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REVIEW PAPER

BIOTECHNOLOGICAL STRATEGIES FOR ENHANCING THE NUTRITIVE AND NUTRACEUTICAL VALUES OF TOMATO (*SOLANUM LYCOPERSICON*)

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Tomatoes are a unique functional food and a natural reservoir of many health promoting nutrients, antioxidants, dietary fibres and chemopreventive nutraceuticals. They are particularly rich in lycopene which has been associated with the prevention of cardiovascular disease and cancers of the prostate and the gastrointestinal tract. As an important vegetable worldwide, tomatoes have drawn the attention of many researchers. Thus, numerous investigations have been conducted and various improvement strategies applied for enhancing the functionality of this medicinal food geared towards disease prevention, global health and well-being. Molecular breeding has produced a number of tomato lines with enhanced levels of lycopene, β-carotene and xanthophylls. Over expression of certain genes have generated tomato fruits with enhanced ascorbic acid levels and folate accumulation up to 25-fold. Plant hormone technology has been used to enhance tomato minerals, antioxidant vitamins, lycopene, β-carotene, flavonoids and phenolic compounds in tomato fruit tissues. Manipulation in soilless culture solutions is valuable for enhancing the antioxidative capacity of tomatoes, vitamin C, flavonoids, lycopene, and β-carotene in fresh fruits. In addition, the spraying of nutrients, such as potassium, in field conditions has a strong stimulatory effect on lycopene contents of tomatoes. Transgenic strategies are also being adopted. These strategies offer a rapid way to introduce desirable traits into the phenotype and differ from other approaches in that novel genetic information is introduced directly into the plant's genome. An important and current trend in the improvement of functional foods is to shift from enhancing single nutritional compounds towards enhancing multiple nutrients and phytochemicals in order to harness their synergistic interactions. This could be achieved by the use of strategies having pleitropic effects such as bioregulators, multigene engineering and regulative genetic elements. However, the full potential of these technologies has not been realised and the relative gap between risk assessment and regulatory threshold desires much attention. These biotechnological strategies for enhancing the nutritive and nutraceutical values of the tomato food crop are discussed in this article.

Keywords: tomato, biotechnology, nutrients, nutraceuticals, health values

Introduction

The well-being and continual human existence of the world populace is wholly dependent on plants or food crops. Modern food technology strategies which are now tailored to induce changes in the synthesis of specific metabolites offer new approaches to metabolically engineer food crops toward producing foods with enhanced health values (Pryme &Lembcke, 2003). These strategies involve either modulating the pattern of gene expression through the use of various biologically active chemical substances or directly manipulating the genetic materials. The tomato is a unique functional food rich in nutrients with numerous health benefits beyond basic nutrition. It has a high content of lycopene and other antioxidants, which have been shown to play chemopreventive roles against prostate cancer (Canene-Adams *et al*., 2005); they also play a pivotal role in inhibiting oxidative stress, improving vascular function, and preventing cardiovascular disease in humans (Agarwal & Rao, 2000; Heber & Lu, 2002; Lin& Xu, 2013). This crop is widely consumed in the world and is suitable for improving human health and wellbeing. Auxins and certain other bioregulators are known to enhance the levels of phytonutrients and some secondary metabolites in tomato fruit tissues (Olaiya & Adigun, 2010). Furthermore, moderate salt stress applied in hydroponic nutrient solutions has also been used to enhance the inner quality of tomatoes, improving the levels of total soluble solids, vitamin C, flavonoids, lycopene, and β-carotene in fresh fruits. In addition, the spraying of nutrients has also been shown to improve the quality of tomato fruits. With the rapid development of gene transfer techniques, it is now possible to improve tomato fruits in order to increase carotenoid contents, thus a number of tomato phenotypes having been generated with enhanced levels of lycopene, β-carotene and xanthophylls (Jackson *et al*., 2008). Flavonoid levels and composition have been manipulated successfully in tomato fruit peel and flesh through different genetic manipulation approaches. Recently, mutation and the over expression of certain genes have generated tomato fruits with enhanced ascorbic acid levels while the over expression of multiple genes have been found more effective in producing the transgenic tomato resulting in folate accumulation up to 25-fold (Zhu *et al*., 2007). Various chemical substances, which may be naturally occurring or synthetic, have been shown to exhibit biological activities and could modulate the growth, development and composition of the plants. These chemicals, when exogenously applied to a plant, tends to modulate the pattern of the expression of various genes with implications on the phenotype of the plant, examples including plant bioregulators which are emerging tools for modulating the expression of various biological responses in plants and which are valuable for crop improvement (Olaiya *et al*., 2013). In conjunction with genetic engineering, these form the basis for modern plant biotechnology in quantitative and qualitative improvement of food crops.

