Chapter 2

β-Carotene Production under Greenhouse Conditions

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Abstract

β-carotene is a secondary metabolite that is a hydrocarbon carotene predominantly located in lower concentrations in PS II functioning as a helper to harvest light pigment during photosynthesis and to dissipate excess energy before damage occurs. As other carotenes, β-carotene is uniquely synthesized in plants, algae, fungi and bacteria. β-carotene is the main diet precursor of pro-vitamin A. Additionally, β-carotene serves as an essential nutrient and is in high demand in the market as a natural food colouring agent, as an additive to cosmetics and also as a health food. Several approaches have been carried out in order to increase β-carotene production in algae, bacteria, fungi and plants using biotechnological and engineering focuses. In the case of plants, such species as the tomato have important β-carotene contents, which are theoretically amenable to management using approaches such as fertilization, growth conditions and mild stress. On the other hand, greenhouse structures can protect crops from wind and rain, and can also protect from insects when fitted with insect exclusion screens. β-carotene production could potentially be improved and enhanced in greenhouse conditions in plants as well as

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in algae, based on the exclusion of the structure, and the possibility of controlling aspects such as climate, fertilization and stress management, among others. Production of β-carotene (and other secondary metabolites) from several organisms in greenhouse conditions should be an interesting future approach, visualizing the greenhouse as a "factory" in frontier technologies such as biotechnology and mechatronics in order to optimize this production.

Introduction

The bright colours found in nature and the molecules that cause them have always fascinated organic chemists. The earliest studies on carotenoids date back to the beginning of the nineteenth century. β-carotene was first isolated by Wackenroder in 1831, and many other carotenoids were discovered and named during the 1800s, although their structures were still unknown. Not until 1907 was the empirical formula of β-carotene, $C_{40}H_{56}$, established by Willstatter and Mieg (Coultate, 1996). The structure was elucidated by Karrer in 1930–31, which was the first time that the structure of any vitamin or provitamin had been established, and he received a Nobel Prize for his work (Middleton et al., 2000). Steenbock suggested in 1919 that there could be a relationship between β-carotene and vitamin A. The concept of provitamins (molecules that are converted into vitamins by the body) was entirely new, and proved to have great significance scientifically and commercially (Coultate, 1996). The first total syntheses of β-carotene were achieved in 1950; various studies were carried out throughout the 1970s–80s to determine its suitability for use in food and its activity in the body. In the early 1980s it was suggested that β-carotene might be useful in preventing cancer, and it was found to be an antioxidant (Burton and Ingold, 1984; Aggarwal et al., 2008). More recently, β-carotene has been claimed to prevent a number of diseases, including several types of cancer, cystic fibrosis and arthritis, and there is a flourishing trade in vitamin supplements containing β-carotene (Coultate, 1996; Middleton et al., 2000; Czeczuga et al., 2007; Veloz-García et al., 2004; Guevara-González et al., 2006; Guzmán-Maldonado & Mora-Avilés, 2006; Aggarwal et al., 2008; Marín-Martínez et al., 2009).

Carotenoids are lipid-soluble yellow, orange and red pigments that are uniquely synthesized in plants, algae, fungi and bacteria (Sandmann, 2001). They are secondary plant compounds that are divided into two groups: the oxygenated xanthophylls such as lutein (3R, 3′R, 6′R β,β-carotene-3,3′diol) and zeaxanthin (3, 3′R-β,β-carotene-3,3′diol) and the hydrocarbon carotenes such as β-carotene (β-β-carotene), α-carotene (6′R,β,β-carotene), and lycopene (Ψ,Ψ-carotene) (Zaripheh and Erdman, Jr., 2002). Carotenoid C_{40} biosynthesis is a branch of the isoprenoid pathway. To begin the process of biosynthesis, isoprene (2-methyl-1,3-butadiene; C_5) is converted into isopentenyl diphosphate (IPP; C_5) which then in turn is converted into dimethylallyl diphosphate (DMAPP; C_5) (see Figure 1). Combining four DMAPP molecules together results in the formation of geranylgeranyl pyrophosphate (GGPP). The first step considered as part of carotenoid biosynthesis is the condensation of two molecules of the C_{20} GGPP to form the first C_{40} carotenoid, phytoene. Desaturation of phytoene then produces in sequence four acyclic compounds: phytofluene, ζ-carotene, neurosporene and lycopene. Cyclization of lycopene can occur on one end, producing monocyclic γ-carotene or δ-carotene, or to both ends, producing dicyclic α-carotene or β-

carotene. Further modification of the pathway can occur with the addition of oxygen functions in the form of hydroxyl, epoxide or keto groups, resulting in the xanthophylls. The carotenoid pathway can be further modified with other structural end groups, such as esterification. Approximately 700 different types of carotenoids have been discovered and characterized (Baransky et al., 2005; Beyer et al., 2002; Hornero-Mendez and Britton, 2002; Niyogi et al., 2001). β-carotene functions in several organisms as an osmotic regulator, protector against irradiance stress, antioxidant agent, and accessory pigment in photosynthesis, among others. In order to increase the carotenoids, especially β-carotene, several approaches have been carried out, including transgenic organisms, gene silencing strategies and fermentation technologies (Diretto et al., 2007; Nanou et al., 2007). Another interesting and promising possibility in this sense is the use of mechatronic methodologies in order to phytomonitor and optimize crop production, especially under greenhouse conditions (van Henten and Bonsema, 1995; Schmitd, 2005; Castañeda et al., 2006). Thus, several strategies, such as process automation (plant nutrition, climate control, light quality, pest and pathogen detection, etc.), sensor development, image analysis of crop and fruit development, among others, together with biotechnological approaches, will promote reaching higher β-carotene (and other secondary metabolites) production in living systems under protected environments such as greenhouses. Finally, understanding and controlling environmental and genetic factors that may contribute to the nutritional value of food (such as production of β-carotene in living systems) will therefore be important when making cultural management decisions in order to increase production.

Role of β-Carotene in Living Systems

Carotenoids are naturally-occurring pigments that are responsible for the different colours of fruits, vegetables and other plants (Ben-Amotz and Fishler, 1998; Raja et al., 2007). So far, more than 700 types of carotenoids have been reported in nature, and about 50 are provitamin-A, which includes α-carotene, β-carotene and β-cryptoxanthin (Faure et al., 1999; Raja et al., 2007). In plants, carotenoids play critical roles in both light harvesting and energy dissipation for the photosynthetic mechanism. Within the thylakoid membranes of chloroplast organelles, carotenoids are found bound to specific protein complexes of photosystem I (PSI) and photosystem II (PS II). β-carotene is the predominant carotenoid in PS I, while it is presented in lower concentrations in PS II (Demmig-Adams et al., 1996; Thayer and Bjorkman, 1992).

Within each photosystem, β-carotene is associated with antenna pigments and photosynthetic reaction centers (Peng and Gilmore, 2003; Taiz and Zaiger, 2003). In PS II complex, β-carotene is highly concentrated close to the reaction center (Niyogi et al., 1997). In humans, carotenoids have been associated with reduced risk of lung cancer and chronic eye diseases such as cataracts and age-related macular degeneration (Raja et al., 2007). Kale (*Brassicae oleraceae* L.) ranks highest and spinach (*Spinacia oleraceae* L.) ranks second among vegetable crops for the accumulation of the carotenoids lutein and β-carotene (Lefsrud, 2006). The results clearly indicate that increasing carotenoid level using different approaches in plants (i.e., spinach and tomato, among others) commonly consumed in the diet

would impart health benefits without changing the dietary habits of individuals (Diretto et al., 2007). In the halotolerant microalgae *Dunaliella*, β-carotene production is enhanced as an environmental adaptation in which β-carotene and glycerol are produced in excess to maintain its osmotic balance in saline stress as well as in irradiance stress (Raja et al., 2007). In this latter case, *Dunaliella* produces β-carotene in excess to overcome irradiance stress and inhibits high photoinhibitory activity when blue light is used, intermediate with white and non-existent with red light (Ben-Amotz et al., 1989; Raja et al., 2007). In addition, β-carotene is a purported anticancer agent that is believed by some to have antioxidant action of a radical-trapping type. However, definitive experimental support for such action has been lacking. New experiments in vitro show that β-carotene belongs to a previously unknown class of biological antioxidants. Specifically, it exhibits good radical-trapping antioxidant behavior only at partial pressures of oxygen significantly less than 150 torr, the pressure of oxygen in normal air. Such low oxygen partial pressures are found in most tissues under physiological conditions. At higher oxygen pressures, β-carotene loses its antioxidant activity and shows an autocatalytic prooxidant effect, particularly at relatively high concentrations. Similar oxygen-pressure-dependent behavior may be shown by other compounds containing many conjugated double bonds (Burton and Ingold, 1984; Meyer et al., 2007; Guruvayoorappan and Kuttan, 2007). The potential ability of β-carotene as an antioxidant, immunomodulatory and anticancer agent led to more active research studies of its application for the prevention of human cancers. During recent years there have been reported elsewhere a myriad of papers in which β-carotene has generally been associated with reducing the risk of lung, gastric, brain and breast cancer, as well as Alzheimer's and heart diseases (Dai et al., 2006; Polus et al., 2006; Voutilanen et al., 2006; Larsson et al., 2007; Czeczuga et al., 2007; Aggarwal et al., 2008).

