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Genetic Engineering of Crop Plants for Enhanced Antioxidants Activity

Gulzar S Sanghera[1], Pawan K. Malhotra[2], Guropkar S. Sidhu[2], Vinod K. Sharma[3,] Brij B. Sharma[4] and Ratna Karan[5]

1. SKUAST-Kashmir, Mountain Research Centre for Field Crops, Khudwani, Anantnag, 192102, J&K, India
2. School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, India
3. Indian Agriculture Research Institute, Regional Station, Katrain, Kullu, HP, India
4. Indian Agriculture Research Institute, New Delhi, India
5. Agronomy Department, University of Florida, Gainesville, USA

Corresponding author: g_singh72@rediffmail.com Tel & Fax: 01931-238246, 09419090403 (M)

Abstract:

The oxidation of simple foodstuffs by cells of most organisms (plants, animals and microbes) is the main way in which they derive energy for use in other processes. However, oxygen consumption and metabolism are fraught with danger. Active oxygen species (AOS) are formed as a result of aerobic metabolism, and these aggressive forms of oxygen are capable of serious damage to cell constituents, including membranes and DNA in plants and animals. The most common natural antioxidants in plants are flavonoids phenols, tannic acid, glutathione, ascorbic acid, carotenoids, and enzymatic antioxidants, SOD etc. that prevent uncontrolled oxidation, regulate electron transport processes, participate in and control enzymatic reactions, and direct acclimation and development through control of gene expression. Nutritional and antioxidative capacity of plants has generated interest among plant scientists and human health researchers alike since nutrition-enriched food has the potential as a preventive strategy to decrease incidence of diet-related diseases. Genetic engineering has been applied to enhance the concentration of useful nutrients in crop plants. Some of these fruits have been tested in animal systems and found to provide protection against diet-related diseases, and improved life span. An attempt has been made in this review to provide a common theme on the nutritional and

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antioxidant capacity of some crop plants, nutritive antioxidants, the potential of engineering enhanced nutritional quality in crops and the contribution of nutritional and quality diet in enhancing quality of life and will be valuable to plant biologists, geneticists and nutritionists.

Key words: Antioxidants, genetic engineering, gene expression phytonutrients, stresses

INTRODUCTION

Antioxidants are first line of defense against free radical damage. A broader definition of antioxidant is "An antioxidant is a molecule that prevents the transfer of electron from oxygen to organic molecules, stabilizes the free radical and terminates free radical reactions." They have the potential to avert the free radicals that induce tissue damage by preventing the formation of radicals, by stabilizing reactive oxidative species scavenging them, or by promoting their decomposition [1], [2]. From their likely source it plays a pivotal role in managing disease. In plant tissues many phenolic compounds (in addition to tocopherols) are potential antioxidants: flavonoids, tannins and lignin precursors may work as ROS-scavenging compounds [3]. Antioxidants act as a cooperative network, employing a series of redox reactions. Interactions between ascorbic acid and glutathione, and ascorbic acid and phenolic compounds are well known [4]. Under oxygen deprivation stress some contradictory results on the antioxidant status have been obtained. Experiments on overexpression of antioxidant production do not always result in the enhancement of the antioxidative defense, and hence increased antioxidative capacity does not always correlate positively with the degree of protection [5]. Nutritional and antioxidative capacity of crop plants has generated interest among plant scientists and human health researchers alike since nutrition-enriched food has the potential as a preventive strategy to decrease incidence of diet-related diseases [6]. A nutritive diet can be beneficial via interactions of several antioxidant components present in food, although the nature of these interactions is still a matter of conjecture. Genetic engineering has been applied to enhance the concentration of useful nutrients in crop plants [7]. Some of these crop plants have been tested in animal systems and found to provide protection against diet-related diseases, and improved life span. Antioxidant supplementation has made significant advances in the substantial reduction in blood pressure, in the elasticity of both small and large blood vessels and also in the long term improvement of blood lipid composition [8]. For a variety of reasons, therefore, research has been directed to understanding the endogenous mechanisms that control antioxidant levels in plants. There are twin aims of improving the resilience of plants to stresses and of enhancing the

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quality of plants used for animal feeds. The ability to generate plants with improved antioxidant contents provides a powerful means of increasing the yield and quality of edible plant products by prevention of oxidative damage to leaves and other tissues. In plants the antioxidative defences guard against oxidative destruction of membranes that causes reduced quality, yield and pre- and post-harvest longevity [9]. Furthermore, we were interested to know the extent in which natural antioxidants and related compounds in plants contribute to, and perhaps synergistically enhance, the antioxidant status. In this review, we have attempted to provide a common theme on the nutritional and antioxidant capacity of crop plants, nutritive antioxidants, the potential of engineering enhanced nutritional quality in crop plants, and the contribution of nutritional and quality diet in enhancing quality of life by reducing disease. It will be valuable to plant biologists, geneticists, nutritionists and academicians.

Need to engineer for antioxidants

Oxygen is essential element for life to perform biological functions such as catabolism of fats, proteins and carbohydrates in order to generate energy for growth and other activities. It is involved in the generation of various kinds of reactive oxygen species (ROS) such as hydroxyl radical ($^{\cdot}OH$), the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), singlet oxygen (O_2), nitric oxide radical ($NO^{\cdot}$), hydroperoxyl radical ($HO_2^{\cdot}$) and various lipid peroxides (Vellosillo *et al.,* 2010). Oxygen being highly reactive is capable of becoming part of potentially damaging molecules commonly called “free radicals” (Pourmorad *et al*., 2006). The main cellular components susceptible to damage by free radicals are lipids (peroxidation of unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids. To protect cells and organs from free radicals, living organisms have evolved an extremely efficient protective system, composed of a group of compounds and enzymes called “antioxidant defensive system”. In nature a wide variety of naturally occurring antioxidants, involves a variety of components, both endogenous and exogenous in origin [9], that function to neutralize free radicals. Free radicals are molecules or atoms of chemical class, skilled of self-regulating persistence with one or more unpaired electrons. In this process, of electrons from additional sources to become stable, they instigate a chain reaction of oxidation; make by damaging DNA, varying biochemical compounds, corroding cell membranes and killing cells completely. Most commons are Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS) ($NO^{\cdot}$, $ONOO^{-}$, etc) or Reactive Sulfur Species (RSS). Some scientists evaluate that such molecular obliteration plays a

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vital role in the expansion of ailments like cancer, heart or lung diseases and cataracts as a result several researchers persuaded that the collective effects of free radicals may escort to ageing, heart disease, cancer, hypertension, diabetes etc. The various ways and means that lead to production of free radicals in the living cells of different organisms include: **e**xposure of ultraviolet light, X-rays and gamma rays, product of electron transport chain, reaction catalyzed by metal etc. by interaction of bacteria and phagocytes etc., addition of e^- to a natural molecule, by loss of single e^- from neutral molecule, cigarette and smoke and environmental pollution.