Improving food crops, especially foods with additional health benefits beyond basic nutrition, is very important as dietary choice is becoming and remains the basis for maintaining a healthy lifestyle and well-being (White & Broadley, 2005). Foods are no longer expected to merely meet an individual's basic physical needs but to also contribute to disease prevention, health and well-being. This concept has given birth to the term 'nutraceuticals', which are food components that provide health benefits beyond the basic nutritional needs of individuals. These phytochemicals are found mid-way of a spectrum that spans from nutrients to pharmaceuticals. They are found in normal levels as part of diets, where they play a role in disease prevention as their major biological function, but can also be incorporated in higher amounts in food during food processing resulting in what is known as functional foods. Fruits and vegetables exhibit nutraceutical properties and reduce the risks of chronic degenerative diseases such as cancers, cardiovascular diseases (CVDs) and diabetes mellitus (Zhao, 2007). They contain essential minerals and vitamins as well as phytochemicals (non-nutritive plant chemicals that have protective or disease preventive properties), which places high values on them in human diets. In developing countries, where population studies have demonstrated inadequate consumption of certain micronutrients, incidence of various nutritional deficiencies as well as threats of high incidence of chronic diseases, it seems opportune to consider approaches to enhance the nutritional quality of certain widely consumed fruits and vegetables. One plausible approach is the development of products with a greater concentration of micronutrients and phytochemicals known to improve health; thus, while consuming fewer servings of produce, the population still has significant exposure to health promoting food constituents. Eating foods with enhanced nutrient and nutraceutical profile could therefore be adopted as a strategy for improving nutrition, health and livelihood. Consequently, there is now a paradigm shift in crop improvement where agricultural food production is no longer geared solely towards the search for high yielding or disease and environmental stress resistant varieties to produce the high yields needed to feed the ever growing world population, but is now also producing varieties with an increased content of essential nutrients and phytochemicals to prevent both malnutrition and disease. Although various approaches have focused on improving the phytochemical contents of crops, such as light effects, temperature, mineral nutrition, water management and irrigation, effects of root stocks, elevated carbon dioxide, as reviewed by Treutterin (2010), only few of them are eco-friendly, relatively cheap and work in a predictable manner. The present article reviews tomato nutritional values and their enhancement using the strategies of chemical and molecular manipulation. Hopefully, this work would be valuable to researchers interested in tomato crop improvement, tomato processing industries and consumers of tomato products.

The tomato as a functional food

Emerging epidemiological evidence on lycopene, tomatoes, and cardiovascular disease has shown promising protective effects with more frequent consumption. Tomato fruits as a natural reservoir of health promoting micronutrients, antioxidant nutraceuticals and colour pigments could therefore be termed a unique functional food (Komal *et al.*, 2011). In addition to being a rich source of vitamins and minerals, it delivers various chemopreventive phytochemicals.

