLHRPYGRVNRKQLK 172
Pepper        DPNPKLIWPNNYGVWVDEFEAMDLLDCLDATWSGAAVYIDDKTTKDLNRPYGRVNRKQLK 170
Tobacco       DPSPKLIWPNNYGVWVDEFEAMDLLDCLDATWSGTVVYIDDNTTKDLDRPYGRVNRKQLK 172
Marigold      DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSSAVVYIDEKSTKSLNRPYARVNRKQLK 179
Arabidopsis   DPSPKLIWPNNYGVWVDEFEAMDLLDCLDTTWSGAVVYVDEGVKKDLSRPYGRVNRKQLK 171
Daffodil      DPNPKLIWPNNYGVWVDEFEDMDLLDCLDATWSGAIVYVDDRSTKNLSRPYARVNRKNLK 175
Maize         DPSPAVVWPNNYGVWVDEFEAMGLSHCLDTVWPSASVFIDDGGAKSLDRPYARVARRKLK 156
              **.* ::************** *.* .***:.*..: *. ::   *.* ***.** *: **

Red_LCYB      SKMLQKCISNGVKFHEAKVIKVIHEEFKSLLICNDGVTIQAAIVLDATGVSRCLVQYDKP 236
CY_LCYB       SKMLQKCISNGVKFHEAKVIKVIHEEFKSLLICNDGVTIQAAIVLDATGFSRCLVQYDKP 236
Citrus        SKMLQKCITNGVKFHQAKVIKVIHEESKSLLICNDGVTIQAAVVLDATGFSRCLVQYDKP 236
Tomato        SKMMQKCIMNGVKFHQAKVIKVIHEESKSMLICNDGITIQATVVLDATGFSRSLVQYDKP 232
Pepper        SKMMQKCILNGVKFHQAKVIKVIHEESKSMLICNDGITIQATVVLDATGFSRSLVQYDKP 230
Tobacco       SKMMQKCILNGVKFHHAKVIKVIHEEAKSMLICNDGVTIQATVVLDATGFSRCLVQYDKP 232
Marigold      TKMLQKCIANGVKFHQAKVIKVIHEELKSLLICNDGVTIQATLVLDATGFSRSLVQYDKP 239
Arabidopsis   SKMLQKCITNGVKFHQSKVTNVVHEEANSTVVCSDGVKIQASVVLDATGFSRCLVQYDKP 231
Daffodil      SKMMKKCVSNGVRFHQATVVKAMHEEEKSYLICSDGVTIDARVVLDATGFSRCLVQYDKP 235
Maize         STMMDRCVANGVVFHQAKVAKAVHYDASSLLICDDGVAVPASVVLDATGFSRCLVQYDKP 216
              :.*:.:*: *** **.:.* :.:* : .* ::*.**: : * :******.**.*******
```

Fig. 28. Alignment of amino acids sequences of *LCYB* gene in canary yellow and red watermelon with other species. '*': amino acids in the column are identical.

```
Red_LCYB      YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLNNNMILKERNSKIPTFLYAMPFSSN 296
CY_LCYB       YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLNNNMILKERNSKIPTFLYAMPFSSN 296
Citrus        YNPGYQVAYGILAEVEEHPFDLDKMVFMDWRDSHLNNNSELKEANSKIPTFLYAMPFSSN 296
Tomato        YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLKNNTDLKERNSRIPTFLYAMPFSSN 292
Pepper        YNPGYQVAYGILAEVEEHPFDVNKMVFMDWRDSHLKNNVELKERNSRIPTFLYAMPFSSN 290
Tobacco       YKPGYQVAYGILAEVEEHPFDTSKMVLMDWRDSHLGNNMELKERNRKVPTFLYAMPFSSN 292
Marigold      YNPGYQVAYGILAEVEEHPFDVDKMLFMDWRDSHLDQNLEIKARNSRIPTFLYAMPFSST 299
Arabidopsis   YNPGYQVAYGIVAEVDGHPFDVDKMVFMDWRDKHLDSYPELKERNSKIPTFLYAMPFSSN 291
Daffodil      YNPGYQVAYGILAEVEEHPFDVDKMVFMDWRDSHLNGKAELNERNAKIPTFLYAMPFSSN 295
Maize         YNPGYQVAYGILAEVDAHPFDIDKMLFMDWRDSHLPEGSEIRERNRRIPTFLYAMPFSPT 276
              *:*********:***: **** .**::*****.**     :.  * ::**********..

Red_LCYB      RIFLEETSLVARPGLQMSDIQERMEVRLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVV 356
CY_LCYB       RIFLEETSLVARPGLQMSDIQERMEVRLKHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVV 356
Citrus        RIFLEETSLVARPGVPMKDIQERMVARLKHLGIKVRSIEEDEHCVIPMGGPLPVLPQRVV 356
Tomato        RIFLEETSLVARPGLRIDDIQERMVARLNHLGIKVKSIEEDEHCLIPMGGPLPVLPQRVV 352
Pepper        RIFLEETSLVARPGLGMDDIQERMVARLSHLGIKVKSIEEDEHCVIPMGGPLPVLPQRVV 350
Tobacco       KIFLEETSLVARPGLRMDDIQERMVARLNHLGIKVKSIEEDEHCVIPMGGSLPVIPQRVV 352
Marigold      RIFLEETSLVARPGLKMEDIQERMAYRLKHLGIKVKSIEEDERCVIPMGGPLPVLPQRVL 359
Arabidopsis   RIFLEETSLVARPGLRMEDIQERMAARLKHLGINVKRIEEDERCVIPMGGPLPVLPQRVV 351
Daffodil      RIFLEETSLVARPGLKMEDIQERMVARLNHLGIRIKSIEEDERCVIPMGGPLPVIPQRVV 355
Maize         RIFLEETSLVARPGLAMDDIQERMAARLRHLGIRVRSVEEDERCVIPMGGPLPVLPQRVV 336
              :**************: :.******  ** ****.:: :****:*:*****.***:****:

Red_LCYB      GIGGTAGMVHPSTGYMVARTLAAAPIVASAIVRCLGSDG-----RFRGDAISSEVWKDLW 411
CY_LCYB       GIGGTAGMVHPSTGYMVARTLAAAPIVASAIVRCLGSDG-----RFRGDAISSEVWKDLW 411
Citrus        GIGGTAGMVHPSTGYMVARTLAAAPIVANAIVRSLSSDR-----SISGHKLSAEVWKDLW 411
Tomato        GIGGTAGMVHPSTGYMVARTLAAAPVVANAIIQYLGSER-----SHSGNELSTAVWKDLW 407
Pepper        GIGGTAGMVHPSTGYMVARTLAAAPVVANAIIQYLSSER-----SHSGDELSAAVWKDLW 405
Tobacco       GTGGTAGLVHPSTGYMVARTLAAAPVVANAIIHYLGSEK-----DLLGNELSAAVWKDLW 407
Marigold      GIGGTAGMVHPSTGYMVARTLAAAPIVAKSIIRYLNNEKSM-VADVTGDDLAAGIWRELW 418
Arabidopsis   GIGGTAGMVHPSTGYMVARTLAAAPIVANAIVRYLGSPSS---NSLRGDQLSAEVWRDLW 408
Daffodil      GIGGTAGMVHPSTGYMVARTLAAAPIVANSIVQYLVSDS-----GLSGNDLSADVWKDLW 410
Maize         GIGGTAGMVHPSTGYMVARTLATAPIVADAIVRFLDTGTGNGMGGLAGDALSAEVWKQLW 396
              * *****:***************:**:**.:*:: * .          *. ::: :*::**

Red_LCYB      PIERRRQREFFCFGMDILLKLDLKGTRRFFDAFFDLEPRYWHGFLSSRLFLPELLLFGLS 471
CY_LCYB       PIERRRQREFFCFGMDILLKLDLKGTRRFFDAFFDLEPRYWHGFLSSRLFLPELLLFGLS 471
Citrus        PIERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLFLPELLVFGLS 471
Tomato        PIERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLFLPELIVFGLS 467
Pepper        PIERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLFLPELIVFGLS 465
Tobacco       PIERRRQREFFCFGMDILLKLDLPATRRFFDAFFDLEPRYWHGFLSSRLYLPELIFFGLS 467
Marigold      PIERRRQREFFCFGMDILLKLDLEGTRRFFDAFFDLEPRYWHGFLSSRLFLPELVTFGLS 478
Arabidopsis   PIERRRQREFFCFGMDILLKLDLDATRRFFDAFFDLQPHYWHGFLSSRLFLPELLVFGLS 468
Daffodil      PIERRRQREFFCFGMDILLKLDLEGTRRFFDAFFDLEPRYWHGFLSSRLFLPELVPFGLS 470
Maize         PANRRRQREFFCFGMDVLLKLDLEGTRRFFDAFFDLEPHYWHGFLSSRLFLPELLMFGLA 456
              * :**************:****** .***********:*:**********:****: ***:
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Fig. 28. Continued.