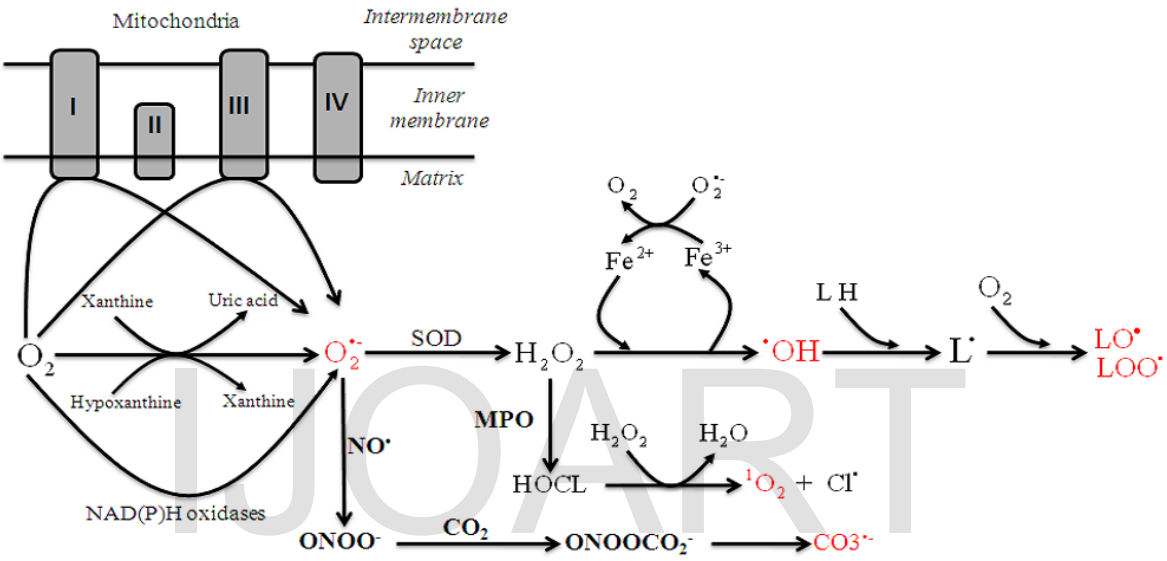

Fig 1 Production of free radicals via different routes [10]

Detrimental effects of free radicals

At high concentrations, ROS are hazardous for cell structures, nucleic acids, lipids and proteins. They react with these biomolecules leading to localized injury and organ dysfunction. Major oxidation reactions are explained as follows:

DNA

Oxidative damage of DNA occurs due to interaction of DNA with ROS. Hydroxyl radical (˙OH) can react with all components of the DNA molecule and damage both the purine and pyrimidine bases and also the deoxyribose backbone. Oxidative damage also leads to the formation of DNA lesions, which is first step involved in mutagenesis, carcinogenesis, and ageing. The interaction of pyrimidine with ˙OH, thymine glycol, uracil glycol, urea residue, 5-hydroxydeoxyuridine, 5-hydroxydeoxycytidine, hydantoin will genrate. Similarly, interaction of ˙OH with purines

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generates 8-hydroxy deoxyguanosine (8-OHdG), 8- hydroxyldeoxyadenosine, formamidopyrimidines etc. [6].

Proteins

Oxidation of proteins by ROS (mainly •OH), involves oxidation of amino acid chains; generate stable and reactive products such as protein hydro-peroxides, which result in fragmentation of proteins. The side chains of all the amino acid residues, particularly cysteine and methionine, are susceptible to oxidation by the action of ROS. Peroxynitrite ($ONOO^-$) is involved in the oxidation of –SH group on proteins. Functionally inactive oxidized proteins are rapidly removed but some can gradually accumulate and result in the alteration of signal transduction process and transport system, ageing, atherosclerosis and many other diseases [6].

Lipid and lipid peroxidation

Cell membrane contains phospholipids which have poly unsaturated fatty acids (PUFA) residues, are highly susceptible to free radical damage. When lipids are attacked by free radicals, it can undergo the highly damaging chain reaction of lipid peroxidation (LP), which is predominantly a destructive and self-prepetuating process, leading to both direct and indirect effects. Lipid peroxidation is broadly defined as "oxidative deterioration of PUFA" [8]. During lipid peroxidation a large number of toxic and mutagenic byproducts; lipid epoxides, lipid hydroperoxides, lipid alkoxyl, peroxyl radicals and unsaturated fatty acids, are formed that act as 'second messengers'. In the process of lipid peroxidation malondialdehyde (MDA) is produced which is a good marker for free radical-mediated damage and oxidative stress. Malonaldehyde can react with the free amino group of proteins, phospholipid, and nucleic acids leading to structural modification, which induce dysfunction of immune systems.

Functions of antioxidants

Mostly free radicals are derived from oxygen and nitrogen and called as reactive oxygen species (ROS) and reactive nitrogen species (RNS). These elements are essential for biological metabolisms but sometimes they are converted into free radicals which are highly unstable and reactive, can start chain reaction and damage biologically relevant molecules such as proteins, lipid or carbohydrates. Antioxidants prevent the oxidation of other compounds. Both enzymatic and non enzymatic antioxidants such as catalase, superoxide dismutase ascorbate peroxidase, ascorbic acid, caratenoids, etc. protect cells from undesired oxidations. Sarvajeet and Narendra [11] reported that the ROS affect many cellular functions by damaging nucleic acid, oxidizing

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proteins and causing lipid peroxidation. Oxidative stress occurs when balance between antioxidants and ROS will disrupt [8]. All antioxidants work together to prevent the damaging effects of free radicals and toxic products of metabolism. However, the antioxidants act to control free radical formation as a coordinated system. Antioxidants can act as: scavenge reactive oxygen species, inhibit the process of oxidation, terminating the chain reactions, repairing damage, convert the radicals to less reactive species, stabilizing or deactivate the free radicals before they attack cells, hydrogen donation by the antioxidant, electron donation by the antioxidant, addition of the lipid to antioxidant, formation of a complex between the lipid and antioxidant, function as peroxide decomposers and enzyme inhibitors [12], [8], [13].

