Improvement of Nutraceutical Value of Food by Plant Symbionts

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Abstract

Consumers and producers have recently shown an increasing interest in health-promoting properties of plant fresh foods, which are currently considered "functional foods." They contain phytochemicals playing a key role in promoting human health by reducing oxidative damages, modulating detoxifying enzymes, stimulating the immune system, and showing chemopreventive actions. Recent findings revealed that the content and composition of phytochemicals in fresh fruit and vegetables is greatly affected by plant genotype, harvest season, soil quality, and agronomic and environmental factors, including mycorrhizal symbioses established by arbuscular mycorrhizal fungi (AMF) with most crop plants. AMF promote plant growth and health and reduce the need of chemical fertilizers and pesticides, leading to less environmental damage and to improved

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food quality and health. They also enhance the biosynthesis of plant secondary metabolites with health-promoting activities, such as polyphenols, carotenoids, flavonoids, phytoestrogens, and activity of several antioxidant enzymes. Recent studies reported a higher nutraceutical value in mycorrhizal globe artichoke and tomato, two plant species largely cultivated for human consumption, suggesting that AMF inoculation may represent a suitable biotechnological tool to be implemented in agri-food chains aimed at producing safe and healthy food.

Keywords

Agri-food chains • arbuscular mycorrhizal fungi • beneficial microorganisms • functional food • globe artichoke • health-promoting compounds • mycorrhizal symbiosis • phytochemicals • plant secondary metabolites • tomato

1 Introduction

Consumers and producers have recently shown an increasing interest in health-promoting properties of plant foods, which represent an important societal issue. Fresh fruits and vegetables are currently evaluated not only for their size, weight, appearance, and flavor but also for their nutritional and nutraceutical value, that is, their content in vitamins, mineral nutrients, dietary fibers, and secondary metabolites. Plant fresh foods are considered “functional foods,” “nutraceutical foods,” or “pharmafoods,” since many epidemiological studies have reported that their consumption may play a key role in promoting human health by preventing chronic diseases and decreasing the risk of mortality from cancer and cardiovascular diseases [1–6]. Though, in most cases, it was not investigated whether the biological activity was linked to a specific plant molecule or to additive and synergistic combinations of phytochemicals [7].

Phytochemicals, represented by thousands of secondary metabolites produced by plants belonging to many different families, genera, and species, are dietary plant molecules which, consumed daily, can beneficially modulate human metabolism [8]. In particular, they represent a rich source of natural antioxidant compounds able to reduce or prevent oxidative damages to different biological molecules, such as lipids, proteins, and nucleic acids [9], damages caused by the action of reactive oxygen species (ROS) deriving from cell aerobic respiration [10–12]. Beyond their antioxidant activity, phytochemicals play a beneficial and functional role in human health by modulating detoxifying enzymes [13] and hormone metabolism [14, 15] and by stimulating the immune system [16], showing also antibacterial and antiviral activity [17].

The most important phytochemicals are represented by polyphenols, widespread compounds functioning as scavengers of free radicals and quenchers of single oxygen formation [18]. Polyphenols are widely distributed in plants and are generally considered beneficial, since they affect different processes in mammalian cells, suggesting an anticarcinogenic and antiatherogenic role [19, 20]. Flavonoids, represented by more than 5,000 bioactive compounds, many of which can be found in food and beverages – such as quercetin in tea, kaempferol in cabbages, and myricetin in blackberry and red vine – have been reported to reduce the risk of cardiovascular diseases, to exert a chemoprotective action, and to have a phytoestrogenic activity [21]. Glucosinolates, phytochemical compounds including more than 130 different molecules mainly occurring in Brassicaceae [22], are considered "plant food protection agents" since a clear correlation has been shown between cruciferous plant consumption and cancer risk reduction [6, 23–25]. Other epidemiological data reported that isoflavones have different health-promoting effects, protecting from cardiovascular diseases [26].

