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PLANT ANTIOXIDANTS AND THEIR NON-TRADITIONAL SOURCES (review)

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Abstract

The viability of eukaryotes largely depends on a biochemical defense system that protects the body from damage. Antioxidants that neutralize free radicals are significant components of biochemical protective system (M.G. Uzbekov, 2014). Oxidative stress underlies many diseases, e.g., oncological, rheumatoid, bronchopulmonary, cardiovascular, and premature aging (S. Miwa et al., 2016; J.G. Geisler, 2019). There are more than 5,000 antioxidants which differ in chemical composition, antiradical and antiproliferative activity. Many studies show the synergism or additive effect of antioxidants (V. Polonsky et al., 2018). That is, to effectively protect the body, the range of antioxidants consumed must be quite broad. In this regard, it becomes urgent to search for new sources of biologically active substances and increase their content in already cultivated species. This work provides a classification of antioxidants. Among exogenous ones, carotenoids, polyphenols (flavonoids), and trace elements are considered in more detail. The various antioxidant activities of these substances are considered. Flavonoids are the most significant antioxidants. The antiradical activity of flavonoids can be 50 times higher than that of many plant substances, vitamins E and C are notably inferior to them (Y. Yao et al., 2010). Black grain rice varieties are rich sources of flavonoids (U.K.S. Kushwaha, 2016). Carotenoids are another effective antioxidants, the distinctive feature of which is interaction with other substances of this nature which increases the biological activity of the compounds (W. Stahl et al., 2004; C. Hu et al., 2020). Sources with high antioxidant potential and significant accumulation of carotenoids can be red grain varieties of rice, momordica, amaranth (Yu. Fotev et al., 2018; D. Shafigullin et al., 2018). The intraspecific diversity observed at the phenotypic level in terms of color characteristics is associated with both regulatory and structural genes (E.K. Khlestkina et al., 2014). The increased content of proanthocyanidins in the seed coat determines resistance to germination on the root, and the presence of anthocyanins contributes to better preservation of seeds after long-term storage and increased plant resistance to stress (T.L. Korotenko, 2018). Antioxidants increase plant resistance to biotic and abiotic stresses. However, this aspect has not been sufficiently studied in rice varieties with colored pericarp. The study of genetic mechanisms that control plant color traits is relevant in connection with the antioxidant and antimicrobial properties of pigments and their colorless precursors (Y. Qin et al., 2018). These compounds provide the prevention of cancer, reduce the risk of cardiovascular diseases, atherosclerosis, type 2 diabetes, increase immunity, improve the synthesis of visual pigments, activate metabolic processes, and slow down aging (C. Xu et al., 2017). Color variations and grain quality traits in rice samples is controlled by 41 loci. The *Ra* (*Prp-b* for varieties with purple pericarp) and *Rc* (brown pericarp and aleurone layer) genes mainly contribute to the phenotypic effect on rice grain color and nutritional quality (Y. Shao et al., 2011). These genes are located on chromosomes 9, 10 and 8 in the regions of the markers RM228 (amplification product size 90-154 bp), RM339 (166-148 bp), and RM316 (160-210 bp) location (T. Furukawa et al., 2007). Molecular characterization of key genes involved in the biosynthesis of the above compounds will allow breeders to control and accelerate selection for color traits, important for improving the nutritional value of functional products.

Keywords: rice, momordica, stained pericarp, flavonoids, carotenoids, antioxidants, anthocyanins, regulatory genes, structural genes, marker-assisted selection, SSR markers

An increase in O₂ concentration on the Earth's surface 2 million years ago stimulated plant and animal evolution to form a defense system capable of protecting from destruction by free radicals in the presence of oxygen in the atmosphere. The system of biochemical protection against free radicals includes substances that neutralize their effects. These substances constitute a system of antioxidants which includes low molecular weight compounds and complex groups of enzymes [1].

Free radicals are formed during redox reactions. In the body of a healthy person, the content of free radicals is quite constant. Disturbances in the functioning of the body or its defense systems provoke excessive formation of free radicals and lead to cell aging [2-4]. When there is an imbalance in the functioning of the antioxidant defense mechanisms, there is an excessive accumulation of free radicals, fat oxidation products, and other peroxidation products, which leads to oxidative stress [5-7].