β-Carotene Production in Several Organisms

As mentioned elsewhere, β-carotene serves as an essential nutrient and is in high demand in the market as a natural food colouring agent, as an additive to cosmetics and also as a health food. It occurs naturally as its isomers, namely all-*trans* (Figure 2a), 9-*cis* (Figure 2b), 13-*cis* and 15-*cis* forms (Wang et al., 1994) and functions as an accessory light harvesting pigment, thereby protecting the photosynthetic apparatus against photo damage in all green plants, including algae (Ben-Amotz et al., 1987).

β-carotene, as a component of photosynthetic reaction centre is accumulated as lipid globules in the interthylakoid spaces of the chloroplasts in *Dunaliella* (Vorst et al., 1994). They protect the algae from damage obtained during excessive irradiances by preventing the formation of reactive oxygen species, by quenching the triplet-state chlorophyll or by reacting with singlet oxygen ($^1O_2$), and also, it acts as a light filter (Telfer, 2002). Only few reports are available on the enzymes and proteins involved in β-carotene regulation. In *Dunaliella*, β-carotene is accumulated into extraplastid lipid globules (García-González et al., 2005), which are stabilized and maintained by a peripherally associated 38 KD protein called carotene globule protein (CgP). Probably, CgP is involved in stabilizing the globules within chloroplast (Katz et al., 1995; Raja et al., 2007). Induction of CgP and deposition of

triacylglycerol are in parallel with β-carotene accumulation (Raja et al., 2007). The biosynthetic pathway for β-carotene has been determined for fungi such as *Phycomyces blakesleeanus* and *Neurospora crassa* (Cerdá-Olmedo, 1987; Rodríguez-Sáiz et al., 2004). It contains three enzymatic activities: 1) phytoene synthase, which links two molecules of geranylgeranyl pyrophosphate to form phytoene; 2) phytoene dehydrogenase, which introduces four double bonds in the phytoene molecule to yield lycopene; and 3) lycopene cyclase, which sequentially converts the acyclic ends of lycopene to β-rings to form α-carotene and β-carotene. A similar biosynthetic pathway is known in all carotenogenic organisms (Lee and Schmidt-Dannert, 2002).
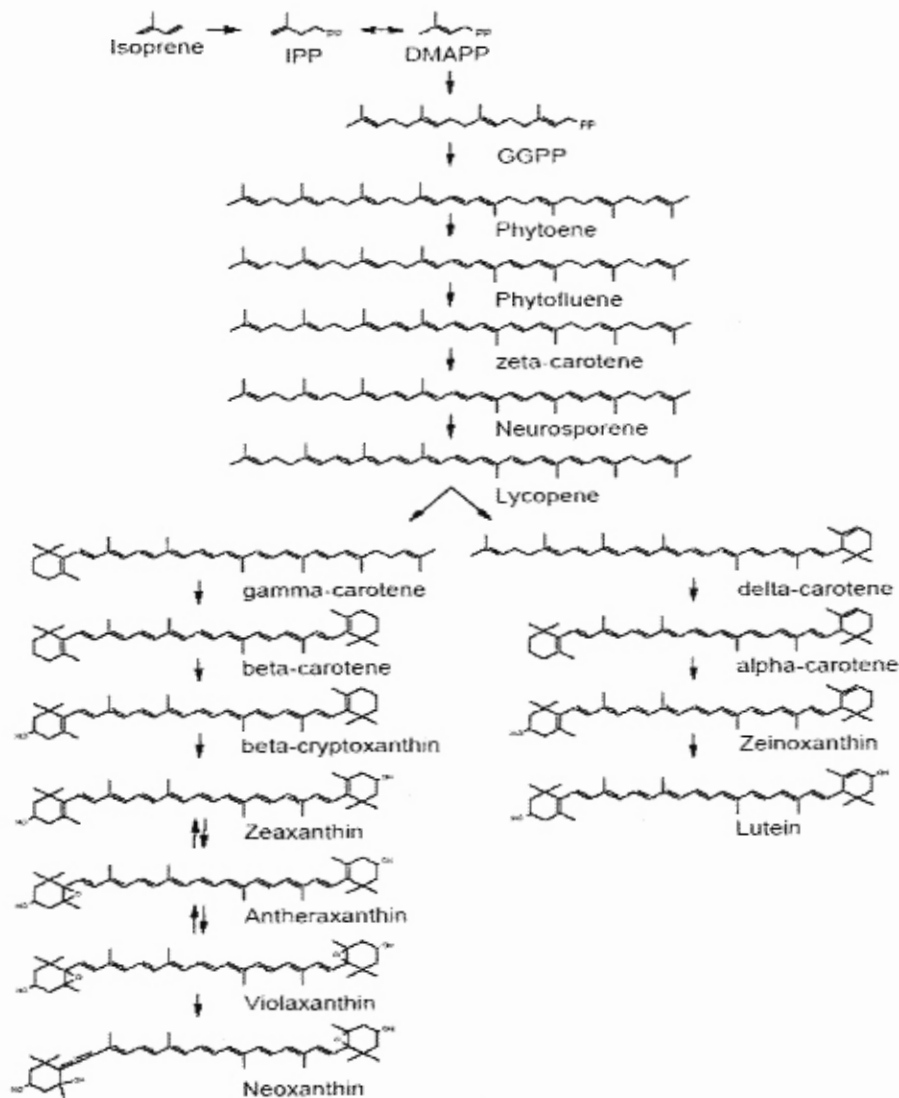

Figure 1. Simplified version of the carotenoid biosynthetic pathway in plants. IPP, isopentenyl diphosphate; DMAPP, dimethylallyl diphosphate; GGPP, geranylgeranyl pyrophosphate.
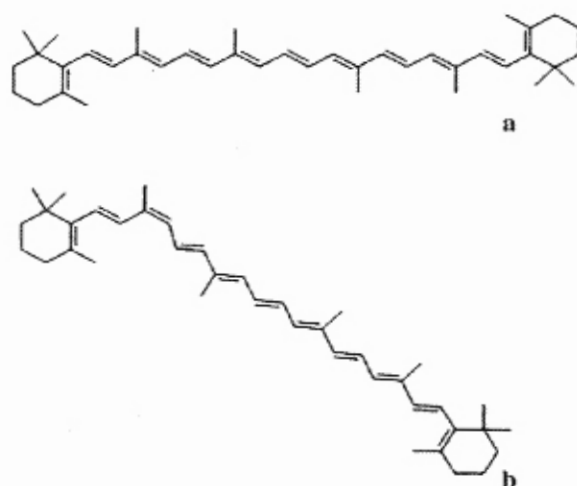
Figure 2. Chemical structures of β-carotene isomers. Panel a, 9-*cis*-b-carotene; Panel b, all-*trans*-β-carotene.