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Red_LCYB      LFSHASNASRLEIMAKGTPSLVNMIGNLVKDRD- 504
CY_LCYB       LFSHASNASRLEIMAKGTPSLVNMIGNLVKDRD- 504
Citrus        LFSHASNTSRLEIMAKGTLPLVNMINNLVQDTD- 504
Tomato        LFSHASNTSRFEIMTKGTVPLVNMINNLLQDKE- 500
Pepper        LFSHASNTSRLEIMTKGTLPLVHMINNLLQDKE- 498
Tobacco       LFSRASNTSRIEIMTKGTLPLVNMINNLLQDTE- 500
Marigold      LFGHASNTCRVEIMAKGTLPLATMIGNLVRDRE- 511
Arabidopsis   LFSHASNTSRLEIMTKGTVPLAKMINNLVQDRD- 501
Daffodil      LFSHASNTCKLEIMAKGTLPLVNMINNLVQDRD- 503
Maize         LFGNASNSSRLEIMAKGTVPLGKMIGNLIQDRDG 490
              **..***:.:.***:*** .*  **.**::* :
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Fig. 28. Continued.

CHAPTER IX

CONCLUSIONS

Comparisons of three diploid (2n = 2*x* = 22) and three triploid (2n = 3*x* = 33) commercial cultivars grown in two irrigation regimes in three distinct Texas locations confirmed that deficit irrigation reduces watermelon marketable yields as seen in different environmental condition. Yield of cultivars and ploidy level (diploid vs. triploid) also varied with three distinctive Texas location due to regional weather condition. Firmness and soluble solids content was higher under deficit irrigation in the triploids. Lycopene content increased significantly within 14 days of maturity, and was relatively constant during the overmature stage of triploid cultivars. Lycopene content did not decline with limited irrigation (0.75 ET) at Uvalde, Weslaco, and Lubbock. Genotypes had a stronger influence on fruit lycopene content than ploidy or the environmental conditions in each location, indicating that selection can be used to enhance health benefits of watermelons. Evaluation of watermelon breeding lines and plant introductions that have the best sources of lycopene and other carotenoids is critical to develop novel colored watermelon varieties with high levels of beneficial compound for human health.

Full-length cDNAs of genes encoding enzymes in the carotenoid biosynthetic pathway were isolated using degenerate PCR and RACE approaches. Two homologous of phytoene synthase were isolated (*PSY-A* and *PSY-B*). In *LCYB*, a 229-bp leader intron was identified, and an unspliced mRNA with leader intron existed dominantly.

Differential expression of *PSY-A* was not detected in different colored flesh. All genes except *PSY-B* were expressed in all flesh colors. *PSY-B* was expressed only in ovary, leaf and root tissue, similar to *PSY2* in tomato. Expression of *CRTISO* was not detected in salmon yellow, which may be due to a mutation reducing enzyme activity of *CRTISO* resulting from a mutation in the promoter region. Two SNPs were detected in a coding region of *LCYB*. These SNPs showed perfect co-segregation with canary yellow and red phenotypes. A codominant CAPS marker was developed from SNP most likely to cause the mutation for the allelic selection of *LCYB* gene. Furthermore, the CAPS marker development will allow breeders economically to distinguish between red and canary yellow watermelon fruit colors at seedling stages. Regulatory mechanisms of carotenogenesis in watermelon are controlled at both the transcriptional and post-transcriptional levels.

LITERATURE CITED

Allen, R.G., L.S. Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration-guidelines for computing crop water requirements. FAO Irrigation and Drainage Paper No. 56. Rome, Italy. p. 300.

Bartley G.E. and P.A. Scolnik. 1993. cNDA cloning, expression during development, and genome mapping of PSY2, a second tomato gene encoding phytoene synthase. J. Biol. Chem. 268:25718-25721.

Bartley G.E. and P.A. Scolnik. 1995. Plant carotenoids: pigments for photoprotection, visual attraction, and human health. The Plant Cell 7:1027-1038.

Bartley G.E., P.A. Scolnik, and P. Beyer. 1999. Two *Arabidopsis thaliana* carotene desaturases, phytoene desaturase and ζ-carotene desaturase, expressed in *Escherichia coli*, catalyze a poly-*cis* pathway to yield pro-lycopene. Eur. J. Biochem. 259:396-403.

Bartley. G.E., P.V. Viitanen, K.O. Bacot, and P.A. Scolink. 1992. A tomato gene expressed during fruit ripening encodes an enzyme of the carotenoid biosynthesis pathway. J. Biol. Chem. 267:5036-5039.

Bramley, P.M. 2000. Is lycopene beneficial to human health? Phytochemistry 54:233-236.

Bramley, P.M. 2002. Regulation of carotenoid formation during tomato fruit ripening and development. J. Exp. Bot. 53(377):2107-2113

Britton, G., S. 1998. Overview of carotenoid biosynthesis, p. 13-147. In: G. Britton, S.

Liaaen-Jensen, and H. Pfander (eds.). Carotenoids Vol. 3 Biosynthesis and Metabolism. Birkhäuser Verlag, Basel, Switzerland.

Buishand, J.G. and W.H. Gabelman. 1979. Investigation on the inheritance of color and carotenoid content in phloem and xylem of carrot roots (*Daucus car*ota L.). Euphytica 28:611-632.

Buishand, J.G. and W.H. Gabelman. 1980. Studies on the inheritance of root color and carotenoid content in red x yellow and red x white crosses of carrot, *Daucus car*ota L. Euphytica 29:241-260.

Chenna, R., H. Sugawara, T. Koike, R. Lopez, T.J. Gibson, D.G. Higgins, J.D. Thompson. 2003. Multiple sequence alignment with the Clustal series of programs. Nucleic Acids Res 31:3497-3500.

Cirulli, M. and F. Ciccarese. 1981. Effect of mineral fertilizers on the incidence of blossom-end rot of watermelon. Phytopathology 71:50-53.

Cunningham, F.X. Jr. and E. Gantt. 1998. Genes and enzymes of carotenoid biosynthesis in plants. Annu. Rev. Plant Pysiol. Plant Mol. Biol. 49:557-583.

De Pascale, S., G. Barbieri, and C. Ruggiero. 1998. Consumptive water use and plant growth of watermelon as affected by irrigation and N fertilization. Acta Hort. 458:49-56.

Erdem, Y. and A.N. Yuksel. 2003 Yield response of watermelon to irrigation shortage. Scientia Horticulturae 98:365-383.

Fambrini, M., M. Salvini, A. Conti, V. Michelotti, and C. Pugliesi., 2004. Expression of the ζ-carotene desaturase gene in sunflower. 138:203-206.

Fish, W.W., P. Perkins-Veazie, and J.K. Collins. 2002 A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. J. Food Composition and Analysis 15:309-317.

Fraser, P.D. J.W. Kiano, M.R. Truesdale, W. Schuch, and P.M. Bramley. 1999. Phytoene synthase-2 enzyme activity in tomato does not contribute to carotenoid synthesis in ripening fruit. Plant Mol. Biol. 40:687-698.

Fraser, P.D., M.R. Truesdale, C.R. Bird, W. Schuch, and P.M. Bramley. 1994. Carotenoid biosynthesis during fruit development. Plant Physiol. 105:405-413.

Fray, R.G. and D. Grierson. 1993. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. Plant Mol. Biol. 22:589-602.

Frecknall, E.A. and G. Pattenden. 1984. Carotenoid differences in isogenic lines of tomato fruit colour mutants. Phytochemistry 23:1707-1710.