Oxidative stress can be provoked by external factors. The formation of free radicals occurs when taking certain drugs or oxygen therapy, irradiation (ultraviolet, laser, radiation therapy), under the influence of environmental factors. In addition, susceptibility to oxidative stress may be genetic [8-10]. Many diseases and pathological processes, including rheumatism, diabetes, heart and vascular diseases, inflammatory diseases, and early aging, begin with the oxidative stress [11-13].

This review examines non-traditional plant sources of antioxidants.

Classification of antioxidants. All antioxidants (AO) are divided into substances of indirect and direct action. Based on their origin, AO are divided into two groups, the enzymatic antioxidants (EAO), e.g., glutathione peroxidase (GP), catalase, glutathione reductase, superoxide dismutase (SOD), and non-enzymatic antioxidants (NEAO) [14-16]. NEAO include substances of endogenous origin, for example, α -lipoic acid, glutathione, coenzyme Q₁₀, and exogenous origin. The latter include carotenoids, vitamins A, C, E, trace elements (selenium), polyphenols (flavonoids) and their synthetic analogues — low molecular weight compounds (ubiquinone, glutathione) [17-19].

EAO are highly specific, their concentration is relatively constant (except for pathological conditions), and they act strictly against activated oxygen metabolites which serve as substrates. Ions of zinc, silver, selenium, manganese, and iron increase the efficiency of reactions [20-22].

Some of the most powerful radical scavengers are phenolic antioxidants. Many of the several thousand known antioxidants, such as phenocarboxylic acids, are of plant origin and enter the body only with food [23-25]. Plants colored in red and brown tones, even black and purple, usually contain carotenoids and flavonoids. Carotenoids are effective antioxidants that scavenge singlet molecular oxygen and peroxy radicals. More than 850 natural carotenoids are known [26-28].

Antiradical activity. Antiradical activity characterizes the effectiveness of a particular antioxidant in neutralizing free radicals. Among natural antioxidants, flavonoids have the highest antiradical activity with a high rate of free radical neutralization [29-31]. AO flavonoids are also capable of inhibiting a number of enzymes that increase oxidative stress [32-34]. It has been shown that the maximum antiradical activity is characteristic of theoflavin, quercetin and cyanidin. The activity of rutin is weaker, and it is minimal in flavones and flavone glycosides [35-37]. When assessing the beneficial properties of plants, special attention is paid to the so-called P-activity, which is largely determined by the phenolic component of the substances they contain. This group includes rutins,

quercetins, isoquercetins, anthocyanins, leucoanthocyanins and catechins. Each plant species and even variety has its own unique composition of antioxidant pools [38-40].

The maximum protective effect is due to both the high antiradical activity of plant metabolites with antioxidant properties and the variety of natural antioxidant substances, even with less antiradical potential, since their targets are often different [41-43].

Sources and properties of flavonoids. Rich sources of flavonoids are plants with dark-colored organs. Their pharmacological value varies depending on the chemical composition of the accumulated substances. Color can be a criterion for the accumulation of flavonoids, for example, anthocyanins or carotenoids in a plant, but it provides little information about the chemical composition of beneficial substances [44-46]. Antioxidants not only protect the human body, but also contribute to the preservation of food, for example, they stabilize food fats, can replace food preservatives, and improve nutritional value [47, 48].

In addition to AO properties, phenolic substances have anti-inflammatory, antimicrobial, and antispasmodic effects [49-51]. It has been noted that mixtures of carotenoids are more effective than each compound alone [52, 53].

Anthocyanins, coloring plant generative organs and fruits, are involved in attracting pollinators and seed distributors. In vegetative organs, anthocyanins are involved in adaptation reactions to environmental conditions. Anthocyanins are able to interact with regulatory proteins and components of signaling pathways, thus modulating physiological processes [54, 55]. The main sources of anthocyanins are dark-colored fruits, including elderberries, chokeberries, pomegranates and blueberries, currants, and black-colored tomatoes [53]. Recently, dark-colored cereals, amaranth, soybeans, grains, and tubers have been considered as sources of anthocyanins [54]. They are even more attractive as sources of these compounds, since they are characterized by longer storage, availability and the possibility of everyday consumption, unlike seasonal berries and fruits. Studies of the consumer characteristics of products made from rice and wheat grains that contained anthocyanins have shown that they are not inferior, and in some parameters are superior to the reference products that do not contain anthocyanins [56-58].

