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### Chapter

# Nutritional Value and Phytochemical Content of Crop Landraces and Traditional Varieties

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### Abstract

Over the years, crops have been improved through breeding, mainly to increase production and, secondly, to introduce resistance to diseases and to achieve tolerance to abiotic stresses, these two latter by resorting to Crop Wild Relatives (CWR). This has resulted, in most cases, in homogeneous and nutritionally poor commercial varieties. Landraces and traditional varieties, barely taken into account, are key resources as they retain nutrients frequently "washed away" in the commercial varieties and also harbour a great genetic variability. They could represent a shortcut when compared to CWR in breeding, saving time and resources. The consumer's growing interest in health and food quality has caused breeders to redirect their attention toward them. This chapter provides information about the content in compounds with health benefits, such as phenolics, minerals, vitamins, etc., of landraces and traditional varieties of the most important crops, which could help to obtain healthier and more nutritious products.

**Keywords:** biofortification, carotenoids, micronutrients, health-promoting compounds, minerals, plant breeding, phenolic compounds, vitamins

### 1. Introduction

# 1.1 Landraces and traditional varieties: similarities, differences and comparison with wild species and commercial varieties

In the wide spectrum of plant material in terms of domestication and/or breeding, the concepts seem to be clear in both extremes, wild forms and commercial varieties. On one hand, the wild plants (either the Crop Wild Relatives, CWR, or those belonging to more distant gene pools) are those that have not been domesticated or subject to processes of artificial (human) selection and breeding. They do not exhibit traits typically present in cultivated plants, like uniform seed germination and homogeneous fruit ripening, or desirable characteristics present in those plants destined to human consumption, mainly related to quality (**Figure 1**). On the other hand, commercial varieties are those obtained by a breeding programme aimed to improve certain traits of the crop and that differ from other existing varieties by distinctive properties, which are uniformly expressed, and transferred in a stable way to the subsequent generations (**Figure 1**).

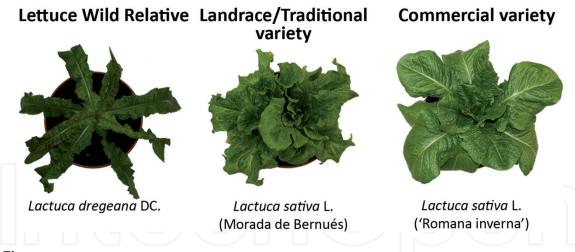


Figure 1.

Examples of phytogenetic resourses within the genus Lactuca: A lettuce wild relative (Lactuca dregeana DC.) and two cultivated forms (Lactuca sativa L.), a landrace/traditional variety (Morada de Bernués), and a commercial variety ('Romana inverna').

In between those two ends, a wide plethora of intermediate forms can be found. That is a grey area with blurred boundaries, what explains the general lack of consensus in even defining the plant material. In many cases, different terms have been used to refer to the same (or similar) type of plant, like ecotype, landrace, race, farmer variety, folk variety, local variety, traditional cultivar, etc. [1]. Even if some definitions are contradictory, there seems to be some recurrent ideas when authors refer to landraces and traditional varieties.

Landraces are profusely described in the literature as autochthonous cultivars or, at least, cultivars that have been grown in a certain area since ancestral times and, hence, are adapted to local growing conditions and uses through natural selection but without any active intervention from farmers. There are several terms difficult to verify in that definition. It does not seem easy to trace back the origin of the cultivars, especially if we take into account that the crop dispersals and the human migrations are inseparable. Besides, even if they have been cultivated in a region for a long period of time and, hence, they are adapted to the predominant environmental conditions, that does not imply that they exhibit a great tolerance to adverse conditions, biotic and abiotic stresses as stated before [1, 2]. Actually, the adverse edaphic, climatic and phytosanitary conditions would be mitigated even by the most traditional low input agricultural systems in comparison to those that the wild plants would have to face in the same region. Finally, it is difficult to defend the idea of farmers growing a cultivar for generations without carrying out any type of selection of the outstanding individuals, even if it is not fully conscious, as stated mainly in the earliest definitions [3, 4]. In fact, the agriculture procedures (seeding, harvesting ...) exert an artificial selection under which the most suitable genotypes for those cultural practises, prosper (and they probably rely on them for their survival, in return). Furthermore, in a scenario in which only the natural selection is acting, the resulting plants would probably be more similar to their wild relatives and less to the bred cultivars (and that is not the case with the landraces). In more recent definitions, the idea of a more or less directed human selection has been embraced [5–7], even if it cannot be considered a formal breeding programme [8].