Different phytochemical molecules have shown a chemopreventive action [27]: for example, the synthetic oleanane triterpenoid, CDDO-methyl ester, is a potent antiangiogenic agent [28]; epigallocatechin-3-gallate from green tea inhibits tumor angiogenesis and vascular tumor growth [29]; polyphenol curcumin prevents hematogenous breast cancer metastases in immunodeficient mice [30]; polyphenol xanthohumol from hop shows antileukemia effects in Bcr/Abl-transformed cells [31]; hyperforin from *Hypericum perforatum* blocks neutrophil activation of matrix metalloproteinase-9 and restrains inflammation-triggered angiogenesis [32]; lycopene from tomato is active in inflammation and in chemoprevention of prostate cancer [27]; polyphenolic compounds contained in red wine are able to inhibit vascular endothelial growth factor expression in vascular smooth muscle cells [33].

Nevertheless, it is important to note that any health-promoting activity of plant food depends on its bioavailability and bioefficacy, which are often related to individual variables, such as microbiome structure and composition, digestive processes, and absorption in the intestine [34–36].

Some phytochemicals, such as isoflavones and other flavonoids, as well as lignans, coumestans, and stilbenes, display estrogenic (and antiestrogenic) activity and are generally called phytoestrogens, notwithstanding the fact that they do not show any structural similarity to naturally occurring estrogens [37]. For example, lignans contained in seeds, sprouts, fruits, vegetables, and whole grains are active in cancer prevention, similarly to isoflavones [38]. These compounds have been studied for their putative preventive role in osteoporosis, menopausal symptoms, arteriosclerosis, heart disease, and cancer, and some were considered alternative to synthetic compounds for therapeutic purposes in humans [39, 40]. They have a complex mode of action via interaction with the nuclear estrogen receptor isoforms ERa and ERb, exhibiting either estrogen-agonist or estrogen-antagonist effects [41]. In particular, antiestrogenic compounds can antagonize estrogen-dependent processes in their target tissues, counteracting the growth of estrogen-related cancers. Moreover, other polyphenols and lycopene have been considered promising pharmacological agents in cancer prevention, as a result of their antiproliferative effects and their inhibitory action on the human estrogen receptors [42–44].

2 Health-Promoting Phytochemicals as Affected by Agronomic Management

Phytochemicals play a major role in ecological interactions between plants and the surrounding environment, being active against pathogens and viruses, in allelopathic interactions, in insect chemoattraction, and in defense mechanisms against biotic and abiotic stresses. As plant secondary metabolites, they can be constitutively expressed but can also be induced by diverse factors, including attack by fungal and bacterial pathogens and herbivores [45]. Recent findings revealed that the content and composition of phytochemicals in fresh fruit and vegetables (FAVs) is greatly affected by other variables, that is, plant genotype, harvest season, cultivation site and techniques, and soil quality, and by agronomic practices, such as quantity and quality of available nutrients and light, irrigation, use of pesticides and chemical fertilizers, and conventional/organic management [46–48]. Such results increased interest in nutraceutical and functional foods, which stimulated scientists to boost research on beneficial plant secondary metabolites and on the best genetic and agronomic approaches to increase their concentration in FAVs [49, 50].

A key reference paper provided a comprehensive database (collected as part of the USDA National Food and Nutrient Analysis Program) of the total phenolic content and antioxidant capacity in over 100 different foods, including fruits, vegetables, nuts, dried fruits, spices, and cereals [51]. Other investigations reported thorough lists of nutraceutical properties of FAVs, depending on different genetic and agronomic variables. Plant genotype was one of the main factors assessed: the antioxidative activity of 92 plant phenolic extracts was found to vary with plant species, which showed highly variable total phenolic content, calculated as gallic acid equivalents (GAE), ranging from >20 mg g^{-1} GAE in berries to <12.1 mg g^{-1} GAE in apples [11]. Two different cultivars of tomato contained 1.0 and 10 mg kg^{-1} of lycopene, whose content ranged from 10 to 100 mg kg^{-1} in the same tomato variety analyzed at two different ripening stages – turning and red – respectively [52]. Plant genotype affected the production of different metabolites, such as ascorbic acid, whose concentrations ranged from 20 to 300 mg kg^{-1} in apple, from 300 to 500 mg kg^{-1} in orange, and from 290 to 800 mg kg^{-1} in kiwi, depending on the cultivar type [53, 54] and glucosinolates in broccoli, which showed huge variability among 50 screened cultivars [55]. In fruit plants, rootstock type affected the nutritional and nutraceutical quality of peel and flesh of peach fruits, which showed the highest antioxidant capacity associated to a high level of carotenoids and phenols in the rootstocks Mr. S 2/5 and Barrier 1, compared with Ishtara and GF 677 [56, 57].