Gallagher, C.E., P.D. Matthews, F. Li, and E.T. Wurtzel. 2004. Gene duplication in the carotenoid biosynthetic pathway preceded evolution of the grasses. Plant Physiol. 135:1776-1783.

Gerster, H. 1997. The potential role of lycopene for human health. Journal of the American College of Nutrition 16:109-126.

Giovannucci, E. 1999 Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. Journal of the National Cancer Institute 91:317-331.

Giovannucci, E., E.B. Rimm, Y. Liu, M.J. Stampfer, W.C. Willett. 2002. A prospective study of tomato products, lycopene, and prostate cancer risk. Journal of the National

Cancer Institute 94:391-398.

Giuliano, G., R. Aquilani, and S. Dharmapuri. 2000. Metabolic engineering of plant carotenoids. Trends in Plant Sci. 5:406-409.

Giuliano, G., G.E. Bartley, and P.A. Scolnik. 1993. Regulation of carotenoid biosynthesis during tomato development. The Plant Cell 5:379-387.

Giuliano, G., L. Gilibero, and C. Rosati. 2002. Carotenoid isomerase: a tale of light and isomers. Trends in Plant Sci. 7(10):427-429.

Guner, N. and T.C. Wehner. 2003. Gene list for watermelon. Cucurbit Genetics Coop. Rpt. 26:76-92.

Handelman, G.J. 2001. The evolving role of carotenoids in human biochemistry. Nutrition 17:818-822.

Henderson, W.R. 1989. Inheritance of orange flesh color in watermelon. Cucurbit Genetics Coop. Rpt. 12:59-63.

Henderson, W.R., G.H. Scott, and T.C. Wehner. 1998. Interaction of flesh color genes in watermelon. J. Heredity. 89:50-53.

Herbinger, K., M. Tausz, A. Wonisch, G. Soja, A. Sorger, and D. Grill. 2002. Complex interactive effects of drought and ozone stress on the antioxidant defense systems of two wheat cultivars. Plant Physiol. Biochem. 40:691-696.

Hirschberg, Joseph. 2001. Carotenoid biosynthesis in flowering plants. Current Opinion in Plant Biology. 4:210-218.

Holden J.M., A.L. Eldridge, G.R. Beecher, I.M. Buzzard, S.A. Bhagwat, C.S. Davis, L.W. Douglass, S.E. Gebhardt, D.B. Haytowitz, and S. Schakel. 1999 Carotenoid

content of U.S. foods: an update of the database. J. Food Comp. Anal. 12:169-196.

Hornero-Mendez, D., R. Gomez-Ladron de Guevara, and M.I. Minguez-Mosquera. 2000. Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. J. Agric. Food Chem. 48:3857-3864.

Ikoma, Y., A. Komatsu, M. Kita, K. Ogawa, M. Omura, M. Yano, and T. Moriguchi. 2001. Expression of a phytoene synthase gene and characteristic carotenoid accumulation during citrus fruit development. Physiol. Plant. 111:232-238.

Imam, M.K. and W.H. Gabelman. 1968. Inheritance of carotenoids in carrots, *Daucus carota* L. Proc. Amer. Soc. Hort. Sci. 93:419-428.

Isaacson, T., G. I. Ohad, P. Beyer, and J. Hirschberg. 2004. Analysis in vitro of the enzyme CRTISO establishes a poly-cis-carotenoid biosynthesis pathway in plants. Plant Physiol. 136:4246-4255.

Isaacson, T., G. Ronen, D. Zamir, and J. Hirschberg. 2002. Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of β-carotene and xanthophylls in plants. The Plant Cell 14:333-342.

Jenkins, J.A. and G. MacKinney. 1953. Inheritance of carotenoid differences in the tomato hybrid yellow x tangerine. Genetics 38:107-116.

Karvouni, Z., I. Johnson, J.E. Taylor, C.F. Watson, A.J. Turner, and D. Grierson. 1995. Isolation and characterization of a melon cDNA clone encoding phytoene synthase. Plant Mol. Biol. 27:1153-1162.

Kato, M. Y. Ikoma, H. Matsumoto, M. Sugiura, H. Hyodo, and M. Yano. 2004. Accumulation of carotenoids and expression of carotenoid biosynthetic genes during

maturation in citrus fruit. Plant Physiol. 134:824-837.

Kim, S., K.S. Yoo, and L.M. Pike. 2005a. Development of a codominant PCR-based marker for allelic selection of the pink trait in onions (*Allium cepa*), based on the insertion mutation in the promoter of the anthocyanin synthase gene. Theor. Appl. Genet. 110:573-578.

Kim, S., K.S. Yoo, and L.M. Pike. 2005b. Development of a PCR-based marker utilizing a deletion mutation in the dihydroflavonol 4-reductase (DFR) gene responsible for the lack of anthocyanin production in yellow onions (*Allium cepa*). Theor. Appl. Genet. 110:588-595.

Kumar, S., K. Tamura, and M. Nei. 2004. MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics 5:150-163.

Laferriere, L. and W.H. Gabelman. 1968. Inheritance of color, total carotenoids, alpha-carotene, and beta-carotene in carrots, *Daucus carota* L. Proc. Amer. Soc. Hort. Sci. 93:408-418.

Lefebvre, V., M. Kuntz. B. Camara, and A. Palloix. 1998. The capsanthin-capsorubin synthase gene: a candidate gene for the *y* locus controlling the red fruit colour in pepper. Plant Molecular Biology. 36:785-789.

Leskovar, D.I., H. Bang, K.M. Crosby, N. Maness, J.A. Franco, and P. Perkins-Veazie. 2004. Lycopene, carbohydrates, ascorbic acid, and yield components of diploid and triploid watermelon cultivars are affected by deficit irrigation. Journal of Horticultural Science & Biotechnology 79:75-81.

Levi, A., C.E. Thomas, M. Newman, O.U.K. Reddy, X. Zhang, and Y. Xu. 2004. ISSR and AFLP markers differ among American watermelon cultivars with limited genetic diversity. J. Amer. Soc. Hort. Sci. 129(4):553-558.

Lewinsohn, E., Y. Sitrit, E. Bar, Y. Azulay, A. Meir, D. Zamir, and Y. Tadmor. 2005. Carotenoid pigmentation affects the volatile composition of tomato and watermelon fruits, as revealed by comparative genetic analyses. J. Agric. Food Chem. 53:3142-3148.

Lincoln, R.E. and J.W. Porter. 1950. Inheritance of beta-carotene in tomatoes. Genetics 35:206-211.

MacArthur, J.W. 1934. Linkage groups in the tomato. J. of Genetics 29:123-133.

MacKinney, G. and J.A. Jenkins. 1949. Inheritance of carotenoid differences in *Lycopersicon esculentum* strains. Proc. Nat. Acad. Sci. 35:284-291

Maynard, D.N. 2001. An introduction to the watermelon, p. 9- 20. In: D.N. Maynard (ed.). Watermelon characteristics, production, and marketing. ASHS Press, Alexandria, Virginia.

Navot, N., M. Sarfatti, and D. Zamir. 1990. Linkage relationships of genes affecting bitterness and flesh color in watermelon. J. Heredity 81:161-165.

Orta, A.H., Y. Erdem, and T. Erdem. 2003. Crop water stress index for watermelon. Scienctia Horticulturae 98:121-130.

Pecker, I., D. Chamovitz, H. Linden, G. Sandman, and J. Hirschber. 1992. A single polypeptide catalyzing the conversion of phytoene to ζ-carotene is transcriptionally regulated during tomato fruit ripening. Proc. Natl. Acad. Sci. 89:4962-4966.

Pecker, I., R. Gabbay, F.X. Cunningham Jr., and J. Hirschberg. 1996. Cloning and characterization of the cDNA for lycopene β-cyclase from tomato reveals decrease in its expression during fruit ripening. Plant Mol. Biol. 30:807-819.

Perkins-Veazie, P., J. K. Collins, S.D. Pair, and W. Roberts. 2001. Lycopene content differs among red-fleshed watermelon cultivars. J. Sci. Food Agric. 81:983-987.

Perkins-Veazie, P., J. K. Collins, S.D. Pair, and W. Roberts. 2002b. Watermelon; Lycopene content changes with ripeness stage, germplasm, and storage, p.427-430. In: D.N. Maynard (ed.). Cucurbitaceae 2002, Naples, Florida. ASHS Press.