In contrast with landraces, traditional varieties (also called folk varieties or farmer-bred varieties), have usually been defined like those that have been maintained by active selection and/or breeding by farmers. And, if this is the main difference between landraces and traditional varieties (as the latter are also cultivated locally and are well adapted to the particular climatic and growing conditions), is it really possible to distinguish them? How do we determine if a certain variety is the

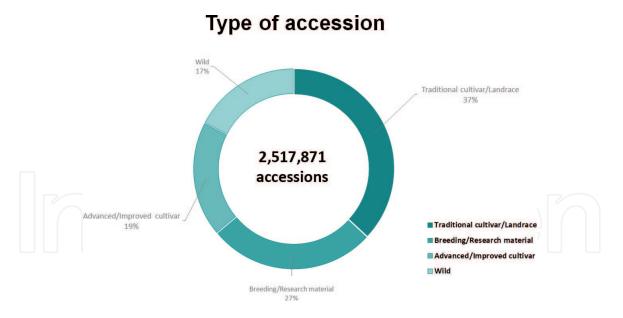
product of merely natural selection or the human intervention has also played a role on it? Is it actually possible to separate both processes? It could be that the question is nowadays irrelevant and the important aspect is that, either if we call them landraces or traditional varieties (**Figure 1**), they consist of dynamic populations that harbour enough genetic variability to show adaptability to local conditions and plasticity to overcome eventual changes, even if they can be fairly uniform for the selected traits. That broad genetic base would explain that, under eventual adverse conditions, they are still able to yield stably (though moderately), as some genotypes within the population will possibly show a better performance. These aspects were early emphasised by the plant breeder Harlan [9] when stated that some of the most important characteristics of landraces are their genetic diversity and dynamism, what has also been adopted in more modern times by other authors [10, 11]. Harlan also pointed out that they are the result of millennia of natural and artificial selection, as a way of integrating these two indiscernible processes. Another approach to overcome this thorny aspect consisted of eliminating the type of selection undergone in the definition of the landraces [12]. Any realistic and updated definition of this type of plant material will have to include the impact of agriculture and, hence, the human influence in their evolution as proposed recently [13].

Another aspect that blurs the lines between landraces and traditional varieties is the gene flow between them. With the availability of molecular markers and Next-Generation Sequencing (NGS) techniques, it is possible to trace the allele introgression from cultivated (all types) to wild plants and *vice versa*. Even if there were landraces exclusively product of natural selection and traditional varieties obtained by men selection, obviously, gene transfer could have also happened between them, especially taking into account that exchanging plant material is a common practise among farmers.

In any case, the main differences when compared to commercial varieties are that landraces and traditional varieties do not always have a traceable origin, they exhibit a great diversity and, precisely for that reason, most of their traits are less uniform within them and less stable through the descendants.