Several conventional breeding programs in different countries, boosted with the aim of obtaining cultivars with enhanced concentrations of phytochemicals, were successful: for example, improvements were registered in tomato lines with 10–25 times increased concentration in β-carotene, compared with conventional varieties [58, 59] and in new peach and plum genotypes rich in phenolic compounds and antioxidant capacities [60, 61]. Such promising and important results allow us to foresee that in the years to come, new breeding programs will lead to the selection of cultivars with enhanced concentrations in phytochemicals.

Beyond plant genotype, agronomic and environmental factors may play a major role in increasing phytochemical content of FAVs, and it is tempting to speculate on the possibility of selecting the best performing growth conditions and techniques to enhance health-promoting properties of crops.

The production of phytochemicals, such as ascorbic acid, phenolic compounds, carotenoids, and glucosinolates, as a result of plant exposure to low temperatures and high light intensity during the growth period, was much variable in quantity and composition in diverse FAVs. However, high light exposure or intensity generally produced positive effects in the concentration of ascorbate, phenolic compounds, carotenoids, and glucosinolates of FAVs, probably due either to enhanced photo-oxidative stress or to increased photosynthesis [50]. The same variable trend was shown by treatments with specific wavelength irradiation, such as red light, blue light, and UV-B on carotenoids concentration in tomato, suggesting that light influences carotenoid metabolism in a very complex way [62, 63].

Other agronomic factors, such as drought and high salinity, are still under investigation for their putative positive effects on phytochemical concentration in diverse FAVs. Interesting responses to water availability were found in grapevine and field-grown olive trees: deficit irrigation regimes increased the concentration of phenolic compounds, improving the quality of grape and virgin olive oil [64, 65]. Other findings suggested that irrigation with saline water may improve carotenoids content and antioxidant activity of tomatoes [66, 67].

It is important to highlight that the extremely high increases in some phytochemicals obtained through genetic selection, conventional breeding, and metabolic engineering – reaching up to 10–25-fold in carotenoids, 20-fold in glucosinolates, and 36-fold in kaempferol-rutoside – raised concerns on their safety [50], since some compounds, such as polyphenols, generally considered beneficial for their anticarcinogenic and antiatherogenic role [19], have proved to be genotoxic at high concentrations [68, 69]. On the contrary, the increases in phytochemical concentration produced by environmental and agronomic factors – ca. twofold – were claimed to "*represent arguably a good balance between effectiveness and safety*" [50].

Such interesting suggestion gives further support to projects aiming at continuing and extending research on the best agronomic techniques and managements to enhance the production of beneficial phytochemicals in crop plants, cereals, fruits, and vegetables in the years to come. One of the most promising agronomic factors affecting plant secondary metabolic pathway is represented by arbuscular mycorrhizal fungi, belonging to Glomeromycota, an important ecological and economical group of beneficial soil microorganisms that establish mutualistic symbioses with the roots of the vast majority of plant species.

3 Plant Beneficial Symbionts: Arbuscular Mycorrhizal Fungi

Mycorrhizal symbioses are beneficial associations between plant roots and soil-borne fungi, occurring in about 90 % of land plants and involving 240,000 plant species and 6,000 fungal species. Depending on host plants and fungal symbionts, many different mycorrhizal types have been observed in nature; nevertheless, their effects on host plants are similar: a larger growth due to a better nutritional status, a higher tolerance to biotic and abiotic stresses, and a general higher fitness, compared with non-mycorrhizal plants [70]. The most widespread type of mycorrhizal symbiosis is the arbuscular mycorrhiza, which is distributed from arctic to subantarctic regions, in temperate and tropical grassland and forests, scrub and desert ecosystems, from sand dunes to alpine sites [71]. Arbuscular mycorrhizal (AM) symbioses occur within all phyla of land plants, in most plant families, except genera and species belonging to Brassicaceae, Chenopodiaceae, and Cyperaceae, and plants which are exclusively hosts of other mycorrhizal fungi.