Perkins-Veazie, N. Maness, and R. Roduner. 2002a. Composition of orange, yellow, and red-fleshed watermelons, p.436-440. In: D.N. Maynard (ed.). Cucurbitaceae 2002, Naples, Florida. ASHS Press

Pier, J.W and T.A. Doerge. 1995. Nitrogen and water interactions in trickle-irrigated watermelons. Soil Science Society of America Journal 59:145-150.

Poole, C.F. 1944. Genetics of cultivated cucurbits. J. Heredity 35:122-128.

Romer, S., P. Hugueney, F. Bouvier, B. Camara, and M. Kuntz. 1993. Expression of the gene encoding the early carotenoid biosynthetic enzymes in *Capsicum annuum*. Biochem. Biophys. Res. Comm. 196:1414-1421.

Ronen, G., M. Cohen, D. Zamir, and J. Hirschberg. 1999. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant *Delta*. The Plant Journal 17:341-351.

Ronen, G., L. Carmel-Goren, D. Zamir, and J. Hirschberg. 2000. An alternative pathway

to β-carotene formation in plant chromoplasts discovered by map-based cloning of *Beta* and *old-gold* color mutations in tomato. Proc. Natl. Acad. Sci. 97:11102-11107.

Sadler, G., J. Davis, and D. Dezman. 1990. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. Journal of Food Science 55:1460-1465.

Salvini, M., A. Bernini, M. Fambrini, and C. Pugliesi. 2005. cDNA cloning and expression of the phytoene synthase gene in sunflower. J. Plant Physiol. 162:479-484.

Sandmann, G. 2001. Genetic manipulation of carotenoid biosynthesis: strategies, problems and achievements. Trends in Plant Sci. 6(1):14-17.

Sauret-Güeto, S. P. Botella-Pavia, and M Rodriguez-Concepción. 2003. Molecular tools for the metabolic engineering of carotenoid biosynthesis in plants. Recent Res. Devel. Plant Mol. Biol. 1:339-363.

Semba, R.D. and G. Dagnelie. 2003. Are lutein and zeaxanthin conditionally essential nutrients for eye health? Medical Hypotheses 61:465-472.

Smajstrla, A.G., B.J. Boman, G.A. Clark, D.Z. Haman, D.S. Harrison, F.T. Izuno, D.J. Pitts, and F.S. Zazueta. 2002. Efficiencies of Florida agricultural irrigation systems. EDIS of UF/IFAS Extension BUL247. p. 1-14.

Tadmor, Y., N. Katzir, S. King, A. Levi, A. Davis, and J. Hirschberg. 2004. Fruit coloration in watermelon: lesson from the tomato. Proc. Cucurbitaceae 2004, the 8th EUCARPIA Meeting on Cucurbit Genetics and Breeding, Olomouc, Czch Republic. p. 181-185.

Taiz, L. and E. Zeiger. 1998. Photosynthesis: physiological and ecological considerations. p. 227-249. In: Plant Physiology. Sinauer Associates, Inc., Sunderland, Massachusetts.

Thompson, J.D., D.G. Higgins, and T.J. Gibson. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research 22:4673-4680.

Thompson, J.D., T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins. 1997. The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research 24:4876-4882.

Tomes, M.L. 1963. Temperature inhibition of carotene synthesis in tomato. Botanical Gazette 124:180-185.

Tomes, M.L. and K.W. Johnson. 1965. Carotene pigments of an orange-fleshed watermelons. Proc. Amer. Soc. Hort. Sci. 87:438-442.

Tomes, M.L., K.W. Johnson, and M. Hess. 1963. The carotenoid pigment content of certain red fleshed watermelons. Proc. Amer. Soc. Hort. Sci. 82:460-464.

Tomes, M.L., F.W. Quackenbush, and T.E. Kargl. 1956. Action of the gene B in biosynthesis of carotenes in the tomato. Botanical Gazette 117:248-253.

Tomes, M.L., F.W. Quackenbush, and M. McQuistan. 1954. Modificaiton and dominance of the gene governing formation of high concentrations of beta-carotene in the tomato. Genetics 39:810-817.

Tomes, M.L., F.W. Quackenbush, O.E. Nelson, Jr., and B. North. 1953. The inheritance

of carotenoid pigment systems in the tomato. Genetics 38:117-127.

Umiel, N. and W.H. Gabelman. 1972. Inheritance of root color and carotenoid synthesis in carrot, *Daucus carota* L.: orange vs. red. J. Amer. Soc. Hort. Sci. 97:453-460.

USDA-National Agricultural Statistics Service. 2004. Statistics of vegetables and melons. p. 33. In: 2004 Agricultural Statistics. USDA-NASS, Washington, DC.

Van den Berg, H., R. Faulks, H. Fernando Granado, J. Hirschberg, B. Olmedilla, G. Sandmann, S. Southon, and W. Stahl. 2000. The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J. Sci. Food Agric. 80:880-912.

Vincze, T., J. Posfai, and R.J. Roberts. 2003. NEBcutter: a program to cleave DNA with restriction enzymes. Nucleic Acids Res. 31:3688-3691.

Zechmeister, L., A.L. LeRosen, F.W. Went, and L. Pauling. 1941. Prolycopene, a naturally occurring stereoisomer of lycopene. Proc. Nat. Acad. Sci. 27:468-474.

APPENDIX A

Full-length cDNA of *PSY-A* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TAG).

5′–
ACGCGGGGAAAGCTGCAGAGAGAAGAGAGGAAGCAAGACCCAATAAAATATTGGGGGGGGGGGGTTTTGTG
GGTAGTTGTGAAGAAG**ATG**TCTGGTGTGAATGCCAACTCTCTGCTGAGCCCCAAGCCAAGAATCAGAATCA
GCAGCAAGCCATTTGGGTCTAGAAGATTGAGTTTCTTTTCTGATGGGGTTTTGGCTTCCTCTGCTGCTGTG
GTGAATCCTTCAAGATCGTCTGAAGAAAGGGTCTATGAAGTTGTGCTGAAGCAAGCGGCTCTTGTGAGAGA
ACCCAAAAGGGATATTCAGAGAGCTTTGGATTGGGAAAAAACCATCCAAAATGAGGGCATCACTGATGGGA
ATCTCTTGTCTGAGGCTTATTCTCGCTGTGGTGAGGTCTGTGCTGAATATGCCAAAACATTTTACTTGGGG
ACACAACTTATGACACCAGAGCGAAGAAGAGCCGTGTGGGCGATTTATGTGTGGTGCAGAAGGACTGATGA
GCTCGTGGATGGACCTAATGCTTCACACATCACCCCTAAAGCTCTTGAGCGATGGGAAAAACGACTAACTG
ATCTATTTGAGGGTCGACCATATGATATGTATGATGCTGCTCTTTCCGATACAGTCTCAAAATACCCTGTT
GACATTCAGCCCTTCAAGGACATGATCGAAGGAATGAGGTTGGACCTGCGAAAATCAAGATATGAGAACTT
TGACGAGCTTTACCTTTATTGCTATTATGTTGCGGGGACTGTGGGGCTCATGAGTGTTCCTGTCATGGGAT
TGGCACCTGAGTCGAAAGCTTCAGTAGAGAGCGTCTACAATGCAGCATTGGCTCTCGGACTCGCCAATCAA
CTCACCAACATTCTCAGAGACGTTGGAGAAGATGCTAGGAGGGGAAGAGTATATCTCCCACAAGACGAGTT
GGCACAAGCAGGGCTATGCGACGACGACATATTCCGAGGGAAAGTGACTGACAAGTGGCGGTTTTTCATGA
AAGGACAGATAAAAAGAGCAAGAAGGTTCTTTGATGAGGCTGAGAAGGGAGTTGCAGAACTTAGTGCAGCC
AGTAGATGGCCAGTGTGGGCATCCCTAATGCTTTATAAGCAAATATTGGATTCCATTGAAGCAAATGACTA
TGACAATTTCACCAAAAGGGCATATGTAGGCAAAGCAAAGAAACTGTTATCCCTTCCCATAGCCTTTGGGA
GAGCTATGGTGGGCCCCTCAAGCTTCAAAGATTTGGTAACAAGA**TAG**TTCCTTTTCCTTTTTTTTTTTTCT
TCTTTTCTTTTCCCCCCTTTTTGTGATTGTTCAATATGTCTAGACAATTTGCTGATTGTAAATTTTAGGTG
TTAGATGTCTTTGAAGCAAATTGACTTCTCCAAAGAAAAAGAATAGCTTGGAAATAGTATAATGGAAAAAA
AAAAAAAAAAAAAAAAAAA–3′

APPENDIX B

Full-length cDNA of *PSY-B* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TGA).