Classification of Antioxidants

In general antioxidants are grouped into below mentioned categories based on their synthesis:

(1) Plant-derived antioxidants and (2) Secondary or synthetic antioxidants.

1. Plant-derived antioxidants

Plants can produce a huge variety of antioxidative molecules. These are mainly phenolics in structures, react with radicals and convert them into more stable products [4]. These are mainly: **Minerals**- co factor of antioxidants enzymes i.e. selenium, copper, iron, zinc and manganese, etc., **Vitamins**- required for metabolic functions i.e. vitamin C, vitamin E, vitamin B and **Phytochemicals**- These are secondary metabolites i.e. Catechins, Carotenoids, Beta carotene, Lycopene, Zeaxantin, Diterpene, curcumin, anthocyanins [14]. According to Members of the Food and Nutrition Board of the National Research Council (USA), dietary antioxidants are substance in foods which decreases the adverse effect of ROS and RNS [15]. Foods not only supply calories but also protect body from the inception of disease. Dietary antioxidants are exogenous. Some dietary antioxidants are presented in Table1.

Table 1: List of food and diet having antioxidants

S. No.	Functional types	Antioxidants	Sources
1.	Vitamins	A, B, C, E, K	Fruits, vegetables, citrus fruits, dairy products, fish, meat,
2.	Alkaloids	Capsaicinoids, berberine, caffeine	Chilli, beverages, herbs
3.	Polyamines	Putrescine, spermidine, spermine	Pea, apple, citrus fruits, spices, liver, meat

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4.	Phytosterols	Campesterol, stigmasterol	Vegetables, seeds
5.	Ferulic acid ester	Oryzanol	Rice bran oil
6.	Isoprenoids derivatives	Carotenoids	carrot, tomato, palm oil
7.	Benzopyran Derivatives (polyphenols/ flavonoids)	Proanthocyanins Anthocyanins, catechins, flavonols, lignans, flavones, isoflavones, isoflavonoids, gallic acid	Grape, apple, pomegranate, banana, pineapple, brinjal, beverages, oil seeds, cucumber, olive oil, pomegranate, green tea
8.	Matals	Zinc, selenium, copper, manganese	Vegetables, meat, egg, dairy products

[Source: 15]

2. Secondary or synthetic antioxidants

Synthetic antioxidants are phenolic compound; they capture free radicals and stop the chain reactions. These compounds are used for the preservation of cosmetic and food products, in pharmaceutical. These are: Butylated hydroxyl anisole (BHA), Butylated hydroxyrotoluene (BHT), Propyl gallate (PG), Tertiary butyl hydroquinone (TBHQ) and Nordihydro guaretic acid (NDGA) [4], [16]. Further, based on the nature of antioxidants, they are classified into two components enzymatic and non-enzymatic systems. The detailed classification of antioxidants is presented in following flow chart (Fig. 1) and explained in subsequent section:

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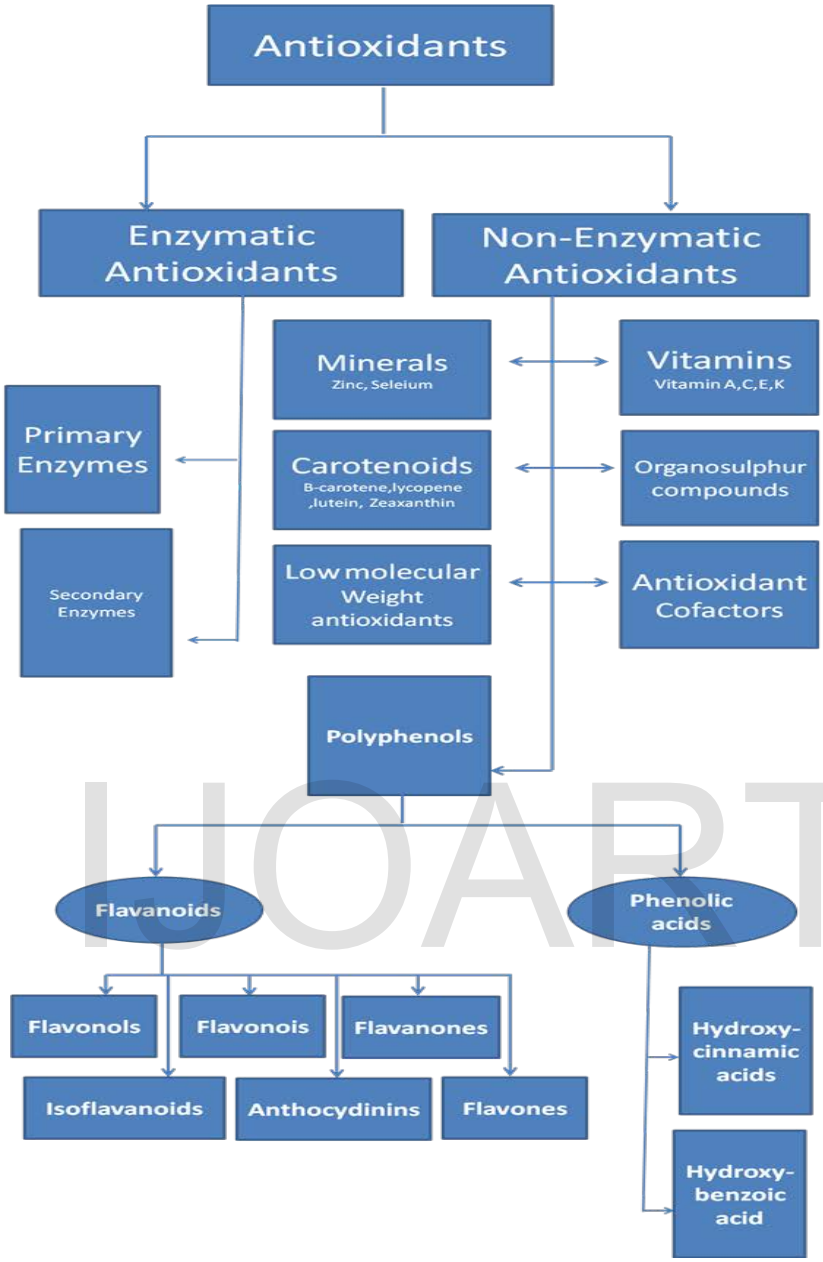