All-*trans* β-carotene is responsible for the light-yellow color of *Phycomyces blakesleeanus*, a heterothallic fungus of the class Zygomycetes and the order Mucorales (Cerdá-Olmedo, 1987; Mehta et al., 1997). In this fungus, many external factors influence the color of the mycelium because they activate or inhibit carotene biosynthesis. Among these factors, sexual stimulation, blue illumination, and the addition of retinol and dimethyl phthalate to the medium represent four separate mechanisms of activation (Mehta et al., 1997). In the red yeast *Xanthophyllomyces dendrorhous* (formerly named as *Phaffia rhodozyma*), β-carotene accumulates as an intermediary of the astaxanthin biosynthesis pathway (Girard et al., 1994; Verwaal et al., 2007). The genes involved in β-carotene production in *X. dendrorhous* have been cloned previously (Verdoes et al., 1999[a,b]). Carotenogenesis in prokaryotes is constitutive or photoinducible. Several prokaryotes, including *Erwinia herbicola* and *Rhodobacter capsulatus* produce Carotenoids constitutively, whereas organisms such as *Myxococcus xanthus*, *Flavobacterium dehydrogenans* and *Sulfolobus* spp., produce carotenoids in a photoinducible manner (Armstrong et al., 1990[a,b]; Burchard and Dworkin, 1966; Grogan, 1989; Weeks and Garner, 1967; Takano et al., 2005). The control mechanisms of carotenogenesis have been studied in phototrophic bacteria such as *Rhodobacter* spp., which revealed the involvement of global signal transduction initiated by light capture in the bacteriochlorophyll (Takano et al., 2005). On the other hand, the molecular mechanism in nonphototrophic bacteria has not yet been fully studied except in *M. xanthus*, a gram-negative gliding bacterium characterized by a unique life cycle (Takano et al., 2005).

Commercial Importance of β-Carotene

The market for ingredients basically found in the food and pharmaceutical area continues to grow much faster than related markets, such as those for feed or industrial chemicals. Examples of highly demanded ingredients are the carotenoids. For many years, the most

prominent representative of carotenoids, β-carotene, was used as a food colorant. Additionally, due to the antioxidative properties of carotenoids, this sector has become one of the fastest growing outlets for such products. In addition, the feed area still is a large sector, demanding all entire range of carotenoids to color fish, broilers and eggs. The worldwide market value of all commercially-used carotenoids was estimated at $887 million for 2004 and expected to rise at an average annual growth rate (AAGR) of 2.9% to just over $1 billion. Specifically, more recent data mentioned that the global market for carotenoids was $766 million in 2007. This is expected to increase to $919 million by 2015, a compound annual growth rate (CAGR) of 2.3% (bcc Research, March 2008; Figure 3). β-carotene has the largest share of the market. Valued at $247 million in 2007, this segment is expected to be worth $285 million by 2015, a CAGR of 1.8% (*Bussiness Wire, 2008; Focus on the Global Market for Carotenoids*).

As mentioned, carotenoids (including β-carotene) are an important group of natural pigments with specific applications as colorants, feed supplements and nutraceuticals; they are also used for medical, cosmetic and biotechnological purposes. A few of the variety of natural and synthetic carotenoids available have been exploited commercially, these includes β-carotene, lycopene, astaxanthin, canthaxanthin, lutein, annatto and capxanthin (Bhosale, 2004; Martín et al., 2008). Although more than 600 different Carotenoids have been described from carotenogenic microorganisms, only a few of them are produced industrially, and β-carotene is the most prominent (Rodríguez-Saiz et al., 2004). β-carotene is still the most prominent carotenoid used in foods and supplements, but due to a changing consumer perception, primarily in Europe, the product is suffering from natural replacements, specifically carrot juice, and market growth in the past few years was much lower than expected. In parallel, the number of producers of synthetic and algae derived β-carotene rose sharply, which added to the imbalance of supply and demand, driving prices down (*Bussiness Wire, 2008; Focus on the Global Market for Carotenoids*). Despite the mentioned before, based on data presented before, it is expected that β-carotene will continue to be the most prominent carotenoid in the market, with an increased value in the next years as the future market projections to the year 2015 (Figure 3).
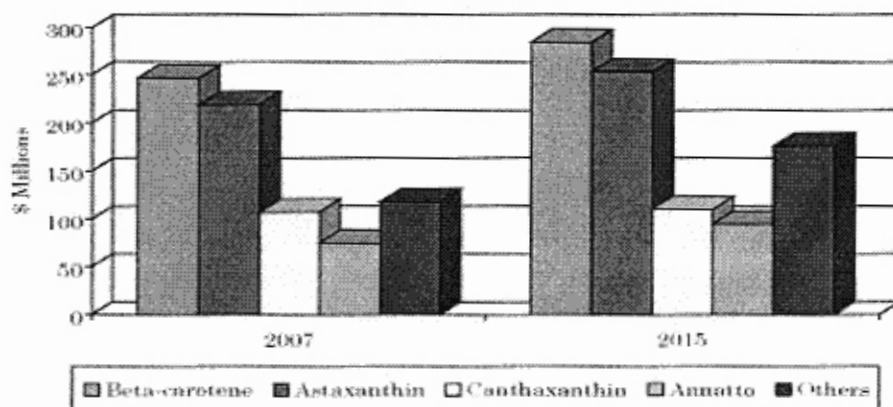

Figure 3. Global carotenoid market value by product 2007 and 2015 (USD $ millions). Source: bcc Research, 2008.

Increasing β-Carotene Production using Biotechnological Approaches

As it has been pointed out previously, the advent of biotechnology has brought the opportunity to accelerate the development of methodologies to investigate and clarify the biosynthesis pathways of carotenoids in general and in particular β-carotene (Umeno et al., 2005). The work and analysis of mutants was a key tool for rough outline of the biochemical reactions that constitute the biosynthetic pathway of carotenoids in the first half of the 1960s (Spurgeon and Porter, 1981). Characterization of biochemical reactions in the cell-free systems and in vivo using radiolabeled precursors generated important information and more details were known. However, it was only possible to capitalize on the knowledge acquired until relevant enzymes involved were identified (Dogbo and Camara, 1987). A concomitant use of this knowledge and the tools of biotechnology have generated methods to increase the amount of β-carotene that occurred in some systems or to produce it in significant levels in biological systems in which this compound is normally not produced (Ye et al., 2000; Kim., et al., 2006; Diretto et al., 2007). Some biotechnological approaches that have been used in order to increase b-carotene contents are the following:

a. Induction of Mutations

One way for increasing the quantity of β-carotene in fungi has been through mutations. As early as 1976, even with emerging knowledge about the route of biosynthesis of carotenoids and the involved genes were reported encouraging results by inducing mutations in *Phycomyces blakesleeanus*. It was handled evidence that there were two genes involved; carS and carA, and β-carotene overproduction was associated with the induced mutation in carS (Murillo and Cerda-Olmedo, 1976). Recessive carS gene mutations abolished the end product regulation of the pathway, and these strains could contain 2 to 5 mg of β-carotene per g of dry mycelium, that is, up to 100 times the wild-type level (Murillo et al., 1978).

b. Methods to Induce Mutations

The most common means to induce mutations have been: N-methyl-N'-nitro-N-nitrosogua-nidine, ethyl methanesulfonate and UV light (An et al., 1989). Nitrosoguanidine (N-methyl-N'-nitro-N-nitrosoguanidine) has been most frequently used for the induction of mutations in a lot of organisms (Gichner and Veleminsky, 1982). The mutations are preferentially produced in DNA regions being replicated at the time of the mutagen exposition in cases such as yeasts and bacteria (Cerda-Olmedo et al., 1968; Dawes, and Carter , 1974; Casadesus and Cerda Olmedo, 1985). In bacteria this results in relatively high rates of closely linked double mutants (Gichner and Veleminsky, 1982). The induction of mutations in bacteria and fungi also has been conducted with the use of UV radiation. However nitrosoguanidine is a more effective mutagen than UV radiation (Casadesus and Cerda Olmedo, 1985). Spores of *Phycomyces blakesleeanus* (strain NRRL 1555) at a

concentration of 1 X 10^7 spores/mL were treated with 0.5 mg/mL of N-methyl-N-nitroso-N'-nitroguanidine in 0.2 M acetate buffer at pH 5 for 80 minutes at 220°C. The suspension was shaken occasionally to prevent the sedimentation of spores. The spores were washed and seeded at a concentration of 50 viable spores per petri dish on agar plates containing glucose-asparagine medium (1/7) supplemented with 0.1 % yeast extract (Meissner and Delbruck, 1968). Mutants were isolated after treatment with 100 ug of N-methyl-N'-nitro-N-nitrosoguanidine (NTG) per ml in pH 7.0 citrate-phosphate buffer, as previously described (Cerdá-Olmedo and Reau, 1970).