5′–
CGTCNATTACCCTCCTAAAGGGACTAGTCCTGCAGGTTTAACGAATTCGCCCTTAAGCAGTGGTATCAACG
CAGAGTACGCGGGGGGGGGCTTTTCTCAAAAGATTTATAAA**ATG**AGTTCTTATATTTGCATCACACCAAAG
CCTAGCATATTCATCAGAGAATGCAAAGGGAAGCTTTTTCCAAAACGATTCACACTTATAATGAGCAAAAG
TGGGGTAATTGCAGCTCCCAAAAACCCTCAGAGATTAAAGTTTCCAACTCTATCAAAACAAGGTATTCCTC
TGGCTGATTTGAACGTCGATGAGATCGTCGAAAGACAATCTCATGCCAACAATTTTTCAAGAGAAGAATCG
TGTAAGAAGAAGCAGCAATTTCACCCTTCATTTCTTGAAGAAGCTTATGAGAGTTGCAGGAAAATCTGTGC
AGAATATGCCAAGACTTTCTATTTG**GGA**ACTCTGCTGATGACAAAGGAGCGACAAAGAGCAATATGGGCAA
TCTATGTTTGGTGCAGGAGAACAGATGAACTTGTGGATGGCCCCAATGCTGTGTATATGAATCCAAAAGTT
CTTGATCGATGGGAAGAACGTTTGGAAGACATCTTTGAAGGGTGTCCCTATGATTTGCTGGATGCTGCTTT
GAGTCACACGGTGTCTAGATTTCCCATAGACATGAAGCCTTTCAAGGACATGATTGAAGGCATGAGAATGG
ACACTAAAAAGTGTCGATATGAGAATTTTGAAGAGTTGTATTTGTATTGTTATTATGTGGCTGGAACTGTG
GGACTAATGAGTGTACCTGTTATGGGAATTGCACCTGATTCTTCACTTCCTACTCAGACTATTTACAGTGC
TGCTCTCCACTTGGGGATTGGAAATCAACTTACCAATATTCTTAGAGATGTTGGAGAGGATGCTATAAGGG
GTAGGATATATCTTCCTCAAGACGAGCTTGCACAGTTCGGGTTATGCGACGACGATATATTGGCTATGAGA
GTGACTGAGAAGTGGAGAGAATTCATGAAAGAACAGATCAAACGGGCGAAGTTCTATTTCAAACTAGCAGA
AAAGGGAGCTTCTCAGCTAGACAAGGCCAGCCGTTGGCCGGTATGGTCATCCTTGATGTTGTACCGAAAAA
TATTGGAAGCAATTGAAGAAAACGATTACAACAACTTCACAAAGCGAGCTTATGTAAGGAGATCCAAGAAA
CTTCTCACACTGCCTCTTGCTTACACTAAAGCTATTTCGGCACCCAGTCTAGTCTTCCAT**TGA**–3′

APPENDIX C

Full-length cDNA of *PDS* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TAA).

5′–
ACGCGGGGGTTCTTGCATACTTCTCTGATCTACCCATTTCTCCACAGTACCGTGGTGGCCGGAGAAGTGTA
CTTCTTGCTCTGGGGGTATACCGTTTATGTGCTTCTAGTGTCTTCTGAGTTGGAATTGCCTTGATATTTAG
GCCTTAAGACAATTCGTGAAGCCTACAAGTGATTTCTGGTCGTGATTCTTGGTTTCATTTCATTCACCGAA
GTAGTTTAGTTTTCTTTCTGGAATTCTGGGTCTTCGGTGAAAGGATTAATTGTTGTCTGCTTCTGTTTATG
AGCTTGTGTATTGAAGGGATTGGAGCTAAGATTTAGTTGTGGGAAGTGGGGTTTTGGTGAA**ATG**TCACTAT
GTGGGTCTGTCTCTGCTCTGAACTTGAGGTGGGAAAAAGGTATTCCAAAAGCAACCTCGAGATGCTGTTCT
CCATTAAGTTGTGAGAAAAGTAATGCTTTAGCGTTTTGGGGGAGTGAGATTGTGGGCGACGGTTTGAAAGT
ATCTGGCAGACATGTTAGTAGGAAACTATCTAAAGGAAACGTACCACTAAAGGTAGTTTGCGTGGATTACC
CTAGACCACAGATAGATGATACTGTTAATTTCATTGAAGCAGCTTCCTTATCTGCTAGTTTTCGTGCTTCT
GCACGTCCCAGTAAACCATTGAAAATAGTGATTGCTGGTGCAGGATTGGCTGGTTTATCGACAGCAAAATA
TCTGGCAGATGCTGGCCACAAACCTGTTTTACTAGAAGCTCGAGATGTTTTAGGTGGAAAGGTAGCTGCTT
GGAAAGATAATGATGGAGACTGGTATGAGACTGGTCTGCACATATTTTTTGGGGCTTATCCCAATGTGCAG
AACTTGTTTGGAGAACTTGGAATCAATGACCGATTGCAGTGGAAGGAACATTCAATGATATTTGCTATGCC
AAACAAGCCGGGGGAGTTCAGTCGATTTGATTTCCCTGAAAAACTTCCTGCACCCGTAAACGGGATATGGG
CTATTTTAAGGAACAACGAGATGCTTACTTGGCCTGAGAAAATTAAATTTGCAATTGGGCTCCTGCCAGCA
ATGCTTGGTGGGCAATCTTATGTTGAGGCTCAAGATAATTTAACTGTGCAAGAGTGGATGAGAAGTCGGGG
AGTACCTGATCGTGTAACAACAGAGGTGTTTATTGCTATGTCAAAGGCTCTGAACTTCATTAACCCCGATG
AACTTTCTATGCAATGCATTTTGATTGCTTTGAATCGATTTCTTCAGGAGAAGCATGGCTCTAAGATGGCT
TTCTTAGATGGAAATCCACCTGAGAGACTATGTGAGCCAATTGTCGAGCATATTCAGTCATTGGGTGGTGA
AGTACGATTTAATTCAAGGATACAAAAAATTGAGTTAAACAATGATGGAACAGTGAAGAGGTTCTTGTTAA
ACGATGGGAATGTAATTGAAGGAGATGCTTATGTATTTGCCACTCCTGTTGATATCCTGAAGCTTCTTTTG
CCTAATGACTGGAAAGCGATCCCATACTTCAAAAAACTGGAAAAATTAGTTGGAGTTCCAGTTATCAATGT
CCACATATGGTTTGACAGGAAACTGAAGAATACATATGATCATTTACTTTTTAGCAGGAGTCCACTTCTTA
GTGTTTATGCTGACATGTCAGTTACATGTAAGGAATATTACAACCCAAACCAGTCCATGTTGGAACTAGTA
TTTGCCCCTGCAGAAGAATGGATTTCCCGGAGTGACTCAGAAATTATTGATGCCACAATGGTGGAACTAGC
TAAACTATTTCCTGATGAAATTTCTGCTGATCAGAGCAAAGCTAAGATTGTGAAATACCACGTTGTTAAAA
CCCCAAGGTCTGTTTACAAGACTGTGCCCGATTGTGAACCCTGTCGCCCCTTACAACGATCTCCTATTGAG
GGATTTTATCTAGCTGGTGACTACACAAAACAGAAGTATTTAGCTTCTATGGAAGGTGCTGTTCTTTCGGG
AAAGCTTTGTGCACAGGCTATTGTAAAGGACTATGAAGTGCTAGTTGCTCGAGAGCAAAGACGAGTCGCCG
AGGCTGGCATTCGTGGACAGGAACTTTTAAGG**TAA**TTTTGAAAAAAAAAAAAAAAAAAAAAAAAA–3′

APPENDIX D

Full-length cDNA of *ZDS* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TAG).