The most important agricultural fodder and grain crops form AM symbioses: from cereals, including rice, corn, barley, and wheat, to legumes and fruit trees including citrus, peach, grapevine, and olive and from vegetables like onion, strawberry, tomato, and potato to economically important species, such as sunflower, cassava, cotton, sugarcane, tobacco, coffee, tea, cocoa, rubber, oil palm, and banana [71]. AM fungi (AMF) show low host specificity and are obligate biotrophs, that is, they cannot be cultivated on synthetic media in the absence of the host. Such inability represents the major constraint to their large biotechnological application. Their life cycle starts with spore germination, originating a short-lived asymbiotic mycelium, which is able to recognize host roots and to differentiate infection structures, the appressoria, on the root surface. Hyphae developed from appressoria grow within the root cortex, intercellularly along the longitudinal root axis, and then penetrate within cortex cells, forming haustoria-like branched structures, the arbuscules, originating from the dichotomous branching of intracellular hyphae which progressively reduce their diameter. Arbuscules are the key structure of the mutualistic symbiosis, representing the site where nutrient exchanges between plant and fungus occur. After establishing the symbiosis, plant-derived carbon is transferred to AM symbionts, reaching up to 20 % of total photosynthate, and then transformed into trehalose and other fungal polyols [72]. Such carbon is essential for the extraradical growth of AMF, which produce large mycelial networks exploring the surrounding environment and efficiently absorbing mineral nutrients from the soil, as a result of the high surface-to-volume ratio of the hyphae [73, 74]. Eventually the fungus is able to complete its life cycle by the formation of new spores.

A large body of investigations showed that AMF have important beneficial effects on plant growth, increasing the transfer of soil mineral nutrients, such as phosphorus (P), nitrogen (N), sulfur (S), potassium (K), calcium (Ca), iron (Fe), copper (Cu), and zinc (Zn), and improving plant tolerance to root pathogens and drought [70]. Differential increases in P and N supply to host plants after inoculation of diverse AMF have been ascribed to phenotypic and functional properties of the extraradical mycorrhizal mycelium [75–79], depending also on the occurrence and differential expression of P transporter and N assimilation fungal genes [80–82].

The mechanism involved in the improved P nutrition of mycorrhizal plants is represented by the efficient soil exploration by fungal hyphae extending beyond the depletion zone caused by the fast absorption of P from the soil solution, which cannot be rapidly replenished, given the poor mobility of P in the soil [83]. Electron microscope studies and chemical analyses revealed that hyphal P translocation is operated through the accumulation of polyphosphate granules within hyphal vacuoles, which are transferred from soil-based to root-based hyphae [84–88]. P is then released to the host cells in the arbuscules by means of polyphosphatases and alkaline phosphatases [89–95]. As AMF are coenocytic organisms, that is, they do not possess cross walls, the high flow rates of protoplasm occurring within their hyphae can explain the rapid mobilization and transfer of P and the other soil nutrients.

Indeed, the inflow rates of P in extraradical hyphae may range from 2 to 20 × 10^{-6} mol m^{-2} s^{-1} [96–99], while bidirectional protoplasmic flow rate, measured on the basis of cellular particles movement (presumably vacuoles, nuclei, fat droplets, organelles, granules), ranges from 2.98 to 4.27 μm s^{-1} [100, 101]. However, it is important to underline that the most important factor affecting the transfer of nutrients from soil to root-based hyphae is represented by the flux of mineral nutrients through appressoria, which for P is 3.8×10^{-8} mol cm^{-2} s^{-1} [102].

A great diversity has been reported among plant species in the extent to which they depend on AMF. For example, some plant species such as *Citrus* spp. are totally dependent on mycorrhizal inoculation during the seedling stage [103], showing no response to P addition, while other species, such as grasses, show a low mycorrhizal dependency [104]. However, the different degrees of mycorrhizal dependency vary with plant species and cultivars, soil P content, root colonization, and efficiency of the inoculated fungal isolate [105].

Moreover, AMF may represent underground communication ways [106], also activating defense pathways before the pathogen attacks. For example, tomato plants colonized by the same AM symbiont showed increased expression of defense-related genes and higher levels of disease resistance enzymes in healthy “receiver” plants after inoculation of “donor” plants with a pathogen [107].