Fig 1 Flow chart showing classification of enzymatic and non-enzymatic antioxidants

Enzymatic antioxidants

Catalase (CAT) is a heme-containing protein based enzyme that catalyses the dismutation of H_2O_2 into H_2O and O_2 [17]. It has high capacity but low affinity enzyme which destroys hydrogen peroxide. Catalase promotes the redox reaction. It is a large tetrameric protein that exists as multiple isoezymes encoded by nuclear genes. They are located mostly in peroxisomes and glyoxysomes, although a specific isozyme Cat3 is present in maize mitochondria. This ubiquitous enzyme has been isolated from different natural sources including animal tissues,

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plants and micro-organisms [8]. CAT reduces H_2O_2 levels in peroxisomes, Ascorbate peroxidase (APX) perform equivalent function in chloroplast and cytosol of plant cell. APX uses ascorbate as a hydrogen donor to break down H_2O_2 to form H_2O and monodehydroascorbate [18]. Superoxide dismutase (SOD) is an intracellular enzyme present in every cell. SOD family is composed of metalloprotein that catalyze the dismutation of O^-_2 superoxide radical to O_2 and H_2O_2. Within the cell, SOD constitutes the first line of defense against ROS. Based on the metal co-factor SOD are classified into three groups: iron SOD (Fe SOD), located in the chloroplast; manganese SOD (Mn SOD), located in the mitochondrion and peroxisome; and copper-zinc SOD (Cu-Zn SOD) located in the chloroplast [19]. The MnSODs and FeSODs are structurally related, whereas Cu ZnSODs show no structural relationship to the other and are thought to have evolved independently. SOD reduced superoxide into H_2O_2. When H_2O_2 concentration increases, it inactivates Cu Zn SOD. Catalase and Glutathione peroxidase reduce H_2O_2 to conserve SOD. Glutathione peroxidase is a group of enzymes containing selenium, which catalyze the degradation of hydrogen peroxide and lipid hydroperoxide, generated during lipid peroxidation, to water using reduced glutathione as substrate [20]. It is cytoplasmic and mitochondrial enzyme which helps in detoxification of peroxides. Dehydroascorbale reductase (DHAR) play an important role in the oxidative stress tolerance of plants. DHAR catalyzed the regeneration of ascorbate from dehyroascorbate [21]. Glutathione reductase (GR) is a low molecular weight thiol compound present in almost all cells. It acts as a disulphide reactant and regenerate ascorbate. It reacts with $^1O_2$ and $OH^{\cdot}$ and protect the thiols group of enzymes. The role of GR in H_2O_2 scavenging has been well estabilished in the halliwell – asada pathway [18]**.**

Non -enzymatic antioxidants

Ascorbic acid (Vitamin C) is a water soluble, electron donor, chain breaking antioxidant, and present in high concentration in many cellular environments, such as the stroma of chloroplasts. It is a monosaccharide, found in both animals and plants [4]. It is capable of neutralizing ROS as well as RNS in the aqueous phase before lipid peroxidation is initiated. It is a reducing agent. It can directly scavenge superoxide, hydroxyl radicals and singlet oxygen and reduce H_2O_2 to water via ascorbate peroxidase reaction [21]. In cells, it is maintained in its reduced form (ascorbate) by reaction with glutathione, which can be catalyzed by protein disulfide isomerase and glutaredoxins [4]. Citrus fruits, potatos, tomatos, green leafy vegetables are the majour source of Vit C. Tocopherols (Vitamin E) is collective a set of 8 related tocopherols (α, β,

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γ and δ) and tocotrienols (α, β, γ and δ) [4]. Relative antioxidant activity of the tocopherol isomers *in vivo* is α >β > γ > δ [22]. Hence, α tocopherol has highest antioxidant activity. TOCs are lipid soluble and chain breaking antioxidant, present in all cellular membranes protecting against lipid peroxidation and prevent chain initiation and propagation of free radical reaction. It is synthesized only by plants and algae and essential component of biological membranes. In cell membrane it protects membrane fatty acids from lipid peroxidation. It reacts with $RO^{\cdot}$, $ROO^{\cdot}$ and $RO^{\cdot}$ derived from PUFA oxidation [17]. Tocopherols act as chemical scavengers of oxygen radicals, especially singlet oxygen, and also physical deactivators of singlet oxygen by chain transfer mechanism [22]. Nuts, seeds, grains and oils are rich sources of vitamin E.

Glutathione (GSH) is an abundant tripeptide (γ-glutamylcysteinglycine) in plant tissue. It is an important water-soluble antioxidant and synthesized from the amino acids, glycine, glutamate and cysteine. It is the main storage form of sulpher. ROS molecules oxidize glutathione, but the reduced form is regenerated in a redox by an NADPH-dependent reductase. In cells, it is maintained in the reduced form by the enzyme glutathione reductase and reduces other enzymes, such as ascorbate in the glutathione-ascorbate cycle, glutathione peroxidases and glutaredoxins [4]. High reproductive potential of GSH is due to central nucleophilic cysteine residue. It scavenges cytotoxic H_2O_2, and reacts non- enzymatically with other ROS: singlet oxygen, superoxide radical and hydroxyl radical [22]. It may help to detoxify many inhaled oxidizing air pollutant like ozone, NO_2 and free radicals in cigarette, smoke etc. In some organisms glutathione is replaced by other thiols, such as mycothiol in the Actinomycetes, or by trypanothione in the Kinetoplastids [4]. Among all carotenoids known, β carotene is the most widely studied molecule. β carotene is inactive form of vitamin A (retinol). These are lipolipophilic organic compounds, present in the chloroplasts and chromoplasts of plants. CARs play many functions in plant metabolism including the role in oxidative stress tolerance [21]. β -carotene prevents lipid peroxidation through quenching the singlet oxygen species and scavenging the free radicals. It is an excellent scavenger of singlet oxygen.