5′–
ACGCGGGGGACAAATTCCCACCCAGCGCATCTTCACTTTCCTGATACCCCCTCTTCCTTTAAGATTCTCAA
AACAAAATTTCAGCCATTAGACTATATTACTTGGAAACTGGCTTCAATTTTATACAGTTGAGCTCCTGGAA
GCTCATTTTCTCCCTCCTTTTCTTCTTCAGTTTACAATT**ATG**GCTTCTGGAATTCTTTTTCCTCCTGTTTC
CTTCACGGGCAAGCATGGCAACTGTCGCAATTTTAGAATTCCTGCTCGTAATTCGGTGGTTCTTCTCAAGG
GTCAAAAGTTTTTGGTTAGATCATCCTTGGACAAAGATGTTTCTGATATGAGTGTTAGTGCTCCCAAAGGA
TTGTTTCCCCCTGAACCTGAACGTTATCGAGGACCCAAATTGAAAGTTGCTATTATTGGAGCTGGGCTTGC
AGGGATGTCAACCGCTGTTGAGCTTTTGGATCAAGGCCATGAGGTTGATATATATGAATCAAGGACCTTCA
TTGGTGGGAAAGTGGGATCATTTGTCGATAAACGTGGAAACCATATTGAAATGGGGCTACATGTATTCTTT
GGTTGTTACAACAATCTTTTTCGTTTAATGAAAAAGGTTGGCGCAGAGAAAAATCTACTTGTGAAGGATCA
TACTCATACCTTTGTAAACAAGGGAGGTGAAATTGGAGAACTTGATTTCCGCTTTCCGATTGGAGCTCCCA
TACATGGAATTCGAGCTTTCTTGGCCACAAATCAGCTCGGGACTTATGATAAAGCAAGAAATGCTTTGGCT
CTTGCCTTAAGTCCAGTTGTTAAGGCTCTTGTTGATCCAGATGCTGCCATGAAGGATATCCGAAATTTGGA
TAGTATAAGTTTTTCAGAGTGGTTCTTGTCTAAAGGTGGCACACGTGCCAGTATCCAGAGAATGTGGGATC
CGGTTGCCTATGCTCTTGGATTTATCGATTGTGACAACATCAGTGCCCGCTGTATGCTTACTATCTTCTCG
TTGTTTGCTACTAAGACCGAGGCTTCTCTATTACGCATGCTGAAAGGTTCTCCAGACGTTTTCTTAAGCGG
TCCCATAAGGAAGTATATCACGGACAGAGGGGGCAGATTCCATCTAAGGTGGGGATGTAGGGAGGTACTTT
ATGACAAATTTGCAGATGGAGAAACTTATATTGCAGGACTGGCAATGTCTAAGGCCACAAATAAGAAAATT
GTGAAAGCTGATGCTTATGTAGCAGCATGTGATGTCCCTGGTATCAAAAGGCTGATCCCATCACAATGGAG
AGAATGGGAGTTCTTTGATAATATTTATAAGCTAATTGGAGTTCCTGTCGTCACCGTCCAACTTCGGTACA
ACGGATGGGTGACAGAATTGCAAGATCTAGAACGTTCGAGGCAGTTAAGGGAAGCTGTGGGGTTGGATAAT
CTCCTTTACACGCCAGATGCAGATTTCTCATGCTTTGCAGATCTAGCGTTAACCTCTCCCGAGGATTACTA
CATTGAAGGACAGGGATCATTGCTTCAATGTGTCCTGACGCCCGGAGATCCTTACATGCCATTGCTAAATG
ACGAGATTATAGCAAGAGTTGCAAAACAGGTCTTGGATTTATTTCCATCATCACAAGGTTTGGAAGTAACA
TGGTCATCGGTTGTCAAGATTGGACAGTCTCTTTATCGCGAGGCACCCGGCAAAGACCCCTTTCGACCAGA
TCAGAAGACCCCTATTAAAAACTTCTTCCTTGCTGGATCATACACAAAACAGGATTACATAGATAGCATGG
AAGGAGCAACATTGTCAGGGAGGCAAGCTTCTGCATATATATGTGATTCTGGTGAGGAATTGATGATGCTA
AGAGAGAAGATTGCTGGCATTGATTCCGAAACTGCCAAATTGAGTGATGAGTTGAGTCTAGTT**TAG**GTTGG
CGCATCTGGATTCACTTCTTATGCTCTAACAAACAAGCATAATGTAAATCATATCATAGTAAAATTATAAT
GCTAGGCCAGCTAATTCTCTTGTATGCCCTGACAGCTCTCTCTACTCTAAATGAATGGCTGATCTTGTCCC
TACAAAAAAAAAAAAAAAAAAAAAAA–3′

APPENDIX E

Full-length cDNA of *CRTISO* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TGA).

5′–
AAGCAGTGGTATCAACGCAGAGTACGCGGGGATCTTCGAAGGAATCATGGGTTGGAATCAAAACCCTCCCT
CTGGTTTATCTTGCCTTGCTGTTCGAAGCGAAAGTTAGCTCAACTACCGAACTCTCCATCATCTGAAGCTG
AAGGATCAGTCAAA**ATG**GTTGTTGTCCGCTCACTTTCCATGCCAGGTTTGATGTTCAATTCTCCATCTGCT
GTTTATAATTCCCATTTTCCCACTGATTACAAGCTCAGTGACTTGGATTTGGGGTGTAAAACTTCTGTGTT
TTCTCATCTGAGCAACGCCCAAATTCTAAATAGAAACAAACCCAGATGCCAAAATCCCAAATTAATCTCCG
ATAAGATTTACAGAAAGCTGTGTGCGAGAGATTCCGAGTTCAATCGCAAGAATTTGGGGCTGTCCAAAACT
CTACAATTGGGGAATATGAAACCCAGAAGTTTACGAGCTAACTTTGTGGATACAGGCTTTTCTGGAGCGAA
TTTGAGGACTGAAAAGTTCATTGTGAAGTCAAAATCAGCATTGGGTGTTGATGAAACTGTGGAGAGAGATG
AAACAACAGGAGGTGGTGAGGAGAAGAGCCTATATGATGCTATTGTTATTGGGTCGGGTATTGGGGGTTTG
GTTGCTTCAACTCAATTAGCAGTGAAAGGAGCCAAGGTTTTGGTTTTGGAGAAGTACGTGATTCCTGGTGG
GAGCTCTGGGTATTACCAGAAGGATGGGTATACTTTTGATGTTGGGTCTTCTGTAATGTTTGGTTTCAGTG
ACAAGGGAAATCTAAATTTAATTACACAAGCTTTGTCAGCCGTTGGTTGTGAGATGCAAGTGATACCTGAT
CCAACCACTGTTCATTTCCATCTACCAGCTAATCTTTCAGTACGGATTCACAGAGAATACAGTGAATTTAT
TGCAGAACTTGTCAGCAATTTTCCCCATGAAAAAGAAGGAATCCTCAAATTCTATGGAGATTGTTGGAAGA
TTTTCAATGCTTTAAACTCATTGGAACTAAAATCACTGGAGGAGCCAATATATCTTTTCGGACAGTTCTTT
CAGAAGCCTCTTGAATGCCTGACACTTGCTTACTACTTGCCTCAAAATGCTGGAGACTTGGCTCGGAAGTA
CATCAAGGATCCCCGTCTGTTGTCCTTTATTGATGCAGAGTGTTTTATTGTTAGCACAGTGAATGCTTTGC
AAACACCAATGATAAATGCAGCCATGGTTTTATGTGACAGGCATTTTGGTGGAATAAACTATCCTATTGGT
GGTGTTGGTGGAATTGCAAAGTCCTTGGCAAAGGGTCTGGTTGATCATGGCAGCTCAATAATGTATAAAGC
AAATGTGACACAGATAATAACCGAAAATGGAAAAGCTGTAGGTGTGAAGCTGTCTGATGGAAGGGAGTTCT
TTGCTAAAACTATCGTATCGAATGCTACCAGATGGGATACCTTTGGAAAGCTGTTAAAAGGAGTGGACCTT
CCCAAGGAAGAGGAAAACTTTCAGAAACTTTATGTTAAGGCCCCATCTTTTCTTTCAATTCATATGGGGGT
GAAAGCTGAGGTTTTACCGCTGGATACAGATTGTCACCATTTTGTGCTTGAGAATGATTGGAGAAGGTTAG
AGGAGCCATATGGAAGCATCTTTCTGAGCATTCCGACTGTTCTCGATGCATCATTAGCTCCAGAAGGATGT
CATATTCTTCACATTTTTACTACTTCTTCCATAGAGGATTGGGAGGGGCTCTCCAGAAAAGAATATGAAGC
AAAGAAGGAGCTGATAGCAGACGAAATCATTACTAGACTCGAGAAGAAGCTATTTCCAGGGCTAAAATCAT
CTATTGATTTTATGGAGGTTGGGACGCCGAAGACACACAGGCGATTCCTAGCCCGTAATAATGGTACCTAT
GGACCAATGCCACGCGGCACTCCTAAGGGATTACTTGGAATGCCATTTAATACAACTGGTATAGATGGGCT
GTATTGTGTCGGTGATAGTTGCTTTCCTGGACAAGGAGTAATCGCCGTAGCCTTTTCCGGAGTGATGTGTG
CGCACCGAGTTGCTGCAGATATTGGGCTCGAAAAGAAGTCCCCCATTTTGGATGCTGCCCTTCTTCGGCTA
CTCGGTTGGTTGAGGACCTTGGCC**TGA**ATTTTGGAGTGAGAATGCACCATATCCGTCGTGCCGCAATAGTT
TTCCAGGGTCAGCCGTGTAACAACCACTGCAATAGTTGTTTAGAGACAGAGGACTAGTAGGCAGCCTCATA
TTCATACAGTTACAATTCATGTGATAGTAACCAATTTGTGAATGAATGAAAAAAGAGTATAGCAAAAAAGC
ACATTTCAACTGAATGTTTCTCTTATAAAAAGAAAAANAANNNNNNCAAAAAAAANACNNCNCT–3′