In conclusion, AMF represent fundamental key factors promoting plant growth and health by processes such as the acquisition of nutrients and water, the modulation of plant hormonal balance, the protection from pathogens, and the abiotic stress protection. Thus, AMF play a major role in organic production of food by reducing the need of chemical fertilizers and pesticides, leading not only to less environmental damage but also to improved food quality and health. Contemporary trends toward low-input, sustainable agriculture consider mycorrhizal inoculation with efficient AM fungal species a promising biofertilization strategy in order to enhance plant growth, yield, and quality.

Recently, AM fungal symbioses have been shown to modify several aspects of host plant metabolism and have been proposed as an environmentally friendly and efficient strategy to enhance plant biosynthesis of secondary metabolites with health-promoting activities.

4 Nutraceutical Value of Mycorrhizal Plants

Many evidences indicate that AM fungal symbiosis may induce changes in primary and secondary metabolism of host plants, for example, increasing the production of secondary compounds – including polyphenols – in the roots of mycorrhizal plants [108–113]. Indeed, the colonization of root cortical cells by AMF causes diverse cytological and metabolic changes: a marked proliferation of plastids, the activation of Krebs cycle, and the modification of plastid biosynthetic pathways during intracellular arbuscule development leading to increased metabolic activity and to higher production of fatty acids, apocarotenoids, and amino acids, such as tyrosine, which, together with phenylalanine, is the main precursor of plant polyphenols in the phenylpropanoid metabolism [114–117]. Interestingly, in *Trifolium repens* plants, accumulation of some flavonoids – quercetin, acacetin, and rhamnetin – was exclusively detected in roots of plants inoculated with *Glomus intraradices* [118].

Several studies investigated also the influence of AM fungal symbiosis on plant shoot metabolic activities. Some authors suggested that accumulation of secondary compounds in the shoots of mycorrhizal plants may represent the main factor conferring resistance to fungal pathogens [119–122]. For example, higher contents of phenolic compounds in mycorrhizal plants were correlated with a reduced severity of diseases caused by *Phytophthora nicotianae* and *Botrytis fabae* in tomato and *Vicia faba*, respectively [123, 124], and with a decrease of *Ralstonia solanacearum* population in tomato [125].

AM fungal inoculation, besides inducing larger plant photosynthetic rates [126, 127], may produce other important biochemical changes, for example, in the non-mevalonate methylerythritol phosphate pathway of isoprenoid biosynthesis, correlated with apocarotenoid accumulation [128], and in physiological mechanisms leading to the accumulation of secondary metabolites, such as phenolic acids, carotenoids, and polyphenols, both in roots and in shoots [129–132]. Moreover, AMF induced also alterations in the activity of superoxide dismutase (SOD) in roots and shoots of different plant species [133–137] and of several antioxidant enzymes in roots of *Phaseolus vulgaris* [138] and in shoots of lavender, rice, and three Mediterranean shrubs [127, 139, 140], suggesting that the induction of plant protective mechanisms could alleviate oxidative damages caused by drought, salt, and other environmental stresses, leading to plant increments in shoot biomass. Accordingly, higher levels of enzymes active in the removal of reactive oxygen species (ROS) in mycorrhizal roots suggested that colonized plants may respond to oxidative stresses by the accumulation of antioxidative enzymes and carotenoids [141–144].

The inoculation of the AM fungal species *Glomus mosseae* and *Glomus versiforme* triggered transient enhancement of the levels of transcripts encoding phenylalanine ammonia-lyase in *Oryza sativa* and *Medicago truncatula* roots, respectively [141, 145], while increased chalcone synthase (CHS) transcript accumulation was found in *M. truncatula* roots colonized by *G. versiforme* [141] and *G. intraradices* [146]. Other effects of AM fungal symbiosis on secondary metabolism, such as phytohormone dynamics, were also reported [147–149].

A recent study found that mycorrhizal red clover showed impaired levels of isoflavones with estrogenic activity, such as biochanin A, formononetin, genistein, or daidzein [150], and natural plant metabolites called “phytoestrogens,” which are believed to play an important preventive role in osteoporosis, menopausal symptoms, arteriosclerosis, heart diseases, and cancer [39, 151] and are investigated as alternative to synthetic compounds for therapeutic purpose in humans [37]. In the species *Arnica montana*, which contains several groups of active secondary compounds, AM fungal inoculation induced sesquiterpene lactones accumulation both in roots and in shoots [131].