Soy is another source of antioxidants, namely isoflavonoids, which are a subgroup of flavonoids. In soybean seeds, the content of isoflavonoids varies from 0.1 to 5 mg/g, depending on the type of isoflavonoids and plant growing conditions [59, 60]. Soybean products have a preventive effect against cancer, inhibiting the growth of cancerous tumors due to the high content of genistein, a natural inhibitor of tyrosine-specific protein kinase, as a result, soybean products are considered functional [61, 62]. The accumulation of isoflavones in soybean seeds in the technical ripeness phase is 0.69 mEq/g (as per quercetin). By the end of the biological ripeness phase, it increases in vegetable soybean varieties to 0.90 mEq/g, which is 9.7% more than in grain varieties.

Sources of antioxidants in the diet are important for improving health and increasing human lifespan. As already noted [11-13], various pathologies are associated with oxidative stress, including the risk of carcinogenesis. Aging may be largely due to the accumulation of oxidants, the by-products of normal metabolism produced by mitochondria [63]. Oxidative damage to proteins and lipid membranes disrupts the structure of key enzymes, and due to lipid peroxidation, the level of mutagenic aldehydes increases [63].

Red grain rice varieties and *Momordica charantia* L. are plants with high antioxidant potential and significant accumulation of carotenoids. According to

the Central Botanical Garden of the Siberian Branch RAS, momordica is capable of accumulating carotenoids in the leaves up to 545.1 mg% per fresh weight, in the arillus of the fruit 68.9-177.6 mg%, in the mesocarp 5.1-9.0 mg%. In the fruits of the orange-fruited tomato variety Top Model (control), the content of carotenoids (1.8 mg%) was more than 300 times lower than that in the leaves of momordica. For comparison, in carrots (a kind of standard for high carotene content among vegetable crops), the amount of carotene on average across 32 varieties was 16.6 mg%, and in the leaves of green vegetable plants, according to a study conducted in India, it was 3.85-130 mg% [44, 64].

The effect of antioxidants on health and life expectancy. Many natural antioxidants are common components of human food, which has made it possible to recommend large doses of them to slow down aging and increase life expectancy. However, half of the planet's population is deficient in one or another AO, which leads to conditions close to radiation aging. It is hypothesized that widespread insufficient AO intake leads to DNA damage through a mechanism similar to the effects of radiation and chemicals [63-65].

It is known that the complex of micronutrients entering the human body with food determines the development of microbial associations in the gastrointestinal tract (GIT), the microbiota of which plays a key role in the homeostasis of the host organism [66]. Dietary polyphenols have prebiotic properties and act against pathogenic gut microbiota, providing benefits in a variety of disorders. In particular, polyphenolic compounds can modulate the composition and function of microorganisms in the gastrointestinal tract, affecting membrane permeability and increasing the susceptibility of bacteria to xenobiotics. When the composition of food ingredients changes, the composition of the microbiota also changes within 24 h, which affects the functional state of the body and its resistance to environmental factors [67, 68].

In mice, antioxidants could change average lifespan by suppressing tumors. The effect of antioxidants on tumors, in turn, has been linked to the involvement of free radicals in the regulation of proliferation and differentiation of both cancer cells and immune cells, as well as their other regulatory functions [69]. Optimizing the content of antioxidants in the diet is a reserve for increasing human life expectancy. There is a connection between demographic indicators and the content of antioxidants in foods that are commonly consumed in different countries. For example, a Mediterranean diet rich in vegetables and fruits has been shown to increase life expectancy [70]. The so-called French paradox, that is, relatively low age-related mortality from cardiovascular diseases combined with high consumption of atherogenic foods in France, is associated with the high content of polyphenolic antioxidants in dark-colored wine. Timely administration of AO, which allows delaying cardiovascular diseases, can in many cases prolong life [71].

It should be noted, however, that the experimental results both confirm the benefits of antioxidants and do not prove their positive effects. Experiments are often carried out without taking into account all the factors influencing the result. More than 5000 antioxidants are known, which differ in chemical composition, antiradical and antiproliferative activity [72]. For many, an effect on proliferative activity was detected in some tumor cell cultures and much weaker in others. Synergism or additive effect of antioxidants is shown. That is, to protect against various forms of cancer, the composition of consumed antioxidants must be quite broad [73, 74]. Diversification of nutrition is necessary to effectively protect the body from the increasingly destructive influence of the environment. Each plant contains its own complex of useful substances. Therefore, limiting the range of fruits and vegetables in the diet reduces the effectiveness of the biologically active substances they contain.