c. Microorganisms with Greater Success in the Production of β-carotene

Among the carotenoid-producing microorganisms, bacteria, filamentous fungi and unicellular algae have been extensively examined in order to evaluate their possible industrial interest. The most successful cases have been with the halophilic alga *Dunaliella*, phycomycetes fungi *Blakeslea trispora*, *Phycomyces blakesleeanus* (Ninet and Renaut 1979; Weete 1980) and the yeast *Phaffia rhodozyma* (Girard et al., 1994).

d. Metabolic Engineering

Summary of the biosynthesis pathway

With genes and cDNAs encoding nearly all the enzymes required for carotenoid biosynthesis in a lot of organisms, sequenced, and their products characterized, it was possible to use genetic engineering to introduce the ability to synthesize the β-carotene in plants or organisms that did not produce it and enhance this capacity in other. In order to place in context the actions undertaken we will briefly describe the general elements of the biosynthetic pathway of carotenoids. Carotenoids as before mentioned, share with the rest of the isoprenoides the basic molecule: isopentenyl pyrophosphate. This is a 5-carbon compound which is the unit of synthesis for the formation of compounds 5, 10, 15, 20 or more carbon (always multiples of 5). By this way arises the skeleton of many isoprenoids through a reduced number of steps of basic reactions (McGarvey and Croteau, 1995). Carotenoids have a skeleton of 40 carbons (C40), which is produced by joining two 20 carbon-molecules of geranylgeranyl pyrophosphate (GGPP). Strictly, the carotenoid biosynthetic pathway begins with the isomerization of IPP to its allylic isomer, dimethylallyl pyrophosphate (DMAPP). DMAPP is the initial, activated substrate in synthesis of long chain polyisoprenoid compounds such as GGPP. The formation of DMAPP from IPP is a reversible reaction that is catalyzed by the enzyme IPP isomerase (EC 5.3.3.2) (Cunningham Jr. and Gantt, 1998.). Introduction of any of a number of different plant, algal, or yeast IPP isomerase cDNAs, or additional copies of the *E. coli* gene for this enzyme, enhances several folds the accumulation of carotenoid pigments within these cells (Sun et al., 1996; Kajiwara et al., 1997). The GGPP molecule is the next precursor in the synthesis of carotenoids; the enzyme that catalyzes its formation is the GGPP synthase (GGPS; EC 2.5.1.29) (Dogbo and Camara, 1987; Ogura et al., 1997). The formation of the symmetrical 40-carbon phytoene (7,8,11,12,7′,8′,11′,12′-

octahydro-ψ,ψ carotene) from two molecules of GGPP is the first specific reaction in the pathway of carotenoid biosynthesis. The biosynthesis of phytoene from GGPP is a two-step reaction catalyzed by the enzyme phytoene synthase (PSY; EC 2.5.1.32).

The next compound in the route of synthesis of β-carotene is lycopene, which originates through four successive desaturation reactions of phytoene (Karvouni, 1995). These desaturation reactions serve to lengthen the conjugated series of carbon-carbon double bonds that constitutes the chromophore in carotenoid pigments, and thereby transform the colorless phytoene into the pink-colored lycopene. The desaturations undergone by phytoene are catalyzed by two related enzymes in plants: phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS). In bacteria and fungi achieve the same result with a single gene product (Armstrong G. A. 1994; Sandmann, 1994). Later lycopene β-cyclase (LCYB), catalyzes the formation of the bicyclic β-carotene from the linear, symmetrical lycopene in plants and cyanobacteria (Cunningham et al., 1994; Cunningham et al., 1996; Hugueney et al., 1996). Xanthophylls comprise most of the carotenoid pigment in the thylakoid membranes of plants. Hydroxylation at the number three carbon of each ring of the hydrocarbons β-carotene and α-carotene will produce xanthophyll pigments zeaxanthin (β,β-carotene- 3,3'-diol) and lutein (β,ε-carotene-3,3'-diol), respectively. Finally, the evidence has shown that a similar biosynthetic pathway is present in all carotenogenic organisms (Sun et al., 1996; Lee and Schmidt-Dannert 2002). The biosynthetic pathway, in all the carotenoid producing organisms follows similar routes with minor variations in the final steps leading to different carotenoids (Umeno et al., 2005). Logically, most of the enzymes involved can be expressed in heterologous host. For more details, several reviews on aspects of biosynthesis and function of carotenoids are available (Hirschberg, 2001; Sandmann, 2002; DellaPenna, 2005). This situation offers multiple opportunities for the use of different approaches using biotechnology tools to increase the production of β-carotene.

e. New Processes and Products Using Genetic Engineering

Although there was not previously carotenoid synthesis capacity, availability of FPP among microorganisms has been utilized at various events which has been successful in producing β-carotene as major carotenoid product in various microorganisms including *Escherichia coli*, *Zymomonas mobilis*, *Agrobacterium tumefaciens* and *Saccharomyces cerevisiae* carrying the *Erwinia uredovora* carotenogenic genes (Figure 4). Expression of β-carotene was achieved through introduction of genes crtE, crtB, crtI and crtY, encoding the four enzymes required, in the genome of the microorganisms mentioned. In these cases, the transferred genes were flanked by promoters and terminators derived from the corresponding organisms (Mizawa et al., 1990; Mizawa et al., 1991; Yamano et al., 1994).

Although the production of vitamin A by chemical means could be cheap, its use as a therapeutic element by oral delivery is erratic and therefore the results are often not as expected (Pirie, 1983). Success might be reached if the provitamin A is integral component of food. As already mentioned, the shortage in the intake of vitamin A is associated with many diseases in developing countries. In some of these countries, rice is the staple food.
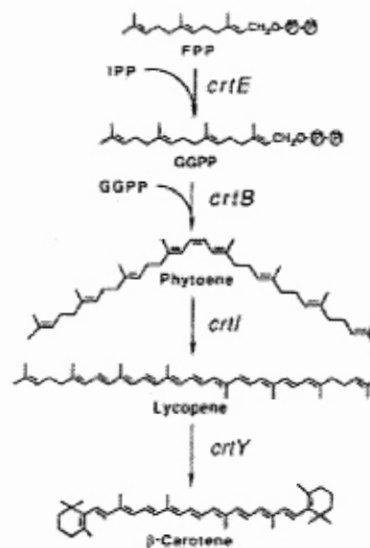
Figure 4. β-carotene biosynthetic pathway of non-photosyntetic bacterium *Erwinia* (Yamano et al., 1994).

Rice cultivars did not produce this provitamin in the endosperm, but immature rice endosperm is capable of synthesizing the early intermediate geranylgeranyl diphosphate; thus, Burkhardt et al., (1997) achieved produce the uncolored carotene phytoene by expressing the enzyme phytoene synthase in rice endosperm. Shortly afterwards, the same group completed the biosynthetic pathway to produce β-carotene in transgenic rice plants (Ye et al., 2000; Beyer et al., 2002). With regard to rice, efforts have been made subsequently to increase the level of β-carotene in the grain (Al-Babili, et al., 2006), and possibly in the near future this effort could be reached. In turn, the potato is a major staple food, and changing its content of provitamin is a possible means of alleviating nutritional deficiencies in the West hemisphere. Potato tubers contain low levels of carotenoids, mainly xanthophylls lutein, antheraxanthin, violaxanthin, and of xanthophyll esters. None of these carotenoids have provitamin A activity. Potato tubers contain low levels of carotenoids, mainly xanthophylls lutein, antheraxanthin, violaxanthin, and xanthophyll esters. Diretto et al., (2006) silenced the lycopene epsilon cyclase, by introducing, via Agrobacterium-mediated transformation, an antisense fragment of this gene under the control of the patatin promoter. The results showed significant increases in β-β-carotenoid levels, with β-carotene showing the maximum increase (up to 14-fold). The same group, achieved through silencing β-carotene hydroxylase increases total carotenoid and β-carotene levels in potato tubers (Diretto et al., 2007).