# 1.2 Importance and conservation of landraces and traditional varieties in germplasm banks worldwide

The great variability harboured by the landraces and traditional varieties is one of their most outstanding characteristics. Historically, all this richness had been preserved and used (a vicious circle of cause and effect) by the agriculturalists. That situation started to change when the erosion of the plant genetic resources became patent for scientists and breeders, not only in the case of landraces and traditional varieties, but also concerning the wild species. Since then, the germplasm banks have assumed a principal role in safeguarding this plant biodiversity [14]. The strategy has revealed itself so successful that, according to the World Information and Early Warning System (WIEWS) on Plant Genetic Resources for Food and Agriculture (PGRFA), approximately 5.4 million accessions are being conserved in over 710 genebanks from 103 countries and 17 international/regional centres [15]. Landraces and traditional varieties represent the heart of the collections, what becomes obvious when the numbers of the different types of plant resources are consulted. As an example, in Genesys, which is a portal that supplies not only seeds, but also characterisation and evaluation data about PGRFA from germplasm banks around the world [16], landraces and traditional varieties account for the highest proportion of accessions (37%), followed by breeding and research material (27%), advanced and improved cultivars (19%), and finally, wild forms (17%) (Figure 2).



#### Figure 2.

Relative amount of the different types of accessions attending to their biological status (excluding the "not specified" material) hold at Genesys [16], the online platform which harbour information about PGRFA conserved in genebanks worldwide.

The high genetic variability exhibited by landraces and traditional varieties obviously translates into characteristics that could be desirable in modern varieties, particularly those related to their nutritional value and content of health-promoting compounds, which is the subject under discussion in this chapter. In modern breeding programmes, flavour selection has prevailed over nutritional quality. That explains why, for instance, modern lettuce varieties have almost lost their ancestral bitterness. That is a direct consequence of the drastic decrease in the content of sesquiterpene lactones, which are not only responsible for the bitter taste but have also beneficial properties for the plant itself and for the animals that feed on it [17]. In other cases, the main objective has not been to eliminate flavours detrimental to the taste but to enhance the pleasant ones. This is the case for sweet corn. Its sugar content has been escalating over the last decades by the selection of varieties with an increasing polysaccharide content: sugar-enhanced varieties, supersweet or extrasweet varieties, high sugar varieties... [18]. The side effect has been the disappearance of non-sweet dark-grain primitive varieties rich in anthocyanins, which happen to be powerful antioxidants with an important role for health by preventing cardiovascular diseases and by presenting anti-cancer activity [19, 20]. The landraces and traditional varieties were shaped under very different criteria, what does not necessary implies that they are better, for instance, from a nutritional perspective, than any commercial variety, though they contribute to increase the agrodiversity and to enrich the diet. In this sense, the germplasm banks can act as gene reservoir to improve crops, allowing us to dive for valuable characteristics to obtain all types of plant material (coming from crosses between different traditional varieties, between traditional varieties and CWR, between traditional varieties and breeding material ...), using both conventional and biotechnological tools.

#### 2. Essential micronutrients

Essential micronutrients are nutrients that must be obtained in the diet as they cannot be synthesised by the human body. They are required in small quantities and usually consist of vitamins and minerals. Micronutrients play vital roles in human

health, so their deficiencies can be devastating. These deficiencies, also known as hidden hunger, are mainly consequence of micronutrient low concentrations in the daily diet, resulting in malnutrition that is considered an important global problem of public health, especially in developing countries. In addition, the impact is more serious in women of reproductive age (especially pregnant women) and under-five children due to their higher micronutrient requirements. In fact, maternal and child malnutrition or micronutrient deficiencies affect approximately half of the world's population [21]. Nevertheless, hidden hunger also affects developed countries due to low quality food or bad habits, like extreme diets to lose weight or alcohol and drug abuse.

Generally, fruits and vegetables are the sources of vitamins and minerals, but their concentrations in most plant foods are not sufficient to reach the daily dietary requirements, even if the recommended amounts are consumed [22]. Besides, micronutrient content usually depends on the plant genotype, among other factors like environmental conditions, time of harvest, etc. Cases in which landraces and traditional varieties of important crops exhibit higher contents of micronutrients than commercial and modern cultivars are described here. They actually could play a key role in human health by supplying an enhanced nutrition.