Importantly, there are experimental data that indicate the limitations of

the free radical theory of aging. Thus, in the absence of oxidative stress, antioxidants did not have a positive effect on aging. Approximately the same result was obtained in mice, in which an increase in life expectancy under the influence of antioxidants was observed only in a conventional vivarium, whereas this did not occur in a pathogen-free vivarium in the absence of stress [65, 75].

Nevertheless, the search for new sources of biologically active substances and increasing their content in cultivated species remain important tasks. When studying the antioxidant properties of substrates, one must take into account the fact that cell cultures are similar to an isolated system not subject to stress, which significantly differs from the conditions inside an organism subject to all kinds of stress. In other words, in some cases, the undetermined effect of antioxidants may be a consequence of an incorrect methodological approach to assessing their properties.

Black and red grain rice as sources of antioxidants. Rice with a black pericarp color was known in China more than 3 thousand years BC. Even then it was served only at the imperial table, since it was believed to ensure health. In recent years, studies have been conducted that have confirmed the healing properties of this rice. Thus, black rice bran contains no less AO than blueberries and currants, and their antiradical activity is higher. Anthocyanins give rice grains their black color. Their benefits in the prevention of cancer and heart disease have been proven [74-77]. Chemical composition of black rice includes phytic acid, B vitamins, microelements, oryzanol, anthocyanins and vitamin E. Dozens of black rice varieties have been created in the world, differing both in the chemical composition of antioxidants and in antiradical activity. The most reactive antioxidants in black rice are cyanidin-3-glucoside and peonidin-3-glucoside. Their content varies over varieties from 19 to 141 mg/100 g and from 11 to 13 mg/100 g, respectively [78, 79].

The color of a sample is directly related to its chemical composition, with darker pericarp indicating higher polyphenol content [80, 81]. The yellow or orange tint is due to carotenoids, aurones, flavones and flavonols, and flavonol glycosides. The red or dark brown color is due to flavonoid compounds — proanthocyanidins and phlobaphenes. The same pigments can determine different colors in different plant tissues due to a tissue-specific system for regulating the synthesis of these compounds [82].

Red grain rice is characterized by the presence of proanthocyanidins, whereas black rice by the accumulation of mainly cyanidin 3-glucoside and peonidin 3-glucoside [83]. Rice with red pericarp contains 166 to 732 mg/100 g of phenolic compounds [84]. Glutinous black-colored varieties accumulate from 260 to 2540 mg/100 g of anthocyanins. Varieties with colored pericarp contain more microelements (zinc, calcium, manganese, iron, and copper) [85]. In terms of total phenol content, red-grain varieties are 8 times inferior to black-grain varieties, in the accumulation of anthocyanins, approximately 60 times inferior, and in antiradical activity 45 times inferior [86].

Functional food products. Flour of a certain composition (from black rice, red rice, amaranth, black wheat, soybeans) is a functional product that helps improve the health. Rice flour can be white (from milled rice) or whole grain (from hulled rice). Polished rice flour is snow-white in color and almost devoid of smell and taste. Coarser whole grain flour is characterized by the presence of a seed germ, darker color and nutty aroma, it contains more vitamins, microelements, and antioxidants. Minimally processed hulled rice is especially beneficial because only the flower scales are removed. Subsequent processing (grinding and polishing) removes the germ and aleurone layer, which increases the shelf life of rice grains, but significantly reduces its nutritional value [87-89].

Rice flour's lack of gluten allows its use as an alternative to wheat flour for those with celiac disease or on a gluten-free diet [90, 91]. Gluten enteropathy, or celiac disease, is a chronic disease in which eating foods containing gluten (wheat, rye, barley) causes poor digestion and absorption due to damage to the mucous membrane of the small intestine.

Rice flour is lower in calories, easier to digest, and acts as a soft sorbent in the intestines, cleansing the body of toxins [92, 93]. The content of essential amino acids in rice flour is higher than in wheat and corn flour, and is slightly inferior to amaranth flour. The leader in their content is soy flour. In appearance, consistency, color, and smell, rice and amaranth flour are similar to wheat flour. Corn flour has a yellow color and a special aroma. Soy flour imparts a bean-like aroma and a brownish tint to bakery [94, 95].