APPENDIX F

Full-length cDNA of *LCYB* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TAA).

5′–
ACGCGGGGAAACATTATCAAACTCTGTTTAAGCAGTGGAGAAAGCAAATTGAGCGAGCGATATTCATTCTC
AGGTCGCTATCAGTTATCTCCACCATTAATTGGCGAGAATATGGAGCCATCTTCCAACTGTGGACGCTGAC
AAACTCCCAATCTTCTTCAATTCCCCTAATTCCATCTCTTGGAGAACAGTGGCCGGCGAAGTCACTTCGTC
CAAATTGGGACTCGTCATTCGCGCTCCACATCCCTCCAATCCATAACAACCAAATGGAGCTCCTTCCCGTC
CTCAGGTTCGCCTCCAAACACACGCCTCTTCATGTTTTAATCACTGAATTCCATTGAAGTTATCCCTGTTC
TTCTGGAGTTCTTGGGGATTTGTTGAAATTTTTGAGCACCCCATTTCGATTCTTCATCTATTGGTTTATTA
CTTAGGTTTGTTTGAGATTTCTGGATATTGGGTCTCTGTAGGGATTCCCTTTTTGACTTTGCTGATAATTC
TGTTTCTGTTGCTCTCTGTAGTTTCATTTGTTTGTTGTAAATCC**ATG**GATACTTTACTTAAAATCAATAAC
AAGTATGGTTTTCTGCAACCATTACATGGGGTTTCGGAAAAAGTGAGTGGTGTGAGGAGTACAAAGTTTCA
GAGTCAGGAATTTGGGTTTGGTCATAGGAAGGGTCGTCTGAAATGGAGGAAAGGGGGTTGTCTTAATGTGA
GAAGTAGTTCTCTTTTGGAGCTTGTTCCTGAAACCAAGAAGGAGAATCTTGAGGTTGAACTTCCCATGTAT
GATCCTTCGAAGGGCCTTGTTGTCGATCTTGCGGTCGTGGGAGGCGGCCCAGCAGGGCTTGCTGTTGCGCA
ACAGGTTTCAGAGGCAGGGCTTTCAGTTTGTGCAATTGACCCATCTCCCAAGTTGATTTGGCCCAACAATT
ATGGGGTTTGGGTGGATGAATTTGAGGCAATGGATTTGCTAGATTGTCTCGACACGACTTGGTCTGGTGCT
GTCGTGTTCACCAATGAGCAATCAACAAAAGATCTTGCTCGACCTTATGCGAGGGTTAATAGAAAGCAACT
CAAGTCAAAAATGTTGCAGAAATGCATTTCCAATGGTGTTAAGTTTCATGAAGCTAAAGTTATTAAAGTTA
TACATGAGGAGTTCAAATCCTTGTTAATTTGCAATGATGGTGTGACCATTCAAGCTGCCATTGTTCTTGAT
GCCACTGGCGTCTCTCGATGCCTTGTCCAATATGATAAGCCTTACAATCCAGGCTACCAGGTAGCTTATGG
GATTTTAGCTGAGGTGGAGGAACATCCATTTGATGTTAACAAGATGGTGTTTATGGACTGGAGAGATTCAC
ATCTGAATAACAATATGATTTTGAAGGAGAGAAATAGCAAAATTCCTACATTTCTCTATGCAATGCCCTTT
TCATCAAATCGGATATTTCTGGAGGAAACTTCTTTGGTAGCTCGACCTGGGTTACAAATGAGCGATATCCA
GGAAAGAATGGAGGTAAGATTGAAGCACTTGGGAATAAAAGTGAAGAGCATTGAAGAGGATGAGCATTGTG
TCATTCCAATGGGTGGACCGCTGCCAGTTCTTCCTCAAAGAGTTGTTGGAATTGGTGGAACAGCAGGGATG
GTGCACCCTTCAACTGGATATATGGTAGCAAGAACTCTAGCAGCGGCACCTATTGTTGCTAGTGCAATAGT
CCGGTGCCTTGGTTCAGATGGACGTTTCAGGGGTGATGCGATATCCTCTGAAGTTTGGAAAGATCTATGGC
CCATCGAAAGGAGGAGGCAGAGAGAATTTTTCTGTTTTGGGATGGATATTTTATTGAAGCTGGATCTAAAG
GGTACAAGAAGGTTTTTTGATGCATTTTTTGATCTTGAACCTCGTTATTGGCATGGATTCTTGTCATCACG
ACTATTCCTTCCTGAGCTGTTACTCTTTGGGCTTTCCTTATTCTCTCACGCATCTAATGCCTCCAGGCTTG
AAATCATGGCAAAGGGAACTCCATCTTTGGTAAACATGATCGGCAATCTGGTAAAGGATAGAGAT**TAA**GAT
GAATATAGAGTTACTGTGTTGTAAGCTAATCACCATACTGATGCACTTGCATCATCACATTTACTTCTGCA
GATGATTGTTCATAAGATTATGAGTTAGCAAAAAAAAAAAAAAAAAAAAAA–3′

APPENDIX G

Full-length cDNA of *CHYB* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TGA).

5′–
GAACAAAGCTCCCCATTTTTCCTGCTCAGGTAATTTTTTCTCCAATACTCCAACTGACAAAAAACTTGTGG
TCTATGTTCCTTCAACCTCCATTTTTAAGCCCTCTTTCCGTCGTCTCTCTCTCCGAGAAGCTTCCCCTTCT
TCCACCGTCTTTTCACTTTTCACCTTCATCTTTCCC**ATG**GCGGCCGGCCTCTCCGCCGCCTTAGTGCCCAA
ACCACTCCATCTCTTCCTTACTTCCTCCCATCTCTCCCCTAAACCTCGAACTCCGTTTCTGTTTCCACCTC
CTGTCTTCCGGAACAGTAGATTCCAATGGAAGATGCGGAGAAAAACTCTGTTCACTGTCTGTGTACTCGTT
GAGGATCAAAATAGTTCCGGTGAGGTGGAGAATCTCTCCGATGAAGGATCGCCGATTGTAATCCCTCAGAT
CCCATCGCCTCATGTTTCAGAAAGATTAGCAAGGAAGAAATCGGAGCGCTTCACTTATCTTGTTGCTGCGG
TTATGTCTAGTTTTGGAATTACCTCCATGGCTGTCATGGCGGTTTACTACCGATTTTACTGGCAAATGGAG
GGCGGAGAGATTCCTTTCTCTGAAATGTTTGGTACATTTTCTCTCTCTGTTGGTGCCGCTGTGGGGATGGA
GTTCTGGGCGAGATGGGCTCATAGGGCTCTCTGGCACTCTTCCTTATGGCATATGCACGAGTCGCACCATA
AACCAAGAGAAGGACCGTTCGAATTGAACGATGTTTTCGCCATTGTCAACGCTGTGCCCGCCATAGCTCTT
CTTTCTTACGGCTTCTTCCATAAAGGCCTTGTTCCTGGTCTCTGCTTCGGCGCTGGCCTTGGAATTACGGT
CTTCGGGATGGCCTACATGTTCGTCCACGACGGTCTCGTTCATAAAAGATTCCCTGTGGGTCCCATCGCCA
ACGTCCCCTATTTCAGAAAGGTCGCTGCTGCTCACCAGCTTCACCATTCAGACAAGTTCAACGGTGTGCCA
TATGGGCTGTTTTTGGGTCCGAAGGAATTAGAGGAAGTGGGAGGCCTAGAAGAATTGGAGAAGGAAATCAA
CAGAAGAATAAAATTGACGGCCCCAAAATCAAACCATGGTTCTTCATCAACCAATATTATG**TGA**AAATGAG
GAGAAAAAGAAAAAAGAAAAAAAAAAAAA–3′