Several studies investigated AM fungal effects on the production of phytochemicals in medicinal and aromatic plants. Higher accumulation of antioxidant compounds (rosmarinic acid and caffeic acid) and essential oils was reported in shoots of *Ocimum basilicum* (sweet basil) inoculated with different *Glomus* species [130, 152, 153]. Essential oil concentration increased in fruits of *Coriandrum sativum*, *Anethum graveolens*, and *Trachyspermum ammi* (+43 %, +90 %, and +72 %, respectively, compared with controls) inoculated with *Glomus macrocarpum* and *Glomus fasciculatum* [154, 155], with differences between the two species of fungal symbionts. The concentration of essential oil showed about 62.5 % increase in *Foeniculum vulgare* seeds produced by plants inoculated with *G. fasciculatum*, compared with non-mycorrhizal controls. Moreover, chemical characterization of the essential oil extracted from seeds produced by mycorrhizal plants revealed an enrichment in anethol concentration [156].

Interestingly, essential oil accumulation may be partly due to a non-nutritional effect of AMF, since mycorrhizal *Origanum* sp. plants produced higher oil concentrations compared with those obtained from non-mycorrhizal plants showing equivalent P content [157].

Root concentration of the alkaloid forskolin, a potent cardioactive and hypotensive diterpenoid compound, was consistently higher in *Coleus forskohlii* plants inoculated with different AM fungal species, compared with non-mycorrhizal controls, with the largest increase (+147 %) when the symbiont *Glomus bagyarajii* was used [158]. Phytochemicals with therapeutic value produced by *Echinacea purpurea* (pigments, caffeic acid derivatives, alkylamides, and terpenes) increased their concentration up to 30 times after inoculation with the AM fungal species *G. intraradices* and *Gigaspora margarita* and the entomopathogenic endophyte *Beauveria bassiana* [159].

An increase in thymol derivatives production was observed in roots of *Inula ensifolia* plants inoculated with *G. intraradices* and *Glomus clarum*: the highest concentration of all the analyzed compounds was found in roots colonized by *G. clarum* [160]. Enhanced concentrations of anthraquinone derivatives (hypericin and pseudohypericin) were reported in *H. perforatum* shoots after inoculation with *G. intraradices* and with a mixed AM fungal inoculum [161].

Interestingly, a positive correlation between AM fungal root colonization of the medicinal plant *Castanospermum australe* and the content of castanospermine – an alkaloid of the indolizidine type – was found both in seeds and leaves, under field and greenhouse conditions, respectively [162].

So far a few studies investigated the relationship between plants, AM fungal colonization, and phytochemicals concentration in plants used for human nutrition. In the greenhouse, anthocyanins, carotenoids, and, to a lesser extent, phenolics occurred in higher concentration in the leaves of mycorrhizal lettuce plants than in non-mycorrhizal controls [163], while AM fungal inoculation of *Allium cepa* plants in pots significantly enhanced the total antioxidant capacity of bulb biomass [164]. In a greenhouse experiment, fruits of tomato plants, inoculated with a microbial mix of AMF and different bacteria, showed higher glucose, fructose, malate, and nitrate contents, while β-carotene, lycopene, and lutein contents increased when the substrate was amended with the microbial mix and green compost [165].

A 2-year field study and a multidisciplinary research reported higher nutraceutical value in mycorrhizal globe artichoke and tomato, plants largely cultivated for human consumption.

4.1 Case Study 1: Globe Artichoke

Globe artichoke, *Cynara cardunculus* var. *scolymus* (L.) Fiori, is an ancient perennial plant species (Asteraceae) native to the Mediterranean Basin, probably domesticated by Romans and diffused by the Arabs in the Southern Mediterranean area during the Middle Ages [166]. Globe artichoke, cultivated for its immature flower heads and for its high contents of phytochemicals, including polyphenols and inulin [167, 168], is considered a functional food, according to the definition of the European Commission on Functional Food Science in Europe (FuFoSE) [169, 170]. Artichoke leaves, rich in polyphenols, are utilized by the pharmaceutical industry for the production of commercial extracts for their choleretic, hypocholesterolemic, and antioxidant bioactivities, as a result of high contents in chlorogenic acid, cynarine, and luteolin [171–173]. Artichoke flower heads are among the richest sources of dietary phenolic antioxidants [174], whose levels depend on cultivar genetic diversity, harvest time, and climatic conditions during plant growth [175, 176].