Colored and white grain rice varieties do not differ significantly in protein content. Amino acids are a material for the synthesis of proteins, the deficiency of which disrupts the synthesis of vitamins, pigments, and hormones. An unbalanced composition of amino acids in food weakens a person's cognitive abilities and reduces immunity. The relationship with the likelihood of diabetes has been established for several amino acids, namely, serine, alanine, arginine. The content of lysine, an amino acid that limits the digestibility of cereal protein, is higher in rice than in wheat, corn and sorghum [92, 96].

The most valuable property of rice flour is its low asparagine content, which reduces the risk of the formation of carcinogenic substances in baked foods. There is evidence that the content of asparagine and soluble sugars is associated with the formation of acrylamide, a substance that causes cancer [96]. The main groups of products in which acrylamide is formed are French fries and chips, coffee, cookies, confectionery and bakery products. Acrylamide accumulates as a result of the interaction of asparagine with sugars (glucose and fructose) at temperatures above 120 °C and low humidity. The amount of asparagine in wheat grain varies from 75.5 to 2150 mg/kg, in oats from 51 to 1390 mg/kg, in corn from 71 to 2900 mg/kg, in rye from 310 to 900 mg/kg, in rice from 14.9 to 24.9 mg/kg. That is, on average, the amount of asparagine in rice is 3 times less than in wheat and corn, and 2 times less than in oats [97]. This property is used by adding rice flour to baked goods and confectionery products to reduce the carcinogenicity of hazardous products. The identified varietal variability by trait makes it possible to increase the usefulness of products with rice flour [94, 95].

Amaranth seed flour has a high protein content, 18.82%, which is 8.5% more than wheat flour. Amaranth seed flour contains almost 7 times more fat than wheat flour, and there is less starch and digestible carbohydrates. It is also characterized by a high potassium content up to 1500 mg%, which is 1378 mg% higher than wheat, and a significantly higher content of iron, calcium and magnesium.

Amaranth flour and flour from colored rice varieties added into the recipe enriches the products with vitamins and microelements [97, 98].

The use of whole grain flour is one of the trends in the production of healthy and functional products. Consuming whole grains reduces the risk of cardiovascular disease, obesity, diabetes, and some types of cancer [98].

Genetic mechanisms regulating the antioxidant properties of black pericarp rice. The study of genetic mechanisms that control color in plants is relevant given the antioxidant and antimicrobial properties of pigments and their colorless precursors. These compounds provide prevention of cancer, reduce the risk of cardiovascular diseases, atherosclerosis, type 2 diabetes, increase immunity, improve the synthesis of visual pigments, and activate metabolic processes [76, 84]. It has been established that intraspecific diversity in pericarp color is due to a complex of regulatory and structural genes [99, 100]. An increased content of

proanthocyanidins in the seed coat is associated with resistance to germination on the root, and the presence of anthocyanins in the pericarp contributes to better preservation of seeds after long-term storage and increased plant resistance to stress [101, 102]. That is, plants with a high content of antioxidants possess significant competitive advantages. Antioxidants increase plant resistance to biotic and abiotic stresses, but this aspect has not been sufficiently studied in rice varieties with colored pericarp [103, 104].

In rice, antioxidant compounds such as oryzanol, tocopherols, and phenolic acids reduce the risk of developing chronic diseases [105]. Among the various phenolic compounds in the grain of colored varieties, there are ferulic acid (56-77%) found in endosperm, bran and whole grains, p-coumaric acid (8-24%), sinapic acid (2-12%), gallic acid (1-6%), protocatechinic acid (1-4%), p-hydroxybenzoic acid (1-2%), vanillic acid (1%) and syringic acid (1%) [105]. Understanding the genetic nature of the traits that determine the antioxidant properties of rice is important for breeding. Variations in grain color and nutritional quality were studied in 416 accessions, including red and black rice. A total of 41 loci were identified for quality-determining traits.