These same phytochemicals impart the visual and taste attributes with implications on the flavour and the eating quality of tomatoes. They are widely cultivated in many countries of the Mediterranean region; China is the world's top tomato grower, accounting for more than one-quarter of the world's tomato acreage. Egypt and India together account for more than one-fifth of the world total; Turkey and Nigeria are also major tomato producing countries. Asia and Africa therefore account for about 79 percent of the global tomato area, with about 65 percent of world output (FAO, 2008). Italy ranks first in tomato processing among countries of the Mediterranean Region. However, with the discovery of nutraceuticals like lycopene, α-carotene, vitamin C, flavonoids and hydroxycinnamic acid derivatives in tomatoes, the consumption has increased tremendously. It has been involved in the protection of humans against diseases such as prostate cancer and cardiovascular disorders (Rein *et al.,* 2006); it also contains valuable minerals such as potassium, phosphorous, magnesium and calcium (USDA, 2008). Many tomato products are rich sources of micronutrients such as folate, and the vitamins A, C, and E (Table 1). Canene-Adams *et al.* (2005) reported that, while tomato products contain similar amounts of potassium and folate compared with other popular vegetables, they are a superior source of alpha-tocopherol and vitamin C and, in comparison with the other regularly consumed vegetables, only carrots are a better dietary source of vitamin A than tomato-based foods. In addition to their micronutrient benefits, tomatoes also contain valuable phytochemicals, including carotenoids and polyphenols.

Table 1. Nutrient and carotenoid composition of tomatoes and tomato products (Source: USDA National Nutrient Database for Standard Reference, Release 16–1. 2004)

	Tomato products (per 100g)					
Nutrients	**Raw Tomatoes**	**Ketchup**	**Tomato juice**	**Tomato sauce**	**Tomato soup**	**References**
Potassium (mg)	237	382	229	331	181	United States Department of Agriculture (USDA), report 2004
α- tocopherol (mg)	0.54	1.46	0.32	2.08	0.50	
Vitamin A (IU)	833	933	450	348	193	
Vitamin C (mg)	12.7	15.1	18.3	7.0	27.3	
Total Folate (μg)	15	15	20	9	7	
Carotenoids(mcg)						
β- carotene	449	560	270	290	75	Lucier *et al*., 2000
α- carotene	101	0	0	0	0	
Lycopene	2573	17007	9037	15152	5084	
Lutein+zeaxanthin	123	0	60	0	1	
Phytoene	1860	3390	1900	2950	1720	Tonucci *et al*.,1995
Phytofluene	820	1540	830	1270	720	

A very important functional food, the tomato is widely consumed in Africa and the rest of the world. It is therefore imperative to adopt suitable methods for their qualitative improvement. Chemical and molecular manipulations have been shown to modulate the biochemical composition of food crops in a predictable manner for their improvement and to produce value-added food crops with high nutrients and phytochemical concentrations (Al-Hakim, 2006; El-Al, 2009; Olaiya *et al.*, 2013).

Molecular manipulation of tomato metabolites

If the basic biochemistry of a food crop is changed, the levels of nutrients very important for health could be altered (Connor, 2000). Plants produce a vast and diverse assortment of organic compounds, the great majority of which seem not to participate directly in growth and development. These substances, termed secondary metabolites, are produced via the secondary metabolic pathways which branch off the primary metabolic pathways. Although variation exists in secondary metabolism in plants, any given plant species only synthesizes a defined and limited range of compounds; genetically related plants accumulating similar compounds often are differentially distributed among limited taxonomic groups within the plant kingdom. For instance, the tomato is known to accumulate lycopene. Strategies targeted at inducing changes in the expression of a trait changing the synthesis of a specific metabolite are referred to as metabolic engineering (Di Matteo *et al*., 2011). Modern food biotechnology provides powerful new approaches to 'metabolically' engineered food crops toward producing foods with enhanced health values. These could be achieved either by directly manipulating the gene or by modulating the pattern of gene expression through the use of chemical substances.