The availability of a large number of carotenoid biosynthetic genes has facilitated the recent progress in the metabolic engineering of carotenogenesis in other plants (Fraser and Bramley, 2004; Taylor and Ramsay, 2005), such as in the cases of Golden Canola seeds (Shewmaker et al., 1999), yellow potato (Ducreux et al., 2005), and plants with high-economic-value carotenoids (Stalberg et al., 2003; Ralley et al., 2004; Lu et al., 2006). Looking ahead, researchers have been highlighted new complementary strategies to enhance expression of carotenoids in general and β-carotene in particular in plants of interest: gene assembly, directed enzyme evolution, and these combined approaches (Umeno et al., 2005).

f. Biotechnology, Greenhouses and β-carotene

Curiously, in countries where there is greater biodiversity, there are also problems of diseases related to deficiency of β-carotene in food (Ye et al., 2000). Under the argument does not affect biodiversity (Quist and Chapela, 2001), the use of genetically modified organisms (GMOs) in these countries is restricted or forbidden, unless it is conducted in conditions of confinement. Greenhouses are an option, in terms of the exclusion, for the production of GMOs, including maize and other crops. Another interest approach using biotechnological knowledge in greenhouses is the elicitation of β-carotene production with specific compounds in no-transgenic systems. The effect of exogenous methyl jasmonate (MeJA) on antioxidative compounds of romaine lettuce (*Lactuca sativa* L.) has been investigated. Lettuces were treated with various MeJA solutions (0, 0.05, 0.1, 0.25, and 0.5 mM) before harvest. Total phenolic compounds content and antioxidant capacity of romaine lettuce significantly increased after MeJA treatments (0.1, 0.25, and 0.5 mM). The total content of phenolic compounds of the romaine lettuce treated with 0.5 mM MeJA (31.6 µg of gallic acid equivalents/mg of dry weight) was 35% higher than that of the control. The increase in phenolic compound content was attributed to a caffeic acid derivative and an unknown phenolic compound, which also contributed to increased antioxidant capacity. The induction of phenylalanine ammonia-lyase (PAL) activity by the MeJA treatment indicated that phenolic compounds were altered due to the activation of the phenylpropandoid pathway. Total content of carotenoids, including lutein and β-carotene, of the MeJA-treated lettuce did not change after 8 days of treatment, whereas the content of the control without MeJA decreased after 8 days (Kim et al., 2007).

Mechatronic Approaches to Improve β-Carotene Production

Mechatronics involves the synergy of mechanics, electronics and computer science applied to the development of electromechanical products and systems by means of an integrated design. Mechatronics is not the union but the intersection of these three fields within the context of system design (see Figure 5).

Originally conceived by engineers from the Yaskawa Electic Company as the conjunction of the words 'mechanics' and 'electronics' [Mori, 1996; Harashima et al., 2005], the mechatronics concept has been redefined to include an interdisciplinary field of engineering now also covering other engineering areas such as electricity, control, computer science, robotics, chemical and nuclear instrumentation and even other areas like medicine and biotechnology. This new approach promotes the design and development of advanced electromechanical systems controlled by embedded systems. The end product is, thus, an advanced electromechanical system whose design is not a sequential process iterating over different fields of engineering but a concurrent process where the product is designed optimally in all these fields of engineering simultaneously. In this sense, the mechatronics is not a new field of engineering but it is the result of its natural evolution towards integral design and implementation of advanced electromechanical systems [Grimheden y Hansen, 2005].
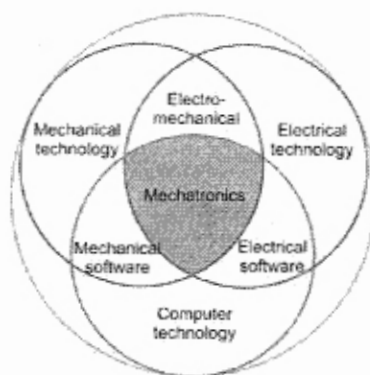
Figure 5. Mechatronics: synergy of mechanics, electronics and computer science.

Mechatronic design has been widely used to provide solutions to diverse problems. For example Lee (1999) developed a device capable of determining certain properties of an object such as shape, texture, temperature, stiffness among others; Tandon et al. (2002) used a predictive model based on an artificial neural network to optimize cutting forces in numerically controlled machine-tools; Xiaoli and Shiu (2000) used *fuzzy logic* techniques along with the wavelet transform to monitor real time wear and breakage conditions by means of current sensors in drilling; Jae and Yun (2005) a highly effective velocity profile generator using polynomial techniques that can produce profiles with different acceleration characteristics; Alaniz et al. (2006) developed a sensorless system to detect tool breakage by means of the wavelet transform applied to monitoring signals of the machine motors; Castañeda et al. (2006) developed an intelligent climate control system for greenhouses based on *fuzzy logic* and FPGAs (*Field Programmable Gate Arrays*) for low cost monitoring.

A typical mechatronic system is structured with a mechanical frame, actuators, sensors, signal conditioning and processing devices, computers, device interfaces and power sources. The sophistication of these systems and the incorporation of new technologies, such as intelligent sensors, control techniques, microelectromechanical systems (MEMS), have provided more maturity to mechatronic solutions. Among the fields where engineering has evolved to integrate areas that did not seem to converge previously is climate control systems or biotronic systems. In this context, biotronic systems is the application of mechatronic technologies to biological systems, especially when referred to intensive production under greenhouses. The application of new technologies and automation systems for biotronic systems has provided engineers with better construction technologies and enhanced designs, intelligent sensors, fitomonitoring and control strategies. This biotronic approach has had a positive effect in critical areas of climate control system for intensive production under greenhouses by increasing its cost-effectiveness:

a. Greater energy efficiency. Having enhanced greenhouse designs and a more precise climate control system can reduce heating and electricity costs.
b. Increased productivity. Automation increases productivity of workers by providing time for more important tasks.

c. Improved administration. Automation offers the value added of real time information to improve administrative decisions and invest time on strategic administration rather than on quantitative administration.

d. Water requirements reduction. Automatic watering systems provide a more precise water dosification control reducing water consumption by providing the right amounts of water on time and in precise amounts.

e. Fertilizer requirements reduction. By having an automated dosification, monitoring and high precision system the amount of required fertilizer can be reduced and used more effectively.

f. Reduced use of chemicals. Having a climate (temperature, humidity and watering) control system for intensive production helps reduce stress in the crops and reduces the risk of diseases caused by pests and, thus, the need for fungicides, pesticides y herbicides.

g. Improved crop quality and uniformity. A climate control system enables the producer to handle the crop properly with uniform grow conditions, simplifying the shipping, handling and commercialization. Moreover, biotronic system provide effective watering and fertilizing systems offering better product quality given the controlled climate and nutrition of the plant that causes it to generate a better content of vitamins, antioxidants and other nutrients. For example, under a controlled climate and nutrition the tomato fruit can be induced to produce a greater content of lycopene and β-carotene.

h. Less equipement wear and damage. A control system with low performance increases the amount of work the artificial climate system requires; on the other hand, a well designed control system simplifies the equipment administration and its lifetime.

i. Continuous monitoring and alarms. A system that automatically informs the producer of inusual situations at an early stage can reduce risks for the crop and the production system.

j. Better decision making. A good control system stores greenhouse climatic data during the crop growth providing feedback for better decisions.

In general, mechatronics applied to greenhouse production (greenhouse automation and mechanization of production processes, climate control system and irrigation system design, greenhouse structural design, etc.) have improved production quality and quantity, and increasing the contents of lycopene y β-carotene in the case of the tomato fruit (Schmidt, 2008). A few examples of relevant work in the field are discussed next.

Greenhouse Mechanisation

International competition demand low cost, high quality and safe horticultural produce, issues that are not new in industrial production (aeronautics and automobile industry, electro domestic etc.). Also, quality management and the improvement of the over-all efficiency of the production process have received considerable attention in industrial production it is not the exception in the greenhouse crop production process which is composed of various steps

(Figure 6). Depending on the crop grown, a whole production cycle may take a few weeks (e.g., lettuce), several months (e.g., tomatoes, etc.) up to several years (roses). In this sense, greenhouse mechanization has impact on the plant production such as seeding and cutting (where the greenhouse crop production process and most of the times seedlings or cuttings are produced by highly specialised companies), grafting (grafting is a necessary step to assure production quality and quantity or prevent diseases) and transplanting (plants are seeded in substrate or soil and then transplanted into or onto the final growing substrate: soil, tezontle, perlite, coco fibre etc.) as well as on the crop production including sorting and packing the harvested produce (the harvested produce is collected, sorted, and packed before shipment to the auction or retailer). In this way crop maintenance and harvest (for single harvest crops the production is finished at harvest time) does not show much automation yet because they are much more difficult to automate (van Henten, 2003).