A recent study reported, for the first time, large increases in total polyphenolic content (TPC) and antioxidant activity, expressed as antiradical power (ARP) in both leaves and flower heads of mycorrhizal globe artichoke plants assessed in microcosm and in the field [177]. Artichoke plants were inoculated in microcosm with the AM fungal species *G. mosseae* and *G. intraradices* and with a mixture of them, then transplanted and grown for 2 years in the field, where plants treated with the inoculum mixture showed large increases of main flower head fresh weight, not only in the first (92.8 %), but also in second year (70.6 %). Such mycorrhizal inoculation responses, persisting for 2 years after field transplant, stimulated further molecular studies, aimed at assessing the persistence of inoculated AMF in the field. ITS rDNA sequences clustering with those of *G. mosseae* and *G. intraradices* were retrieved only from inoculated plant roots.

Leaves of mycorrhizal artichoke grown in microcosm showed enhanced phenolic complement, depending on the inoculum composition. TPC and ARP were significantly higher (50 % and 33 %, respectively) in plants inoculated with the *Glomus* mixture, compared with control plants (Fig. 85.1). In the field, inoculated plants showed higher phenolics content in the edible parts (flower heads), compared with controls, with the highest values detected in plants inoculated with the

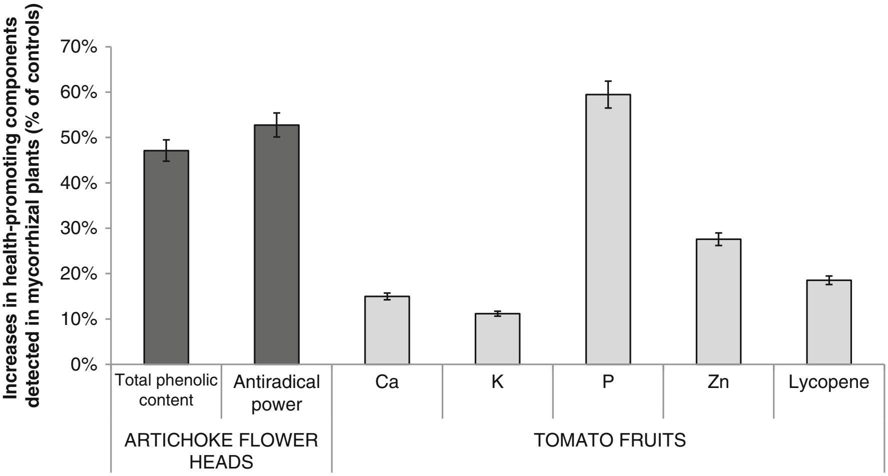

Fig. 85.1 Increases in health-promoting compounds detected in artichoke flower heads and in tomato fruits produced by mycorrhizal plants, compared with non-mycorrhizal controls. Increases are statistically significant ($P < 0.01$)

combination of the two AM fungal species. Higher TPC values were found in secondary flower heads (second year of field cultivation) compared with main flower heads (first year of field cultivation). Antioxidant activity of flower heads followed the same pattern of phenolics accumulation: ARP increases (52 % and 32 % in the first and second year in the field, respectively) were higher in plants inoculated with the mixed AM fungal inoculum. As mycorrhizal inoculation has been proved to induce changes in host plant secondary metabolism, including the phenylpropanoid biosynthesis, further studies should investigate the role of mycorrhizal symbiosis in the activity of two hydroxycinnamoyltransferases, recently isolated and functionally characterized in globe artichoke [178, 179].

4.2 Case Study 2: Tomato

Tomato (*Solanum lycopersicum* L.) is extensively cultivated worldwide, and its fruits have assumed the status of “functional foods” as a result of epidemiological evidence of reduced risks of certain types of cancers and cardiovascular diseases [180, 181]. They are a reservoir of diverse antioxidant molecules, such as lycopene, ascorbic acid, vitamin E, carotenoids, flavonoids, and phenolics, and may provide a significant part of the total intake of beneficial phytochemicals, as a result of their high consumption rates. Among carotenoids, lycopene has a strong antioxidant activity and is able to induce cell-to-cell communications and modulate hormones, immune systems, and other metabolic pathways [182].