Antioxidants production potential in plant system

Plants contain a wide variety of free radical scavenging molecules, such as polyphenols, dietary glutathione, vitamins and endogenous metabolites. These natural products observed as antioxidants. A vast number of plants have been studied for their potential sources of antioxidants. Plant-derived antioxidants act as singlet and triplet oxygen quenchers, peroxide

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decomposers, enzyme inhibitors and synergists. Plants serve as one of the cheapest available source of antioxidants. Among different parts of the plants, leaves receive special consideration, e.g. *Etlingera genus* [23], *Olea europaea* [24], *Ligustrum vulgare* [25], *Acacia confuse* [26], *Populus tremuloides* Michx [27], root of *Medicago sativa* [28] and *Carissa spinarum* [29] were also reported to contain antioxidants. Besides dietary sources, antioxidants can be obtained from food processing industries and agricultural-by-products. The respective by-products are seeds, peels, bark, mill wastes and trimming wastes. The industrial-by-products, like peel and seed, are demonstrated to have high antioxidant level which is even higher than the flesh and other parts of the fruit with the presence of high polyphenol content, some are; mangosteen peel [30] and grape seed and skin [31]. Since, plant have many antioxidants and screening of plant antioxidants is done through various *in vitro* models like DPPH methods [32], Nitric oxide method [33], DMPD method [34], ABTS method [35], ORAC method [36], TBARS assay [37] and via various *in vivo* models [38] using rats and mice [20]. Some plants and fruits are listed in Table 2 and 3which possess antioxidant property with their chemical constituents and biological activity.

Table 2: list of plants showing antioxidant property

Plant species	**Family**	**Biological behaviour**	**Chemical/ active constituents**
Acacia catechu (L.f.) Willd.	Mimosaceae	Diarrhoea, cough, skin eruption, asthma, check nasal bleeding dysentry, bronchitis	Catechin and quercetrine [39]
Bauhinia forticate	Caesalpiniaceae	Hypoglycemic,blood purifier and diuretic	Kempferol-3,7-O-dirhamnoside
Dolichos biflorus Linn.	Fabaceae	Hypolipidemic effect	Flavonoids
Prunues domestics	Rosaceae	The juice from the bark applied on body swellings and contusions and oils from kernels applied on neuralgic pains	Prunetin, genistein and quercetin
Foeniculum vulgare Mill.	Apiaceae	Stimulant, aromatic, carminative, purgative, diuretic, in venereal diseases, vermicide, useful in chest, spleen and kidneys troubles, estrogenic. Fennel oil antioxidant	Volatile oil, fenchone, anethole, limonene, anisaldehyde, estragole.
Ocimum sanctum Linn.	Lamiaceae	Expectorant, in catarrh, bronchitis, ringworm and other	Volatile oil, terpenoids, eugenol,

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		cutaneous diseases, stomachic, gastric disorders of children, in earache	thymol, estragole
Glycyrrhiza glabra Linn.	Fabaceace	Diuretic, emmenagogue, in vomiting, asthma, bronchitis, in acute conjunctivitis, for curing wounds, in peptic ulcer	Glycyrrhizin, flavonoids, liquiritin, isoliquiritin, rhamnoliquiritin, 2-methylisoflavones
Solanum nigrum Linn.	Solanaceae	Diuretic, laxative, dropsical affections, virulent gonorrhoea, malaria, dysentery, hepatoprotective	Polyphenolic compounds, flavonoids, steroids.
Withania somnifera Dunal	Solanaceae	Analgesic, increase immunity, hepatoprotective	Steroidal lactone, withanolides, glycene, withanine
Punica granatum L.	Punicaceae	Fever, cough, pimples, boils, throat disorder, dysentery, diuretic, cardiac problems, vomiting and bronchitis	Punicalin [40], [41], [42]
Mangifera indica Linn.	Anacardiaceae	Leuchorrhea, dysentery, bronchitis, biliousness, urinary discharges, in haemorrhage from the uterus, lungs or intestine	Cyanogenetic glycosides, polyphenlos, vitamin A and C, mangiferin, β-sitosterol, quercetin, ellagic acid, gallic acid

Table 3: List of some fruits showing antioxidant activity

S. no.	Fruits	Active constituents	References
1.	Berries	Flavanols hydroxycinammic acids, hydroxy-benzoic acids, anthocyanins	[43]
2.	Strawberries	Vitamin c, bioflavonoids	[44]
3.	Cherries	Hydroxycinnamicacids, anthocyanins	[45]
4.	Blackgrapes	Anthocyanins, flavonols	[46]
5.	Grape	Alanine, ascorbic acid, a tocopherol, b carotene, histidine, methionine, palmitic acid, b sitosterol, selenium	[13]
6.	Citrus fruits	Flavanones, flavonols, phenolic acids	[47]
7.	Olives	Polyphenols	[13]
8.	Plums, prunes, apples, pears, kiwi	Hydroxycinnamic acids, catechins	[45]

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In vitro production of antioxidants

In vitro cultured plant cells synthesize, accumulate and exudate many secondary metabolites which have significant potential as antioxidants. Antioxidants are compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate. The antioxidant compounds can be recycled in the cell or are irreversibly damaged, but their oxidation products are less harmful or further converted to harmless substances [48]. The antioxidant activity of plant secondary metabolites has been widely demonstrated in *in vitro* systems. *In vitro* technology offers several benefits, i.e. simpler extraction and purification from interfering matrices, independence from climatic factors and seasons, shorter and more flexible production cycles, easier fulfillment of the high-profile pharmaceutical production demands etc.