Nowadays, seeding is a highly mechanised process. A fully automated seeding line consists of a tray washer, a tray de-stacker, a tray filling machine, a seeding machine, a machine covering the trays, a watering machine and a tray stacking machine and to improve product quality and uniformity as well as efficient use of the production area, camera assisted tray inspection and filling machines are used to achieve 100% filling with good quality plant material. Rose cutting production robotic that use machine vision and industrial manipulators is in the market (Rombouts and Rombouts, 2002). The development of Geranium and Chrysanthemum cutting sticking robots were reported by Simonton (1990) and Kondo and Monta (1999), respectively. Grafting is a delicate process which requires a high degree of skill and the operation is physically and mentally demanding. A grafting robot was developed by Nishiura et al. (1996). The grafting robot achieved a success rate of 97% at a speed 10 times faster than human workers (Kondo and Ting, 1998). This machine is commercially available and can be used for grafting cucumber, water melon, melon, tomato and egg-plant at a capacity of 800 plants per hour. A robot for sorting and transplanting of Orchid seedlings in tissue culture was reported by Kaidu et al. (1998) and Okamoto (1996). For seedling production, transplanting machines are common practice nowadays. The development of transplanting robots has been reported in the USA, Korea and Japan (Yang et al., 1991; Sakaue, 1996; Ryu et al., 2001). Mobile growing systems and internal transport systems have been developed especially for potted plants and seedling production throughout the last two decades. More, recently also roses and gerbera's are produced at relatively small scale in a mobile growing system (van Henten, 2003). Automatic in row pesticide sprayers are commonly used in greenhouse vegetable production. Automatic over head pesticide sprayers are used during the production of seedlings, flowers and potted plants. Hand-tool has been developed to attach the plants to the wire with a copper ring significantly reducing the amount of labour needed for this task. Machines for lettuce and radish harvesting are commercially available. A robot prototype for leaf picking of cucumbers in greenhouse was reported by Van Henten et al. (2004). There are some researches of harvesting robots for harvesting cucumbers (Arima and Kondo, 1999; Van Henten 2003), tomatoes (Kondo, 1996; Monta et al., 1998) without having these machines commercially available. Automatic grading lines for tomatoes (colour, weight and diameter), cucumber (weight) and sweet pepper (weight) are commonly used in combination with automated box fillers and stacking machines. Sorting lines consisting of buffered feeders, maturity measurement, length and thickness measurement and automatic bunching (Van Henten 2003).
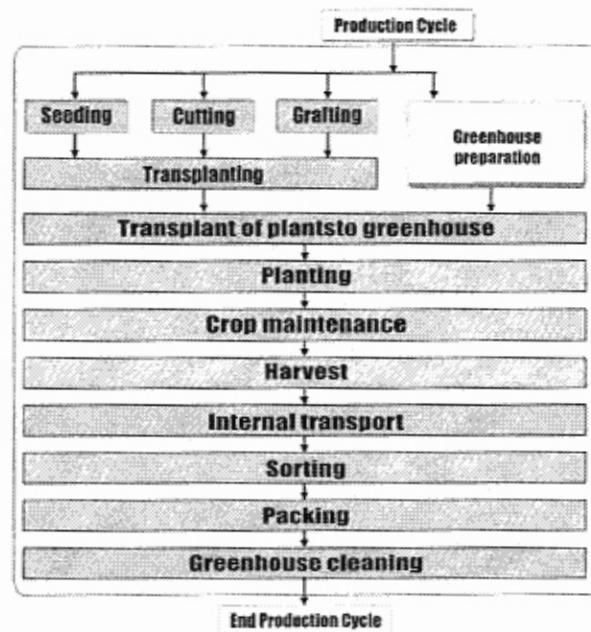
Figure 6. Greenhouse crop production process (Van Henten, 2003).

Mechanical and Structural Design of Greenhouses

Greenhouse design, structure, topology and cover provides a barrier between the external environment and the crop, which creates a microclimate that protects crops from wind, rain, pests, diseases, weeds and animals. Likewise, such protection enables control of the climate variables (temperature, humidity, CO_2, etc.) and allows to effectively implement chemical and biological monitoring to protect the crop. All these characteristics make the production under greenhouse have higher yields than conventional open field production.

Research related to the design of the structures of greenhouses and fluid mechanics involved in them have been conducted from a mechanical and structural point of view as a means of improving the climate inside the greenhouse (Critten, 2002). Baptista et al., (1999) mention that ventilation is one of the most important tools for controlling the climate in the greenhouse. In order to understand more clearly the physical processes associated with natural ventilation several research works have been carried out. Earlier works were merely experimental, employing mainly the tracing gas technique (Boulard & Draoui, 1995; Baptista et al., 1999). On the other hand, purely theoric approaches have also been used, as in the case of energy balance models (Seginer, 1997; Roy et al, 2002). However, these techniques provide information only about the rate of overall ventilation without giving details of the spatial distribution of lines of air flow, temperature or humidity inside the greenhouse. In recent years, computer simulations have reinforced the theoretical and experimental research. The numerical simulations made using the CFD technique have always been accompanied by an experimental stage that has served to give certainty (validating) the results obtained by

CFD. Recent advances in CFD programs facilitate the study of vector and scalar fields in the present climate in the greenhouse through the solution of the equations governing the flow of fluids (Navier-Stokes equations). This technique also allows consideration of all the climate variables (temperature, relative humidity, CO_2 concentration), as well as the geometry of the greenhouse together with the crop in question (Molina-Aiz et al., 2004). Norton et al. (2007) reviewed the use of CFD for modelling and designing of ventilation systems for farm buildings. Bartzanas et al. (2002) carried out an analysis of the process of ventilation in a tunnel greenhouse equipped with anti-insect mesh on the side windows. Fatnassi et al. (2003) studied the patterns of air flow, temperature and humidity in a tomato greenhouse using a three-dimensional model. Campen y Bot (2003) employed a three-dimensional model to study the ventilation of a "parral" type greenhouse taking into consideration the presence of the surrounding greenhouses. Molina-Aiz et al. (2004) carried out measurements of velocity and temperature inside an Almeria type greenhouse to determine the effect of wind speed on natural ventilation. Ortiz (2004) carried out the numerical simulation of natural ventilation in a greenhouse of 10 Colombian ships, without considering the presence of the crop. The results showed the impact of exterior climate upon the pasive climate control of greenhouses using natural ventilation. Rico-Garcia et al. (2006) studied the effect of wind on the ventilation of two configurations of greenhouse using two-dimensional simulations and experiments to validate the simulations. Ould Khaoua et al. (2006) a two-dimensional model used in CFD to study the effect of wind speed and the configuration of the vents on the patterns of ventilation and temperature inside a greenhouse of glass.

Currently, there are greenhouses on the market with different topologies and cover materials (glass, plastic, etc.) developed in different countries (Mexico, Netherlands, France, Israel, etc.), which have been successfully applied to production in greenhouse in different climates, however, innovation and technological development in this area is vital for efficient energy use and improve the quantity and quality of production.

Modeling and Climate Control of Greenhouses

In particular, climate control in a greenhouse is one of the tools used for the improving of the production. According to Challa (1990), strict control of different climate variables (temperature, radiation, the amount of CO_2 in the atmosphere, humidity, etc.). which have an effect on plant growth, results in increased production and quality. The climate control in a greenhouse should be considered as part of general management rather than an isolated activity. The administration can be defined as a collection of activities aimed at achieving certain goals. One of the goals of the farmer as a businessman, is generally maximize the profits. The optimal control of the climate of a greenhouse has been the subject of many philosophical thoughts throughout the past two decades. One of the first qualitative analysis on this subject dates from 1978, when Udink ten Cate, Van Dixhoorn and Bot considered the climate control of a greenhouse and the administration of the crop from an integral point of view (Udink ten Cate, 1978). Their ideas changed in different ways during the following years (Challa et al., 1988; Challa and Van Straten, 1991; van Henten, 1994, 2000, 2003), but the fundamental concept is still the same. Within this concept of integral control, a

greenhouse is seen as a process in which multilevel hierarchical levels are distinguished by different time scales. The main reason for this hierarchical decomposition of the administration of a greenhouse is the inherent complexity of the process being considered, there are a lot of variables in the process related to the cultivation and the climate of the greenhouse, and their complex interactions, which would inevitably demand decomposition into sub-problems, which is more desirable in the design of control systems; this hierarchical decomposition in a greenhouse can be compared to that found in industrial production systems where automatic control concepts can be applied.