Ra, *Prp-b* genes for varieties with purple pericarp and *Rc* (brown pericarp and aleurone layer) were confirmed to be major contributors to the phenotypic effect on rice grain color and nutritional quality [106, 107]. These genes are localized on chromosomes 9, 10 and 8 in the regions where markers RM228 (90-154 bp amplification product), RM339 (166-148 bp), RM316 (160-210 bp) are located. A total of 11 markers were identified for four pericarp color traits and one marker (RM346) is associated with phenolic content. The *Wx* gene locus was identified as a chromosomal region that determines color intensity. The identified markers can be used to improve the beneficial properties of rice via marker-assisted selection (MAS) [108].

Another study identified QTL (quantitative trait loci) for 5 traits of color, phenolic content, flavonoid content and antioxidant capacity [109]. Correlation analysis showed that the color traits of rice, namely, color intensity (L), red tint (a), yellowness (b), color tint (C) were interrelated. Phenol content positively correlated with flavonoid content and antioxidant capacity ($p < 0.001$), while flavonoid content was not associated with antioxidant capacity, but positively correlated with L color intensity. Three QTL located between markers GA285 and CT580 on chromosome 2, were associated with parameters L, b and C; the last two traits were also influenced by QTL on chromosome 8. Two other QTL on chromosome 2 (qPH-2 and qFL-2-1) flanked by markers CT87 and G1234 were identified as loci for the content of phenols and flavonoids with additive effects determining 16.91 and 12.71% of the phenotypic effect. Three QTL located in the same region of chromosome 7 between markers G379A and CT360 affected the color parameter a and antioxidant capacity. They may be allelic for the *Rd* gene which is responsible for pigmentation in brown rice [110].

To increase the nutritional value of products from functional rice varieties with colored pericarp, it is important to combine selection for traits that determine adaptability, quality and productivity [102, 111].

Genetic mechanisms regulating carotenoid biosynthesis in *Momordica charantia* L. Carotenoids were quantified in various organs of *M. charantia*, and genes responsible for the accumulation of carotenoids were identified. Using the momordica transcriptome database, identification was performed of a cDNA fragment clone encoding geranyl-geranyl-pyrophosphate synthase (McGGPPS2) and several clones of full-length cDNA encoding geranyl-geranyl-pyrophosphate synthase (McGGPPS1), zeta-carotene desaturase (McZDS), lycopene beta cyclase (McLCYB), lycopene epsilon cyclase (McLCYE1 and

McLCYE2), beta-carotene hydroxylase (McCHXB) and zeaxanthin epoxidase (McZEP). In various organs of *M. charantia* (leaves, flowers, roots, fruits) and at four stages of fruit ripening, the expression of mRNA encoding these eight putative enzymes of carotenoid biosynthesis, as well as the accumulation of lycopene, α -carotene, lutein, 13Z- β -carotene, E- β -carotene, 9Z- β -carotene, β -cryptoxanthin, zeaxanthin, anthraxanthin and violaxanthin. The transcripts constitutively express at high levels in leaves. Taken together, these results indicate that the enzymes McGPPS2, McZDS, McLCYB, McLCYE1, McLCYE2, and McCHXB may be key factors to control carotenoid content in momordica. In the future, over-expression of carotenoid biosynthesis genes in *M. charantia* can be used to increase the yield of these nutritionally and medically important antioxidants [46, 111].

Thus, our analysis of scientific publications showed the promise of using black and red rice, momordica and amaranth as non-traditional sources of antioxidants and microelements. The high content of antioxidants in these crops and their high antiradical activity have been confirmed in many studies. Cyanidin-3-glycoside and piodin-3-glycoside are the main antioxidants of black rice. For red rice, momordica, and amaranth, high antiradical activity is associated with carotenoids. In black rice, flavonoids are among of the main antioxidants. The antiradical activity of flavonoids can be 50 times higher than that of many plant antioxidants; they are significantly inferior to vitamins E and C. Carotenoids are also effective antioxidants. The distinctive feature of carotenoids is their interaction with other substances of this nature, which increases the biological activity of the compounds. The intraspecific diversity in color traits observed at the phenotypic level is associated with both regulatory and structural genes. In addition to nutritional value, the increased content of anthocyanins contributes to better preservation of seeds after long-term storage, prevents germination on the root, and increases the adaptability of plants to biotic and abiotic stresses. Studying the genetic control of the biosynthesis of flavonoids, carotenoids and other-er antioxidant compounds will facilitate breeding for target traits to produce functional foods.

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