Recent breakthroughs in gene science have allowed the precise identification of genes that produce individual nutrients, phytochemicals, flavour or toxic substances/anti-nutrients compounds found in natural plants thereby making possible the manipulation of specific components in a natural food material. The understanding of the various biochemical pathways leading to the synthesis of these bioactive compounds has been used and still holds great potential for the chemical and molecular manipulation of crops. Strategies for achieving the redirection of metabolic fluxes include the manipulation of a single step in a biosynthetic pathway to increase or decrease the metabolic flux to target compounds so as to block competitive pathways or to introduce shortcuts that divert the metabolic flux in a particular way (Mehta *et al.*, 2002). However, this strategy is limited in value because the effects of modulating single enzymatic steps are often absorbed by the system in an attempt to restore homeostasis. Recently, strategies aimed at targeting multiple steps in the same pathway are attracting increasing interest because they seem to control metabolic fluxes in a more predictable manner (Zhu *et al.*, 2011). This might include up-regulating several consecutive enzymes in a pathway; up-regulating enzymes in one pathway while suppressing those in another competing pathway; or employing regulatory genes, such as transcription factors (TF), to establish multiple control over one or more pathways in the cell (Zhang *et al.*, 2009). Since technical hurdles limit the number of genes that can be transferred to a plant and the sequential transfer of genes for a single target is an extremely time-consuming approach, researchers have developed new transformation methods to introduce multiple transgenes into plants and express them in a coordinated manner (Naqvi*et al*., 2009). It should be understood that quite a number of these approaches to metabolic engineering involve the direct manipulation of the genome. Genetic engineering has not been widely accepted by the public because of the limited knowledge of the safety of these genetically modified foods and the concerns that the scientific community does not completely understand the ramifications for altering the genetic material (Kuzma & VerHage, 2006). More recently, chemical approaches for improving the yield and quality which modulate the phenotypic expression of plant genome are providing attractive biotechnological tools for crop improvement. Such tools include the exogenous application of plant bioregulators, spraying of the plant with nutrients and the introduction of salt into the growing medium to achieve mild salinity stress in tomatoes (El-Rokiek *et al.*, 2012; Olaiya *et al.*, 2013).

The knowledge about the key enzymes in biochemical pathways has been valuable for directing the biosynthesis of the secondary metabolites. Chemical and molecular manipulations have largely shown modulatory effects on the biochemical contents of nutritional importance in tomato fruits. One instance is the recent development of a purple tomato produced by scientists in the United Kingdom and other European countries, containing high levels of anthocyanin which is responsible for this purple colour. This tomato in a pilot test significantly extended the lifespan of cancer susceptible mice that were fed with the new tomatoes compared to mice that were fed with normal tomatoes (Butelli *et al.*, 2008). The team of scientists have taken Delila (Del) and Rosea1 (Ros1) genes, two transcription factors from the snapdragon plant *Antirrhinum majus* snapdragon, inserted them into the tomato plants through genetic engineering, thus producing a tomato with high levels of anthocyanin comparable to that in berry fruits, such as blackberry and blueberry, where anthocyanins occur naturally at high levels.

More recently, a relatively simple and rapid approach is the improvement of crops through the exogenous applications of plant growth hormones generally called plant bioregulators. One of the goals of bioregulator application is to enhance the flavour and nutritional quality traits of food crops (Table 2). These traits are attributed to the joint actions of many genes such as genes coding for chalcone isomerase, chalcone synthase, flavanone hydroxylase, which are responsible for the accumulation of flavonoids and lycopene β-cyclase (tLcy-b) for lycopene (Yokoyama & Keithly, 1991; Thakur*et al*., 2005; El-Gaied *et al*., 2015). However, the phenotypic effects and nutritional manipulation of the application of bioregulators have been widely reported as shown in Table 2. For instance, Nandi *et al*. (1995) reported an increase in amino acid and protein contents in tea shoots and oak tissues following exogenous application of bioregulators. Presowing seed treatment with auxin bioregulators at 100mg/l has also been shown to modulate some biochemical indices of the nutritional qualities of tomatoes (Olaiya &

Adigun, 2011). They have been found to increase the levels of β-carotene, flavonoids, vitamins, minerals and sugars in tomato fruits, especially at a 100 mg/L concentration (Olaiya & Adigun, 2010; Olaiya, 2011).