The main objective of this kind of hierarchical control is the treatment of the raw information produced by the sensors, this treatment can be addressed with simple empirical relationships or through simulation models of the relations established between climate and the physiological response of the crop. The commercial greenhouse control systems and research in this area revolve around this outline of the production system in a greenhouse. Research has been conducted at all levels of the hierarchical pattern, either in every particular level, or as a whole, i.e., covering two or all levels. At the lowest level is the shorter-term monitoring of climatic conditions of the greenhouse, which operates with a time scale of seconds or minutes. This level has to do with the efficient operation of the valves of both heating and CO_2, and the mechanisms of the ventilation system, etc., which has to do with controlling climate variables in the greenhouse, such as air temperature, concentration of carbon dioxide and moisture. In this lower level, research in climate control of greenhouses have been mainly focused on the control of air temperature by applying different control strategies. Classic control schemes were used by Kamp (1996) and Bontsema (1994) studied predictive control schemes, which are based on measuring the disturbance and act immediately on the process before it affects the variable that is controlling. For this kind of control it is necessary to have a model of behavior of the process to calculate the control action necessary to compensate the effect of the disturbance. Tanatu (1989) showed that with the use of models to predict the impact of changes in outside weather conditions on the interior environment, it is possible take corrective actions in advance rather than waiting until the change in internal climatic conditions is detected. Young (1994) showed that substantial improvements could be made on the controllers if the PID control algorithm directly took into account the real response of the greenhouse. At this level, studies have been conducted on more advanced control schemes based on changing controller behavior under new circumstances such as the open loop adaptive control (Kamp, 1996) and model-based closed loop adaptive schemes (Udink ten Cate, 1983; Rodriguez, 1996). Davis (1984) designed an algorithm to control the temperature with ventilation which performs better than with a PI controller with fixed parameters. Ehrlich (1996) studied the use of an intelligent controller to control the temperature of a greenhouse, and based on this concept used two approaches for modifying and determining the set points.

The middle level is responsible for the control of vegetative development, where the time scales are set by physiological processes and can range from one hour to several days. The main task at this level is to generate the optimal trajectory of climate variables that are controlled at the lower level to achieve the required outcome at the top level. It is at this stage where contributions have been reported regarding the increase in lycopene and β-carotene content in the production of greenhouse tomato (Schmidt, 2008). Of course, there is the need

to have information on the plant, either through growth models and development of the plant or fitomonitoring techniques, and we must optimize an objective function so as to maximize production and minimize the economic costs. Most research at this level have been developed around the interaction of the crop with the climate in the greenhouse to get the ideal conditions for the development of the crop in an optimal way. This second level, essentially open-loop, may become a closed loop process when the growth of plants is quantified using sensors or machine vision (van Henten, 1995; Schmitd, etc.). This way, it is possible to modify the lower level set points to improbé the overall behavior. Kozai (1985) and Jacobson (1988) studied greenhouse control on the basis of expert systems, where the response of crops to their environment is indirectly represented by means of the preset set points as knowledge rules. In learning based control (based on the model of decision of a farmer) both logic and decision rules made by the farmer have a paramount importance. This integration of a subsystem of decision or model of "decision-action" is aimed at evaluating the interventions of human beings in the biophysical sub-system. Clouaire (1996) studied this kind of heuristic modeling (expert systems) based on artificial intelligence techniques. At this stage Hashimoto (1985) proposed a scheme of control depending on the response of the plant (speaking-plant), where the plant is considered as a black box and identification techniques are used in the system to determine the responses of plants to changes in the microclimate. This type of approach has been being conducted due to advances in the field of sensors and hardware technology, which allowed for continuous monitoring of the dynamic response of the plant (Schmitd, 2003). Some experimental studies have been released independently on the variables that affect the development of the plant. To cite some examples, the ratio of wind in controlling the temperature has been studied theoretically (Bailey, 1985), this being one of the few approaches that has been formally proven in practice in commercial greenhouses in production (Chalabi, 1996). This concept is based on the performance of the crop, which uses a simple model which only defines a band of temperature that does not lead to production losses and a physical model that deals with the energy balance of the greenhouse and includes solar radiation, wind speed and the exchanges of radiation in the greenhouse. The physical model is used to determine the trajectory of the operation point of warming that minimizes the total consumed heat loss. There is also work done on the use of models applied to the control of humidity based on transpiration control strategies (Stanghellini, 1992), where the idea is that the requirements of the quality of the crop are based on the rate of transpiration. Schmidt (2008) developed an study on levels 1 and 2 by means of fitomonitoring and Mollier diagrams, where the comparision between different controllers is shown and this is one of the first works where the importance of humidity on the quality of the fruit is quantitatively determined. Results show a significant increase in the average fruit yield per plant, as well as a considerable increase in the content of Lycopene and β-carotene in the tomato fruit.

At the highest level, decisions are made regarding the planning of the overall production of the crop, where the scale of time is likely to range from weeks to months. Models are used for the development of the crop, and even market models are used to optimize profitability. Based on the experience of the manager or purely economic criteria, the implications of changes in dates of harvest are explored, of reducing production costs, and so on. This level communicates the middle level the production goals so that it generates paths to be followed by the climatic variables to meet the main objective. At the highest level is where decisions

are made regarding the planning of the overall production of the crop. Only in some researchs has been considered a global approach, which tends toward the optimal integration of climate control. This approach is fairly complex, first because it considers different time scales: 1 to 2 months at the level of cultivation, 1 to 2 days at ground level and some processes in the cultivation, and 10 to 20 minutes at the level of greenhouse climate. And, secondly, the rapid dynamics of the system is influenced by the strong interactions of the greenhouse climate and the rapid fluctuations in the external environment, especially natural radiation. Moreover, accurate predictions of climate over the long term are not usually available. A final problem is the need for measurements of the crop online. To make optimal control suitable for the production of crops in a greenhouse all these difficulties must be considered and resolved. Most research studies have dealt by parts with the optimal control of production under greenhouse conditions. Sengier (1998) considered the problem of the slow sub-system, whereas Hwang (1993) focused efforts on the rapid sub-problem. Chalabi et al. (1996) and Tchamitchian et al. (1992) optimized part of the greenhouse production system. Seginer (1997) studied the optimal control based on neural models and neural controllers. Among the first ones to address the problem of optimal control in general is Van Renten (1994, 2003), who presented a methodology to decompose the system in two different time sacles despite the presence of strong influences and rapid fluctuations of the external inputs. According to this methodology, long-term problem must first be resolved, and then using the results of it, the problem of short-term can be addressed. To practically apply the methodology of Van Henten, two problems must be solved, the first is related to exogenous inputs, that is, the climate must be known over the full range of optimization for calculating the optimal control; secondly, because that the best control is essentially open-loop, feedback is required to deal with initial states, modeling errors and flaws in climate prediction. There is still a problem inherent in this type of control proposals, namely, the practical solution. Tap (2000) used the decomposition in two time scales proposed by Van Henten and combined it with two kinds of climate prediction and online adaptive optimal control, based on the use of an integrated model of the greenhouse-crop (tomatoes) to solve the problems of the methodology proposed by Van Henten. The combination of these approaches (two times-scale receding horizon optimal control algorithm) represents the state of the art when it comes to implementing optimal control in the production of vegetables in greenhouses.