The levels of lycopene and other beneficial phytochemicals in fresh tomato fruits may be affected by many agronomic factors, such as cultivars [58], cultural practices and ripening stage [183], and also by cultivation conditions, such as the establishment of mycorrhizal symbioses in plant roots. Recent works reported that mycorrhizal inoculation improved tomato growth and the production of fruits, which contained significantly higher quantities of ascorbic acid and total soluble solids [184].

A recent multidisciplinary work for the first time investigated the nutraceutical value and safety of mycorrhizal tomato fruits produced by mycorrhizal plants inoculated with the AM fungal species *G. intraradices*, by assessing the antioxidant, estrogenic/antiestrogenic, and genotoxic activity of tomato fruits [185]. The data obtained showed that mycorrhizal inoculation positively affected the growth and mineral nutrient content of tomato fruits, with enhanced uptake of soil mineral nutrients, that is, Ca, K, P, and Zn, whose concentrations in tomato fruits produced by inoculated plants were higher than in the controls. Interestingly, fruit P and Zn contents were 60 % and 28 % higher than those of controls, suggesting an important role of the symbiosis in improving the nutritional value of tomatoes, in particular for Zn, which is considered a key human mineral nutrient [186].

Lycopene content of tomato fruits from mycorrhizal plants was 18.5 % higher than that of controls (Fig. 85.1). Such an evidence of plant secondary metabolism modification was not linked with the production of putative unsafe compounds, such as mutagenic ones, since tomato extracts induced no in vitro genotoxic effects. Indeed, the two genotoxicity tests used, that is, the Ames *Salmonella*/microsome mutagenicity assay (the “first line” for detection of gene mutation) and the human lymphocyte MN test (a “first line” for detecting chromosome aberrations), excluded the presence of any putative phytochemical with DNA-damaging activity. Given the chemoprotective/chemopreventive effects of lycopene, it is tempting to suggest that the higher lycopene content detected in mycorrhizal tomato fruits could represent an important factor in neutralizing the DNA-damaging activity of any possible mutagenic compound occurring not only in tomato fruits but also in other foods consumed together with tomatoes.

Moreover, tomato fruit extracts – both hydrophilic and lipophilic fractions, originating from mycorrhizal plants – strongly inhibited 17-β-estradiol-human estrogen receptor binding, showing significantly higher antiestrogenic power, compared with controls. As lycopene represents a promising pharmacological agent in cancer prevention on account of its antiproliferative effects and inhibitory action on the human estrogen receptors [42, 43], the data obtained allow us to suggest that tomato fruits and those produced by mycorrhizal plants at a higher rate could antagonize the estrogen-like activity elicited by several environmental-industrial xenobiotics to which humans are exposed through the food chain [185].

5 Conclusion

Many epidemiological studies have reported that the consumption of FAVs may play a fundamental role in promoting human health by preventing chronic diseases and decreasing the risk of mortality from cancer and cardiovascular diseases. Thus, plant fresh foods are considered “functional foods,” not only because of their nutritional properties but also for their content in phytochemicals, which may vary depending on environmental and agronomic conditions, including plant mycorrhizal status. AM symbionts are ecologically and economically important beneficial fungi playing a major role in sustainable food production systems: they determine higher plant growth rates, increase resistance to biotic and abiotic stresses, and reduce the need of chemical fertilizers and pesticides, allowing a safe production of high-quality food. As they enhance the biosynthesis of many different compounds with nutraceutical value in leaves, roots, and fruits of plants used for human nutrition, the inoculation of selected AM fungal species and isolates may represent a suitable and environmentally friendly biotechnological tool to be implemented in agri-food chains aimed at producing safe and healthy food. Further studies are needed to answer questions as to whether different AM fungal species and isolates may differentially modulate plant secondary metabolism and the production of phytochemicals; whether inoculation time, technique, and protocols may change fungal performance affecting healthy compounds accumulation; and whether agronomic managements and practices may influence the persistence of AMF in the field and their efficacy in inducing favorable changes in the biosynthesis of secondary metabolites with health-promoting activity.

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