APPENDIX H

Full-length cDNA of *ZEP* gene in the carotenoid biosynthetic pathway was isolated using degenerate PCR and RACE. Bold letters are start codon (ATG) and stop codon (TGA).

5′–
ACGCGGGGACAATACCCACCGTCTCCATTTCTTCTTAACTGTTCATCATCCTCTTCCTTTTTCTCTGCAGA
TTCTTCAAGATCAGTGGCTTTTGATACTGACCCATC**ATG**GCTTTGACCAGATTTCACAACCCTTTTAATCT
TTCCTCCTCTGGTTTGTCAAGAACATGTTTCCCAGTTCCAGCTTTTCGGGAATACCTAGTTGAGATTTCGC
CTTCTCAAAGGATTGGGTGTAATTTTGCGGGAAAATCAACTTGTGGGCGGCGGAAGAAAGTGACCCAAGTG
AAAGCCGCCGTCGCAGAGGCGCCACCGGCGGAAGGGGAGGCCGGAGAAATCAGCCGGAGCTTGCCGACGAA
GAATGTTCGGGTACTTGTGGCTGGTGGTGGAATTGGGGGTTTGGTTTTTGCTTTGGCGGCGAAGAGGAAAG
GGTTCGATGTGGTGGTTTTCGAGAAGGATATAAGTGCTATTAGAGGAGAGGGGCAGTACAGGGGGCCGATT
CAGATACAGAGCAATGCTTTGGCGGCTTTGGAAGCCATTGATTTGGGGGTTGCTGAGGAAGTTATGAGAGT
GGGTTGTATTACTGGTGATAGGATTAATGGGCTTGTTGACGGGGTTTCTGGAAATTGGTACATCAAGTTTG
ACACGTTCACTCCTGCAGCGGAACGAGGACTTCCGGTCACTAGGGTAATCAGTCGAATGGCATTGCAACAA
ATTCTTGCTCGTGCTGTGGGTGATGATGTGATTATAAATGGTAGTAATGTTGTTGACTTTGAGGATAATGG
AAACAAGGTCAAGGTGACTCTTGAAAATGGACAGCAACATGAGGGCGATCTCCTGGTTGGAGCAGATGGTA
TATGGTCAAAGGTTAGAAAGAACTTGTTTGGTCACTCAGAAGCAGTATATTCTGGCTACACTTGCTATACA
GGTATCGCAGACTTCATTCCAGCTGACATCGAAACTGTTGGGTACCGTGTGTTTCTGGGACACAAACAATA
CTTTGTTTCCTCAGACGTCGGTGCAGGAAAGATGCAGTGGTATGCATTTCACAAGGAACCACCTGGTGGCA
CTGATCCCCCTAACAGCAAGAAGGAGAGACTGTTCAAAATTTTTGAAGGTTGGTGCGACAATGTGATAGAT
CTTATACATGCCACTGATGAAGATTCTGTTCTTCGACGTGATATATATGATCGCACGCCCATTTTCACATG
GTCGCTAGGTCGCGTAACTTTGCTTGGGGATTCTGTACATGCCATGCAGCCAAATATGGGTCAAGGGGGAT
GCATGGCGATTGAGGATGGTTATCAACTTGCACTTGAGCTAGATAAAGCATGGAACAAAAGCGTAGTCTCA
GGATCTCCTATTGACATTGTCTCATCGTTGAAGAGTTATGAGAGTAGTAGAAGAATACGGGTTGCTGTAAT
TCATGGAATGGCAAGAATGGCTGCATTAATGGCTTCCACATATAAAGCTTATTTGGGAGTTGGACTTGGCC
CCCTTTCGTTTTTGACACAGTTCAGAATACCACATCCTGGGACATTTGGTGGAAGGTTTTTTATTGATCTG
GCAATGCCCTTGATGCTTAATTGGGTCCTTGGCGGTAATAGTTCAAAATTAGAAGGGAGGCCCCCAGCTTG
CAGACTCTCAGACAAAGCAAACGATCAGTTACGCCAATGGTTTGAAGATGATGATGCCTTGGAGCGAGCTA
TTAATGGAGATTGGTTTCTATTACCACAAGGAGGCGAAGCTAGCGTTTCACATCCTATTTGCCTACCCAGA
GACGAGAACCAGCCCTGCTTGATTGGAAGTGTGGAGCAAGAAGTAGATTCAGGGTTATCGATTGCTATACC
GTTGCCTCAGGTTTCAGAAAAGCACGCCCGTATTCATTACAAAGATGGGGCCTTCTTCTTGACTGATCTGA
GGAGTGAACATGGTACCTGGCTCTCTGATCACGAAGGACGACGGTACCGTGTACCTCCGAATTTTCCAGTA
CACTTCCATCAATTCAACATTATTGAATTAGGTTCTGATAAGAAGGCAGCATTTCGTGTGAAGGTGATAAG
ATCTTCAGTTGAATATGACAGAGAAAAAGTAAAGATGAACTCA**TGA**AGCAATGTGGGAAATCTCCATATTG
AGTTCTTGTTAATTCTAGAGATATGTACAGATTATTGAAGGCAGTGGAGCACTCATCTACTGATTTTGAAT
GCAACAGATACAGAGTGATTTGTATTTCTCACTCACTTTTTGAGTGTAAAGATGCATACAACTCCCCACTT
CTGTAGTGATTTTTAATAGACCAAAATGTTAGTATACCCCAAAAGTTTTAGATTAAAACAAAGATGTACTA
TTATATGTTTCTTATACTTCAAGAAAAGAAATATATATGCTCAATTAGTTGAAAAAAAAAAAAAAAAAAAA
AAAA–3′

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M.S.	Horticulture, KyungHee University, Republic of Korea, 1996
B.S.	Horticulture, KyungHee University, Republic of Korea, 1993

Experience

2001-2005	Research Assistant, Horticultural Sciences, TAMU
1996-2001	Research Technician, Horticulture, KyungHee University
1997-2000	Teaching Assistant, Horticulture, KyungHee University
1999	Editorial Assistant, *Proceedings of the International Symposium on Quality of Fresh and Fermented Vegetables*, Acta Horticulture 483
1994-1995	Teaching Assistant – Horticulture, KyungHee University
1993-1996	Graduate Research Assistant, Horticulture, KyungHee University

Publications

Bang, H., D.I. Leskovar, D.A. Bender, and K. Crosby. 2004. Deficit irrigation impact on lycopene, soluble solids, firmness and yield of diploid and triploid watermelon in three distinct environments. Journal of Horticultural Science & Biotechnology 79(6): 885-890.

Leskovar, D.I., H. Bang, K.M. Crosby, N. Maness, J.A. Franco, and P. Perkins-Veazie. 2004. Lycopene, carbohydrates, ascorbic acid and yield components of diploid and triploid watermelon cultivars are affected by deficit irrigation. Journal of Horticultural Science & Biotechnology 79(1): 75-81.

Leskovar, D.I., H. Bang, K. Kolenda, P. Perkins-Veazie, J.A. Franco. 2003. Deficit irrigation influences yield and lycopene content of diploid and triploid watermelon. Acta Horticulturae 628: 147-151.