On the other hand, the biggest drawback for the development of techniques for monitoring, lies in the need for an appropriate model of the greenhouse climate, as the algorithm design is based on prior knowledge of the model and is independent of it. The kindness of the controller will depend on the differences between the actual process and the model used. This is the reason that justifies the great efforts being undertaken to design, calibrate and validate a good model of the greenhouse climate. During the last two decades, a large amount of scientific knowledge has been accumulated and expressed in mathematical models with regard to greenhouses. In the literature, several climate models have been submitted, some are based on the physical laws involved in the process (thermodynamic properties), models in which the processes responsible for the transfer of energy and mass are to be examined. Such models provide a detailed description of the climate in a greenhouse in connection with the weather outside, the physical properties of the greenhouse and its equipment, and therefore are of a high order, such as the models by Bot (1983), Zwart (1996),

Tap (2000), Tavares (2001), and Castañeda et al. (2007). On the other hand in this kind of models, there are simplified models based on the linearization of the exchange of sensible and latent head, such as the models by Boulard (1993 and 2000). There are other models based on transference functions, in which the nonlinear greenhouse system is linearized by selecting an operation point and assuming first order outcomes, such as in models by Udkin Ten Cate (1985b). Some others are black box models, which are based on analysis of data into and out of the process such as the model by Boaventura et al.(1992), López et al. (2007) or special cases of black box models based on neural networks such as the model by Seginer (1994) or based on fuzzy logic (Boaventura et al., 2006). There are research studies of simplified physical models, where the greenhouse is considered a solar collector and its thermal performance can be described by an equation of energy balance. These kinds of models are based on the linearization of the Exchange of sensible and latent heat and use only a limited number of parameters (Boulard and Baille, 1993; Boulard and Wang, 2000; Castañeda et al., 2002).

A large number of solutions have been proposed to improve climate control in the direction of the requirements set above. However, these solutions have been only partial ones, and have not been fully implemented commercially and all have been developed around a hierarchy of a decision-making process. Climate commercial controllers are mainly based on looping only at level 1 of the hierarchical schema. A few commercial controllers have incorporated heuristic rules and some other more advanced commercial systems that— although also rely on a lot of heuristic knowledge and are focused only to control the climate in the greenhouse without taking into account the physiological processes or the second level of the hierarchical schema—unlike the first ones, allow us to make more efficient use of energy and integrate advanced models in which the climate control system takes into account the climatic conditions of both the exterior and interior control system, and the ventilation is based on a model that calculates the energy balance. It is worth to mention that the option of integrating models, is not widespread because it works only for certain structural designs of glass greenhouses, so it can not be applied to other types of greenhouses with different types of covers.

Irrigation and Dosage of Nutrients in Greenhouses

The contribution of adequate water and fertilizer is one of the key elements in improving production and quality of the crop in the greenhouse. Providing the plants with the right amount and enough water and nutrients requires an irrigation system with the following four components: System for feeding nutrients and water, keeping a record of events, sensors and a method for making decisions. If any of the four components is missing, then the efficiency of the system is reduced and the yield and quality of production decreases (Waller, 2004). With a proper irrigation system the quality and quantity of crops can be considerably increased (Dominguez A., 1996). An optimal irrigation is a complex process: the amounts of water and nutrients must be enough to avoid stoping the photosyntesis process and, thus, the growth, but at the same time an excessive amount of water and nutrients can cause an exaggerated vegetal growth damaging the product among other consequences (Howard M,

1992). Work has been carried out on the determination of the optimum amount of water to be used in greenhouses (Guang-Cheng et al 2008; Chun-Zhi et al, 2008) and, in the development of automatic control systems for fertigation (Michels & Feyen, 1994; Kell et al., 1999; Yunseop et al., 2008). Likewise, work on intelligent controllers for fertigation has been developed (Bahat et al., 2000; Caprarico et al., 2008). However, most of the current commercial fertirrigation equipment provide a medium degree of automation based on classic controllers, and allow control over the mixture of fertilizer and water, controlling the pH and the electrical conductivity through feedback from sensors that return an online measurement of these two variables and not for each nutrient (e.g., calcium, potassium, phosphorus, etc.). Moreover, these systems usually have some type of feedback (pressure, radiation sensors, etc.) to determine the amount of water to supply.

Other Technologies Applied to Agriculture

The integrated pest management has a direct relationship with production in the greenhouse. The pest detection and monitoring activity is a tedious and time consuming task (Thomas, 1995; Stansly et al., 2004). IPM is a management strategy to optimize dealing with the pests in an economic and environment-friendly way (Koumpouros et al., 2004). In practice, the most common detection procedure is the visual exploration of plants or the use of sticky colored screens able to trap flying insects (Tang, 2008; Smith et al., 1983) and it is done once a day, it requires a number of well trained and well equipped personal. Some works related with the development of automatic systems based on machine vision for pest detection have been reported (Neethirajan et al., 2007; Zayas et al., 1989; Zayas and Flinn, 1998; Ridgway et al., 2002). However, the vast majority of these works have not been brought to commercial products.

Other important aspects of electronic technologies applied to agriculture are ruggedness and low cost which are a necessity for its implementation (Zhang, 2002). In this regard, new technologies such as FPGAs are presented as a good alternative for many real-life applications, a technology that has been under a lot of contributions in different fields of application in image processing and signals (Reyneri, 2004; Sklyarov's, 2004), multimedia (Ramachandran, 2004), robotics (Sridhara, 2004), telecommunications, cryptography (Daly, 2004), network systems (Martins et al., 2005; Moon et al., 2005) and computing in general (Salcic, Z., 1997; Gschwind et al., 2001; Chains and Megson, 2004; Ali et al., 2004). There are successful applications in detecting fracture and wear inserts in machine-tools (Troncoso, 2004), real-time monitoring of climatic variables (Mendoza-Jasso, et al., 2005) and intelligent climate control in greenhouses (Castaneda et al., 2006).

Greenhouse Possibilities in
β-Carotene Production

Greenhouse production systems are an important tool for improving the quantity and quality of production (considering the increase in β-carotene as part of the quality). As

mentioned, there has been a lot of work on the system of production in greenhouses and today there is a vast area of opportunity in different areas of engineering of greenhouses to increase the quality and quantity of production.

Having reviewed the various areas that are closely related to improvement in quality and quantity of crop production in greenhouses—such as the design and development of structures, the production process of growing in a greenhouse, and climate control systems and other related technologies—it is interesting to note that only the synergy of different fields will enable us to improve the quantity and quality of production, in particular climate control and irrigation (Castañeda et al., 2006). In this sense, from the analyzed fields follow some discussions and future prospects.

In the same vein, a new control system must meet certain requirements in relation to the goal of climate control according to the following criteria: production, quality of the crop, product quality, time of the production process, costs and risks of production. In addition, at the same time it must eliminate the drawbacks and deficiencies of the current controllers. However, monitoring systems have evolved into complex systems in which a large quantity of knowledge is implemented: control algorithms, instrumentation and various climate processes. Despite the great success of these automated systems for control of greenhouses that are now available, the control systems today are focused on environmental monitoring; information about the plant growth as a function of climate variables is used only in an indirect way through a generic pattern of the desired trajectory of operation, which are sometimes changed every day depending on the performance of the crop. If knowledge about the physiology of the plant and the physical processes can be incorporated, new improvements in control systems can be achieved (Castaneda et al., 2005). In this sense, progress in the coming years in the fitomonitoring technology, artificial intelligence, sensors and mechanical systems, modeling and control techniques will enable the development of control systems that involve the hierarchical control scheme of production in greenhouses.

In terms of fertigation systems, most of the current specific ion sensors are only available for off-line measurements; in this sense, the coming years—with the development of specific ion sensors (e.g., phosphorus, potassium and calcium) for online measurements, as well as the improvement of mechanical, hydraulic and electronic systems for fertigation—will allow a more precise dosage of each nutrient. Likewise, advances in fitomonitoring systems allow the control system to make decisions on the amount of water and nutrients based on the best response of the plant.

Concluding Remarks

Taken together, the data presented in this review display an interesting future in the production of β-carotene (and other secondary metabolites) exploiting methodologies based on biotechnology and mechatronics. Biotechnology approaches must be used in order to increase β-carotene content in living systems and even produce it in those in which it is not naturally possible. Plant or algae systems are obvious candidates to be used as "β-carotene factories" in greenhouses. Thus, mechatronics tools will be fundamental in order to control and optimize β-carotene production. Nowadays, the available machines are largely based on

principles of industrial automation. It is expected that the next generation of technologies for greenhouse production will be the result of combined innovations in the field of biotechnology, mechatronics and robot technology, mechatronic sensing and control hardware and software, electronics technologies, ion selective sensors, machine vision pest scouting sensors, cultivation systems, plant physiology and plant breeding, among others.

Acknowledgments

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