#### 2.1 Organic micronutrients: vitamins

Vitamins are a diverse group of organic molecules that are essential in trace quantities for a proper metabolism of all living organisms and are mainly synthesised by plants and microorganisms. Vitamins can be classified into fat-soluble (A, D, E and K) or water-soluble (vitamin B-complex, C and H) compounds. Their main function is to act as cofactors for many enzymes and as natural antioxidants, both in plants and animals. In addition, some vitamins play specific roles, for example, in human vision (vitamin A) [23] or as hormones implied in calcium and phosphorus homeostasis in the blood stream (vitamin D) [24], and many of them are indispensable to prevent chronic diseases [19, 20].

Plants, mostly fruits and vegetables, are the main source of vitamins for humans. However, their concentration in the edible portions of most important crops is usually below the recommended daily intake, which entails important implications for global human health [24]. Interestingly, some landraces exceed these minimal requirements or, at least, they are richer than commercial cultivars in these micronutrients, especially for vitamins A, C and E.

#### 2.1.1 Vitamin A

Vitamin A is a fat-soluble vitamin group that includes retinol and its derivatives, like retinoic acid and retinal, among many others [25]. Besides, among the large group of compounds known as carotenoids, there are some that can act as precursors of vitamin A, known generically as provitamin A, such as  $\alpha$ -carotene,  $\beta$ -cryptoxanthin and  $\beta$ -carotene, the most abundant and nutritionally active within them all. The richest sources of vitamin A are from animal origin, whereas carotenoids are synthesised mainly by plants, but also by some fungi and microorganisms.

Carotenoids play important roles in plant metabolism: acting as pigments in different tissues, mediating plant–animal interaction for pollination or seed dispersal, participating in cell photoprotection against photooxidative damage and heat stress, and protecting membranes from lipid peroxidation thanks to their antioxidant activity [26]. In humans, provitamin A is involved in vision, immune responses, cellular growth, development and reproduction [23]. Vitamin A deficiency is one of the micronutrient deficiencies with more devastating consequences for health. It is the main cause of preventable blindness in children and pregnant women, especially in low-income countries, and raises the risk of suffering several diseases and of dying as a result of severe infections. Between 250,000 and 500,000 vitamin A deficient children become blind every year, half of them dying 12 months later [27]. Therefore, it is a question of the utmost importance to know what plant-based foods contain high levels of provitamin A.

The  $\beta$ -carotene content was measured in two Spanish landraces of tomato (Solanum lycopersicum L.) and in the commercial variety 'Moneymaker' [28]. A higher concentration of this carotenoid was found in green fruits of the two landraces when compared to 'Moneymaker', whereas in ripe fruits, only the landrace Negro Yeste had a higher amount, even more than double. Also in comparison with the commercial variety 'Moneymaker', three tomato landraces, two from Italy and one from Guatemala, showed a significantly higher  $\beta$ -carotene content [29]. In other study carried out in melon (*Cucumis melo* L.), landraces of different origins exhibited the highest levels of  $\beta$ -cryptoxanthin and  $\beta$ -carotene compared with commercial melons [30]. In an analysis of the  $\beta$ -carotene content of mungbean (Vigna radiata L. Wilzeck), the landrace VI000323 B-G happened to have grains significantly richer than two improved mungbean lines at their maturity stage [31]. Though modern wheat (*Triticum* spp.) varieties were not analysed, old varieties (from the 1900–1960 breeding period) were included as reference, and the average values obtained for  $\beta$ -carotene and  $\beta$ -cryptoxanthin were significantly higher in the wheat landraces than in the old cultivars [32]. Also in landraces of pepino (Solanum muricatum Ait.) from the Andean region [33] and in the landrace G-4615 of sweet potato (Ipomoea batatas (L.) Lam.) from Solomon Islands [34], higher contents of  $\beta$ -carotene than in improved varieties have been obtained.

#### 2.1.2 Vitamin C

Vitamin C is a water-soluble vitamin that comprises ascorbic acid (AA), the main biologically active form, and its oxidation product, dehydroascorbic acid (DHAA), easily convertible into AA in the human body [35]. In plants, vitamin C plays relevant roles in metabolic and defence processes, as it is an important anti-oxidant in the ascorbate-glutathione pathway, it protects enzymes with prosthetic metal ions, it is a cofactor for many enzymes (including those involved in cell wall synthesis), it is involved in photosynthesis and respiration, etc. [36].