Polyphenols

Polyphenols are easily oxidizable phenolics, highly desirable as dietary and therapeutic free radical scavengers. The excessive production of polyphenols in tissue culture results from unfavorable or suboptimal culture conditions. Polyphenols in plants derive mainly from the shikimic acid pathway through aromatic carboxylic acids—cinnamic or benzoic. Some antioxidant polyphenols are: phenolic acids (caffeic acid derivatives), lignans, hydrolysable tannins (gallotannins and ellagitannins), stilbenes and xanthones. Phenolic acids are present in free form as glycosides in plants. They are common as despised-the intermolecular ester of two or more units composed of the same or different phenolic acids such as: caffeic, coumaric, ferulic, gallic and syringic. Rosmarinic acid (RA) found in many Lamiaceae and Boraginaceae species, a depside composed of two caffeic acid molecules. It can accrue in cell cultures in larger amount than in intact plants. The suspension cultures producing rosmarinic acid were generated from *Eritrichum sericeum* [49], *Lithospermum erythrorrhizon* [50], Boraginaceae and *Lavandula vera* [51], [52], [53], *Oci-mumbasilicum* [54]. Extracts from lavender cells accumulating rosmarinic acid also have superior radical scavenging properties [55]. Lithospermic acid B is a tetrameric depside, accumulating especially in the hairy root cultures of *H. officinalis* and elicited *S. miltiorrhiza* as well as in *Lithospermum erythrorhizon* suspension cultures [56]. Flavonoids are tricyclic phenyl benzo pyran structure but biosynthetically come from phenylalanine and malonyl-CoA in the phenylpropanoid pathway Subclasses offlavonoid includeflavonols, flavones, isoflavonoids, flavanons, flavanols, proanthocyanidins (catechins),

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and anthocyanidins. Its antioxidant properties depend on the hydroxylation pattern. Oligomeric proanthocyanidins (condensed tannins) are considered to be the most powerful antioxidants of all the flavonoids owing to their structure (procyanidin B1). They are responsible for the antioxidant activity of wines, teas and fruits. The induction and isolation of oligomeric proanthocyanidins have been reported in the cell cultures of *Vaccinium pahalae* [57] and *Vitis vinifera* [58]. Another flavonoid polyphenols is anthocyanins. Pigments are useful as products because their accumulation can easily be visually evaluated. Anthocyanins are obtained from *in vitro* cultures of *Fragaria ananassa* [59], *V. vinifera* [58], *Rudbeckia hirta* [60] and others. Biological activity of anthocyanins varies, due to their structural diversity. Stilbenes are bicyclic polyphenols characterized by resveratrol. It is a phytoalexin and the red wine antioxidant. Although it present in appreciable amount of natural sources like grapes and some Polygonaceae species, it has been also induced in elicited hairy root cultures of peanut (*Arachis hypogea*) and recovered expeditiously from the liquid medium [61].

Isoprenoids

These are produced from isopentynyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) in plant cells. They are lipophilic and play important role in protecting membrane lipids. IPP and DMAPP are subsequently fused to yield enormously variable compounds in numerous groups of terpenoids (mono, sesqui, di, and triterpenes and their derivatives), carotenoids, and steroids [62]. Carotenoids are most powerful antioxidants. Both β-carotene and lycopene are recognized as dietary chemopreventive agents, protecting against cancerogenesis. Crocin derived from saffron (stigmata of *Crocus sativus*) has been confirmed as a powerful antioxidant [63]. Abietane diterpenes are present in many Lamiaceae medicinal plants and in some gymnosperms. It can be obtained from *in vitro* cultures of *R. Officinalis* [64] and *S. officinalis* [65]. Light plays a crucial role in stimulating the diterpene biosynthesis *in vitro* [64] even though in natural conditions they can only be found in the underground parts of plants. Volatile terpenoids responsible for aromatic properties of plant organs serve as a multipurpose chemical defense weapon against fungal and bacterial pathogens along with long distance chemical communication e.g. attracting pollinators. Tissue culture technique is able to produce volatile constituents of essential oils [66].

Other antioxidants

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Tocopherols broadly used in human nutrition as vitamin E and in food conservation. It is powerful free radical scavengers and protects plant cells against oxidative damage in a lipophilic environment. The extraction from plant oils usually yields a mixture of β, γ, δ and α-tocopherols and tocotrienols; therefore plant tissue culture is an alternative source to obtain pure α-tocopherol. Several culture systems of *Helianthus annuus* (sunflower) have been established for this purpose.

Betalains is nitrogen containing compounds, widely used as non-toxic food colorants. The major benefits of betalains as dietary antioxidants are their bioavailability, which is greater than most flavonoids, and their superior stability in comparison to anthocyanins [67]. Red beetroot (*Beta vulgaris*) is a rich natural source of betacyanins. Beetroot pigments have been produced by cell suspensions and transformed roots in bioreactors [52]. Kinobeon A is a novel, red-colored compound isolated from Carthamus tinctorius cell cultures, distinctively combines the quinoid aromatic character with several conjugated double bonds. It efficiently quenches various reactive oxygen species, inhibits lipid peroxidation and supports the survival of mammalian cells during oxidative stress [68].

Genetic engineering of crop plants for antioxidants amelioration

The antioxidant activity and nutritional value of fruits vary with the species, cultivar, amount as well as various tissues of the fruit (e.g. peel, flesh and other tissues) and form of consumption (fresh, juice or processed) (69].

To increase antioxidant level of biofortify food attempts have been made by using traditional breeding strategies [70]. These methods comprise crosses and back crosses for several generations which require high genetic variations of the trait and heritability, and it is a highly time consuming process [71]. Conventional breeding approach also has several limitations. In cultivated tomato, the expression of chalcone isomerase (*CHI*) gene is responsible for the production of flavonoids. *CHI* gene expression is blocked in the peel and inhibited in the pulp affecting the production and accumulation of flavonoid–quercetin. Wild *Lycopersicon* accessions express the *CHI* gene. Wild *Lycopersicon* was crossed with a cultivated tomato species to develop a non-transgenic, metabolic strategy to produce flavonoid-rich hybrids [72]. Hybrid tomato showed 11.8-fold increase in flavonoid– quercetin in F1 hybrids. However, further analysis of progeny was not possible because the hybrids were consistently seedless. Genetic engineering is a powerful tool to introduce favorable modifications in metabolic pathways of

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plants to ameliorate the quantity and bioavailability of phytonutrients with particular attention to antioxidants [7]. Various vegetable and fruit crops have been transformed to improve the concentration of a useful nutrient metabolite [73] and are explained below:

Tomato is a major dietary source of lycopene and comprises other antioxidants, vitamin C, flavonoids and tocopherols. Lycopene accumulation in tomato fruit is associated to a differential regulation of phytoene synthase (PSY), phytoene desaturase (PDS), lycopene beta (LCY-b) and lycopene epsilon (LCY-e) cyclases [74]. During ripening expression of the genes encoding PSY and PDS proteins, and lycopene production increase whereas LCY-b and LCY-e, which convert lycopene to either β-or δ-carotene, declines hastily [73]. Various research groups [75] have successfully used LCY β-cyclase gene under fruit-specific promoters to increase the β-carotene content of tomato fruit though its silencing via antisense expression doubled the increase in lycopene [76]. PDS-crtI expression also increases level of b-carotene [77]. Bacterial crtB gene encodes PSY gene when engineered with the fruit poly-galacturonase promoter led to 5–10-fold increase in PSY enzyme activity and a significant increase in phytoene, carotene and lycopene [78]. A regulatable promoter with heterologous genes dominates endogenous control of carotenoid pathway genes, enabling higher accumulation of carotenoids during fruit ripening [7]. Expression of yeast SAM decarboxylase (ySAMdc) fused to the ripening-inducible promoter E8 in homozygous lines of tomato led to higher polyamines accumulation, which caused enrichment of fruit juice quality and 2–3-fold higher lycopene accumulation compared with the controls [79]. Fruit-specific Pds promoter with lycopene b-cyclase (b-Lcy) and b-carotene hydroxylase (b-Chy) genes increase 12-fold in b-carotene over the control [80]. RNAi-mediated suppression of an endogenous photo-morphogenesis regulatory gene, *DET1* increases carotenoids and flavonoids without affecting other fruit quality parameters [81].

Tomatoes also contain small amounts of flavonoids, mostly located in the peel of the fruit [82]. Total phenolics (flavonoids and anthocyanin) in tomato have been increased successfully by using transgenic technology. Constituent expression of petunia chalcone isomerase chi-a gene in tomato fruit resulted in a 78-fold increase in flavonols, mainly rutin [82]. Similarly, crypto chrome *cry2* gene caused several-fold increase in flavonoids and non-flavonoids [83]. Cryptochrome *cry2* gene is involved in the regulation of flowering time and photomorphogenesis under low fluence light in *Arabidopsis*. Isoflavone synthase GmIFS2gene of soybean catalyses the formation of genistin from substrate naringenin in isoflavone biosynthesis pathway, its

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constitutive expression in tomato led to an increase of genistin and quercetin glycoside in fruit peel [84]. The transgenic plants displayed altered antioxidant profiles. From different plant sources, genes encoding flavonoid biosynthesis pathway enzymes were used to produce transgenic tomato fruits, resulting in the accumulation of stilbenes (resveratrol and piceid), deoxychalcone (butein and isoliquirtigenin), flavones and flavonols [85]. Constitutive expression of chalcone isomerase and flavone synthase (*CHI/FNS*) gene under the control of CaMV double 35S promoter cause 3-fold higher antioxidant capacity in tomato fruit peel. Synthetic GTP cyclohydrolase I (*gchI*) gene under the control of a fruit-specific promoter, was overexpressed in tomato, resulting in 2-fold and 3–140-fold increases, respectively, in folate and pteridine contents while p-aminobenzoate (PABA) content was reduced [86]. Expression of *arabidopsis* aminodeoxychorismate synthase (ADCS) gene resulted in 19-fold more PABA accumulation than in the controls. A genetic cross between the pteridine and PABA over-expressing transgenic lines led to 25-fold more folate in the progeny [86].

The *Erwinia* PSY *crtB* gene fused to a tuber-specific promoter was introduced in potato and increase in the contents of violaxanthin, lutein and β-carotene was attained [87]. Antisense silencing of potato β-carotene hydroxylase *CHY1* and *CHY2* genes (metabolize β-carotene) led to 4–7-fold increase in lutein but b-carotene level was enriched by 38-fold [88]. *Or* gene (*orange* gene) mutation is responsible for β-carotene accumulation in cauliflower, when introduced in potato led to a 6-fold higher level of total carotenoids in transgenic against the controls [89], [90]. Potato plants were transformed with a modified MYB transcription factor to increase the content of health promoting polyphenolic compounds such as kaempferol and chlorogenic acid. Constituent expression in potato of petunia dihydroflavonol reductase (DFR) gene (key enzyme in flavonoid biosynthesis) significantly enhanced anthocyanins and phenolics [91]. The activation of the flavonoid biosynthetic pathway resulted in 100-fold increase in kaempferol content and 4-fold increase in phenolic acids [92]. Tocopherols are potent antioxidants with anticarcinogenic properties and also have a potential to be used as chemotherapeutics [93]. Overexpression of *crtB* gene in potato tuber led to significant increase in the α-tocopherol content [87].

Grape stilbene synthase gene, *Vst1*, was expressed in apples for stilbene biosynthesis. This led to a several-fold accumulation of piceid (*trans* resveratrol glucoside) [94]. The increase in piceid consistently led to higher amounts of hydroxycinnamic acid and flavonols in the transgenic

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apple. Stilbene synthase gene from three different *Vitis* species when expressed constitutively in kiwi fruit led to resveratrol glucoside (piceid) [95].

The NADPH-dependent D-galacturonate reductase, encoded by *GalUR*, converts D-galacturonic acid to ascorbic acid. Ascorbic acid content is correlated with *GalUR* expression pattern in strawberry fruits [96] and overexpression of GalUR resulted in 2–3-fold higher levels of ascorbic acid. Banana is a good source of vitamins B6 and C. There are wide diversity and genetic variability of banana in fruit provitamin A carotenoids, lutein and other micronutrients [97]. Genetic engineering has the potential to further enhance nutritional quality and revenue, and make banana a more appreciated source of antioxidants [98]. Overexpression of *Arabidopsis* γ-tocopherol methyl transferase gene (under the control of constitutive promoter) in lettuce increase the levels of α-tocopherol compared with the wild-type control [99].