Table 2. Bioregulators involved in enhancement of nutrients/nutraceuticals in food crops

Bioregulator	**Nutrients/ Nutraceuticals**	**Food Crop**	**References**
Auxins, Gibberellic acid (GA_3)	Total soluble solids	Tomato	Khan *et al.* (2006); Olaiya *et al.* (2010); Ali *et al.* (2012).
Auxins,	Crude fiber	Tomato fruits	Olaiya *et al.* (2010)
Auxins,	Crude proteins	Tomato fruits	Khan *et al.* (2006), Olaiya *et al.* (2009).
Auxins, GA_3	Minerals	Tomato fruits, tomato shoot	Khan *et al.* (2006); Olaiya & Adigun (2010)
Auxins, GA_3	Vitamin A		Olaiya & Adigun (2010); Olaiya (2011)
Auxins, GA_3, salicylic acid	Vitamin C	Tomato fruits	Graham & Ballesteros (1980); Olaiya (2011); Ali *et al.* (2012).
Auxins, GA_3, 2-(4-methylphenoxy) triethylamine, (MPTA) 2-(4-chlorophenylthio) triethylamine (CPTA), salicylic acid	Carotenoids	Tomato fruits	Yokoyama (1977); Khan *et al.* (2006); Olaiya & Adigun (2010); Ali *et al.* (2012).
Auxins	Flavonoids	Tomato	Olaiya & Adigun (2010).
Benzothiadiazole	Flavonoids	Grape vine	Fumagalli *et al.* (2006)
Methyl-Jasmonate,	Flavonoids	Raspberry, grape berry	Wang & Zheng (2005);

Although the elucidation of the action mechanisms of auxins is in developmental stage, giant strides have been made in understanding the general influence of some other bioregulators on carotenogenesis in plants and the use of DCPTA has contributed to understanding the effects of bioregulators in tomato plants at the molecular level. This bioregulator possesses two distinct properties, indirect stimulation of the synthesis of carotenoids and direct inhibition of the transformation of the acyclic to cyclic carotenoids, thus the pre-treatment of tomato seeds with DCPTA causes the accumulation of carotenoids. This observation suggests that they may act through a common route of derepression of genetic material (Figure 1).

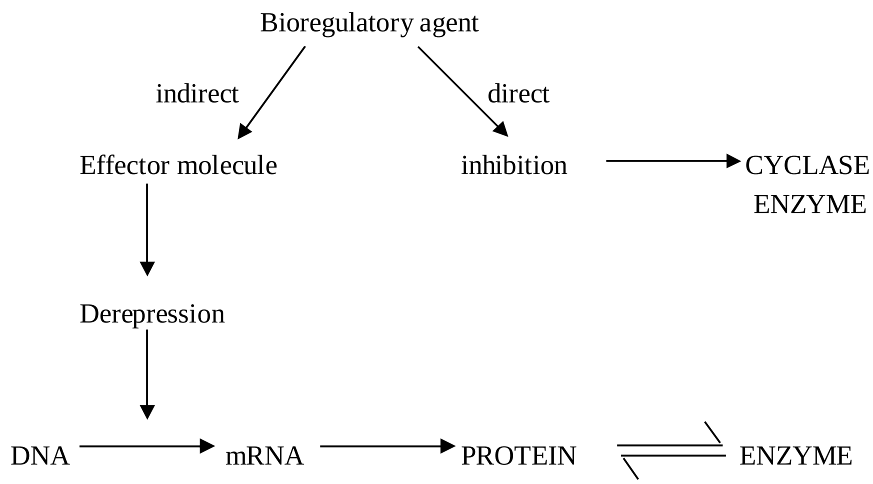

Figure 1. Scheme for modes of action of a bioregulatory agent (Adapted from Yokoyama & Keithly, 1991)

Conclusion

The biotechnological modification of plants serves as a valuable tool for research and commercial applications. Currently, the deployment of biotechnological strategies for enhancing the tomato food crop value is advancing and the possible use of bioregulators on a commercial scale is gradually gaining support. Bioregulators hold a great promise in tomato crop improvement and research activities on their use, as well as the full elucidation of their mechanisms of action in producing value added tomato fruits, is still a subject of investigation in the scientific community.

Conflict of interest

None declared

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