In humans, it is crucial in some metabolic processes as it participates in collagen formation and inorganic iron absorption, and contributes to a healthy state by reducing the cholesterol levels, preventing chronic diseases and enhancing the immune system by its antioxidant action [37]. The main consequence of vitamin C deficiency is scurvy and, although relatively few people suffer this deficiency, the benefits of the micronutrient are evident, so it is important to find vitamin C-rich plant food.

Some studies have reported a higher content in vitamin C in crop landraces with respect to commercial varieties. For example, 17 Spanish melon traditional varieties were evaluated and most of them had significantly higher values of AA when compared with 10 commercial accessions of reference, in some cases even doubling the AA values of the commercial variety within the same market class (Piel de Sapo, Yellow, Ananás...) [38]. Traditional varieties of lettuce (*Lactuca sativa* L.) from Aragón (Spain) have also been reported to have higher average contents in vitamin C than commercial varieties, especially AA content [39, 40]. Some Spanish

landraces of eggplant (*Solanum melongena* L.) had also a higher concentration of both, AA and DHAA, than commercial hybrids [41]. In other experiment, four to seven traditional varieties of tomato contained higher concentrations of vitamin C than the commercial variety 'Baghera', with significant differences for the traditional varieties CIDA-62 and BGW-004123. In addition, CIDA-62 fruits showed the highest antioxidant activity, whereas the lowest was observed in the commercial variety [42]. Other authors also reported 10 indeterminate tomato landraces that exhibited significantly higher AA contents than the commercial variety 'Moneymaker' [29]. In analyses of the AA content in accessions of garlic (*Allium sativum* L.) from Plugia region (Italy), the six landraces evaluated had a higher content than the commercial cultivar used as reference [43]. Higher contents of total vitamin C have also been obtained in grains of the mungbean landrace VI000323 B-G from Taiwan [31], in the Greek onion (*Allium cepa* L.) landrace Vatikiotiko [44] and in two rare landraces of Italian turnip (*Brassica rapa* L. subspecies *rapa*) [45] when compared with commercial and improved varieties.

#### 2.1.3 Vitamin E

Vitamin E is a fat-soluble vitamin group made up of tocopherols and tocotrienols, a group of lipid-soluble compounds. Both tocopherols and tocotrienols can present four different methylated forms,  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ , and although all of them are antioxidants,  $\alpha$ -tocopherol is the most abundant form and has the highest activity [46].

In plants, the main function of vitamin E is as antioxidant, quenching and scavenging singlet oxygen, controlling the extent of lipid peroxidation, preserving the integrity of the membranes, and protecting against photoinhibition and photo-oxidative stresses [36].

In humans, vitamin E also acts as a potent antioxidant and it is involved in multiple physiological processes, such as regulation of gene expression and cognitive performance. Besides, vitamin E plays a key role in maintaining a healthy state by controlling the inflammation, enhancing the immune function and preventing light-induced pathologies of the skin and eyes, and degenerative disorders like cardiovascular diseases, atherosclerosis and cancer. Its deficiency is common in developing countries and affects mainly children and the elderly, and can cause haemolytic anaemia in premature babies and neurological and ophthalmological disorders as well as myopathy in children. In developed countries it is rare and only appears in some stages of development, such as in premature babies, and specific conditions, like in digestive and genetic pathologies [24].

A total of 28 Korean accessions of soybean (*Glycine max* L.) were evaluated and the highest total tocopherol contents were observed in the seeds of the 7 landraces analysed, especially in HaNagari, in comparison with the modern cultivars developed by cross-breeding, in which paradoxically the content decreased gradually with the year of registration [47]. Furthermore, a strong negative correlation between tocopherol contents and lipid peroxidation was observed (what demonstrates the vitamin E role in oxidative stress tolerance), with the soybean landraces showing the lowest lipid peroxidation. In wheat, higher contents of tocopherols and tocotrienols were obtained for some landraces in comparison with modern cultivars when individual genotypes were analysed [48]. Hazelnut (*Corylus avellana* L.) is also a good source of vitamin E and an Argentinian landrace has been reported to have the highest total tocopherol content in comparison with different commercial cultivars [49]. The total contents of tocopherols and tocotrienols, as well as total vitamin E, were higher in traditional red rice (*Oryza sativa* L.) varieties than in three light brown new-improved varieties [50].