Synthetic codon-optimized GTP cyclohydrolase I (*gchI*) based on indigenous *Gallus gallus* gene when introduced into lettuce improved folate levels. The transgenic lines had folate content that ranged from 2.1- to 8.5-fold higher than the non-transgenic lines. Carrot roots have high levels of α-carotene and β-carotene, but are scarce in ketocarotenoids. Introduction of a β-carotene ketolase gene from alga *Haematococcus pluvialis* into carrot root lead to in ketocarotenoid accumulation [100]. Transformation of bacterial PSY (*crtB*) gene using seed-specific napin promoter in canola seed resulted in orange coloured seed with a 50-fold higher total carotenoid level than the wild-type [101]. Transgenic canola seeds that co-expressed *crtI* and *crtB* gene were found to accumulate lycopene and β-carotene [102]. Flax seed is rich in α-linolenic acid and lignans. Lignans are sources of phytoestrogens and act as antioxidants due to the presence of α-linolenic acid, a ω-3 fatty acid, which is essential for human health [103]. Overexpression of bacterial *crtB* gene in flaxseeds caused an increase of 7.8– 18.6-fold in the carotenoid levels of the seeds [104]. Sweet potato is a rich source of vitamin A, ascorbic acid and carbohydrates. A tobacco microsomal fatty acid desaturase gene (*NtFAD3*) was overexpressed using a modified CaMV35S promoter in sweet potato to increase the levels of unsaturated fatty acids. Transgenic sweet potatoes thus obtained had increased amounts of linolenic acid [105].

Overexpression of the *Arabidopsis* genes involved in the biosynthetic pathway of α-tocopherol. *VTE4* (γ-TMT–γ tocopherol methyl transferase) gene in combination with the *VTE3* (2-methyl-6-phytylbenzoquinol methyltransferase) gene, increased by 8-fold the α-tocopherol content in

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soybean seeds [106]. Similarly, *Perilla frutescens* γ-TMT produced higher levels of α-tocopherol and β-tocopherol [107].

Table 4 List of genetically engineered crops with enhanced target phytonutrients (antioxidants)

S.No	GM fruit	Target nutrient	Gene introduced	Reference
1.	Tomato	β-carotene	β *Lcy/Arabidopsis*+ β *Chy*/Peppe	[80]
			DET1/Tomato (RNAi)	[81]
			β -*Lcy*/*Arabidopsis*	[7]
		Lutein	*CRY2*/Tomato	[73]
		Anthocyanin	*Del*/Snapdragon+*Ros1*/Snapdragon	[108]
		β -cryptoxanthin, zeaxanthin	B-*Lcy*/Arabidopsis+ β *Chy*/Pepper	[80]
		Lycopene	*ySAMDC, Spe2/Yeast*	[79]
		Phytoene	*CrtB/E. uredovore*	[78]
		isoflavone/genistin	*GmIFS2*/Soybean	[84]
		Stilbenes	*STS/Vitis vinifera*	[85]
		Flavonoid/quercetin kaempferol	*CHI*/Petunia	[82]
		p-coumeric acid	*CRY2*/Tomato	[83]
		Flavonoid	*DET1*/Tomato (RNAi)	[81]
		Flavonol	*MYB12/Arabidopsis*	[109]
		Campestero β- sitosterol Cycloartenol	*hmgr1/Arabidopsis*	[110]
		trans resveratrol, Caffeic acid	*StSy/V. vinifera*	[111], [112]
2.	Potato	Carotenoid	*Or*/cauliflower	[89], [90]
		Carotenoids	*CrtI*, *CrtB* and *CrtY/E. uredovora*	[88]
		β-carotene: carotenoids	*LCY-e*/potato (antisense)	[113]
		α-tocopherol	*crtB-TP/E. uredovora*	[87], [114]
		Amino acids/total protein	*AmA1/Amaranthus Hypochondriacus*	[115]
3.	Brassica	β-carotene, Lutein	ε-*CYC/Brassica napus* (RNAi)	[116]

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		Carotenoid	*crtB*/bacteria	[101]
		β-carotene	*crtI +crtb*/bacteria	[102]
			DET1 (RNAi suppression)	[117]
4.	Canola	Carotenoid	*idi + crtW+ crtZ* (synthetic)/marine bacteria + *crtE + crtB + crtI + crtY/Pantoea ananatis*	[118]
5.	Oilseed	α-tocopherol, γ tocopherol	γ-TMT/Arabidopsis	[99]
6.	Flax	Carotenoid	*crtB/P. ananatis*	[102]
7.	Carrot	Ketocarotenoid	*CrtO/Haematococcus pluvialis*	[100]
8.	Lettuce	Folate	*Gch1/avian*	[119]
		Iron	*pfe/Soybean*	[120]
		Tocopherol	*γ-TMT/Arabidopsis*	[121]
		Resveratrol	*STS/Parthenocissus henryana*	[122]
9.	Soybean	α-tocopherol	*AtVTE3+AtVTE4/Arabidopsis*	[106]
		β-tocopherol	*γTMT/Perilla frutescens*	[107]
10.	Sweet potato	Linolenic acid	*NtFAD3*/Tobacco	[105]
11.	Apple	trans-piceid	*Vst1/V. vinifera*	[114]
12.	Kiwi	Piceid	*Stibene synthase/Vitis* spp	[94]
13.	Papaya	Piceid	*Vst1/V. vinifera*	[94]
14.	Strawberry	*p*-coumaryl alcohol *p*-coumaryl-1-acetate	*CHS/strawberry* (antisense)	[95]

Conclusion

Cellular damage arises due to imbalance between reactive oxygen species and antioxidant defense system. The main cellular components (DNA, protein and lipid) are susceptible to free radicals damage and cause irreversible damage that can lead to cell death. It may lead to various diseases i.e. Cancer, heart disease, ageing, neurodegenerative disease, diabetes. Antioxidant molecules defend the body from harmful effect of free radicals. Plants having variable phytochemical compounds including vitamins (B, C, E and beta-carotene), folates, lycopene, flavonoids, isothyocyanates, glucosinolates, polyphenols, glutathione and minerals contribute to the antioxidative capacity of vegetables, fruits, nuts and various herbs. However, the levels of

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antioxidants present in crop plants are low and significantly influenced by genotype/cultivar, growth condition and developmental stage. Genetic engineering has come to the rescue as a refined tool to increase the antioxidant and nutrient capacity of fruits to the levels favorable for not only for a highly nutritional diet but also to enable in-depth studies on the relationships between diet, genetics and metabolism. Together with modern biotechnology, deciphering transcriptome-proteome-metabolome of the new transgenics should provide new knowledge to ease the concerns of the society and open the market for genetically engineered crops. This knowledge will also help to develop precise strategies for redesigning metabolic pathways so that desired levels of a particular phytonutrient (antioxidant) in a fruit or other crops can be achieved.

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