#### 2.2 Mineral micronutrients

Mineral micronutrients are inorganic elements required in small quantities to play vital functions in both, plants and animals. The nutrient classifications are dynamic and, sometimes, even contradictory. That is because, on one hand, the limit between small and big quantities that determine the inclusion of an element in the micronutrient or macronutrient list can result arbitrary. On the other hand, new discoveries about the participation of some elements in important physiological mechanisms cause their transfer from the "nonessential" to the "essential" list. Magnesium (Mg) is a clear example of discrepancies on the first criteria as, depending on the author, is described as micronutrient or macronutrient as ranks in an intermediate position in terms of recommended daily allowances [51]. Regarding the second criteria, some minerals like boron (B) have been known to be essential for plant nutrition for a long time but it has not been until a few decades ago that its important effect on human nutrition was noted [52].

In plants, mineral micronutrients participate in different physiological processes of primary and secondary metabolism, like photosynthesis, electron transfer, activation of enzymes, cell defence, hormone perception, gene regulation... So, mineral deficiencies affect the plant life cycle seriously, causing even plant death in the severest cases [53].

In humans, more than 22 mineral elements (altogether micro- and macronutrients) are essential and they can be obtained with an appropriate diet [51]. Nevertheless, mineral deficiencies are very common, especially in developing countries, and their consequences, such as learning disabilities in children, increased morbidity and mortality, low productivity at work..., are detrimental for humans. Iron (Fe), zinc (Zn) and iodine (I) are the mineral elements most frequently lacking in the diet and their deficiencies, together with vitamin A deficiency, are responsible for about 12% of the deaths among under-five children globally [21]. Fe is important for oxygen transport and haemoglobin formation, and its deficiency is the main cause of preventable iron-deficiency anaemia, poor cognitive development, and maternal and child deaths [54, 55]. Zn plays a central role in growth, development and in the normal functioning of the immune system, so its deficiency hampers growth, alters immunity and also causes diarrhoea among children [56, 57]. Moreover, both deficiencies are also associated with childhood stunting. I is a component of the thyroid hormones and a strong antioxidant. Its deficiency can also cause growth impairments and, in addition, thyroid enlargement (goitre), hypothyroidism, pregnancy loss, infant mortality and cognitive and neuron psychological impairments [58]. On the other hand, manganese (Mn), copper (Cu) or selenium (Se) deficiencies are not a global issue, but they are common in some populations of developing countries, specifically in parts of China, India and Africa [51].

Many landraces of horticultural crops are reported to present higher contents of minerals and oligoelements than commercial varieties. In a study carried out with seeds of Turkish lentils (*Lens culinaris* Medik.), the average values of all the micro-minerals quantified (Cu, Fe, Mn, and Zn) were higher in the landraces than in the commercial cultivars, being Kahmar1 the richest in Zn and Cu, Diykub in Fe, and Kahmar2 in Mn [22]. Also in Turkey, higher contents of Zn and Se have been observed in common bean (*Phaseoulus vulgaris* L.) landraces than in modern varieties, especially in the landrace LR05 [59]. The Greek onion landrace Vatikiotiko [44] and a Greek garlic landrace [60] were both richer in Zn, Mn and Fe than well-established onion cultivars and hybrids commercialised in Greece and a garlic commercial cultivar used as control, respectively. In addition, the mineral content of the onion landrace was even higher than the suggested by USDA (United States Department of Agriculture) for raw onions as a standard reference, especially for

Fe. Results obtained in chickpea (*Cicer arietinum* L.) revealed that landraces from Kyrgyzstan presented higher average values for Fe, Mn, Cu and Zn compared with a breeding line [61]. In Andean landraces of pepino [33], in several eggplant landraces from Spain and Cuba [62], and in landraces of mungbean [31], higher contents in Fe and Zn than in commercial and modern varieties have also been reported.

For cereal crops there are also several studies in which landraces are reported to be richer in mineral micronutrients than commercial varieties. Wheat is one of the most important cereal crops worldwide and there are many studies on wheat landraces. The maximum contents in Fe, Cu, Zn, Mn, and Se were observed in wheat landraces from Canary Islands in comparison with the commercial cultivar 'Vitrón' [63]. Other authors [64] also reported landraces and old cultivars of wheat with a higher average concentration of B, Cu, Fe, and Zn, and of Cu, Fe, Zn, and Mn, respectively, than modern cultivars. Similarly, the average content in Fe, Zn, Mn, Cu, and strontium (Sr) in wheat grain was reported to be significantly higher in 12 Sicilian landraces than in 3 modern cultivars [65]. Other study found seven Afghan wheat landraces with higher content in Fe than reference lines in three different locations [66]. In the case of rice, two Indian landraces showed a higher content in Zn in brown and even polished (considered a poor source of micronutrients) grains than the commercial variety 'BPT 5204' ('Samba Mahsuri'), very appreciated for its high yield and quality [67].

#### 3. Health-promoting phytochemicals

Plant-based foods are rich in different phytochemicals with health-promoting properties for the human body, in spite of not being essential nutrients. Polyphenols and carotenoids are the most important ones among these plant phytochemicals. Unlike micronutrients, their deficiencies in humans are not devastating, but their health benefits are very significant.

#### 3.1 Polyphenols

Phenolic compounds (monophenols and polyphenols) are one of the most abundant and widely distributed groups of chemicals in plants, with more than 8,000 structurally-different compounds currently identified [68]. Particularly, polyphenols are characterised by the presence of aromatic rings with one or more hydroxyl groups and, depending on the basic chemical structure, they are classified in at least 10 different types. However, there is a growing tendency to group them in 2 main categories: flavonoid (flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins) and non-flavonoid (phenolic acids, stilbenes, lignans, xanthones, and tannins) compounds. In plants, polyphenols are involved, on one hand, in crucial biological processes, such as cell division, development, hormonal regulation, reproduction, photosynthesis, pigmentation and pollinator attraction, and, on the other hand, in protection mechanisms against oxidative damage due to radiation or biotic stress (pathogens), among other causes, thanks to their antioxidant properties [69].

Polyphenols seems to be the main contributor to the total antioxidant activities of fruits, with flavonoids being the most abundant in human diets. The health-promoting effects associated with phenolic compounds include the elimination of free radicals, as well as the prevention of chronic diseases, such as cancer, diabetes and cardiovascular and neurodegenerative diseases [68].

There are a number of studies in which different polyphenols are more abundant in horticultural crop landraces than in commercial cultivars. This could be because

some polyphenols contribute to the bitterness and astringency of the food, what could have been negatively selected in modern breeding programmes. Tomato is one of the most important crops worldwide and it is very rich in polyphenols. Several Italian and Spanish landraces have been reported to have higher contents of total phenolic compounds than the commercial varieties 'Brigade' and 'Moneymaker', with significant higher levels of the flavonoids quercetin-3-rutinoside, kaempferol-O-rutinoside and kaempferol-O-glucoside in the case of the Spanish landraces [29, 70]. Nevertheless, polyphenols are abundant in many other crops. For example, different Spanish landraces of eggplant exhibited the highest average and individual contents of total phenolic compounds when compared with several commercial cultivars in two independent studies [41, 62]. Other study found higher levels of chlorogenic acid in three Italian landraces of carrots (Daucus carota L.) in comparison with a commercial cultivar used as reference [71]. Landraces of pepino from the Andean region also exhibited a higher average content of total phenolics than commercial cultivars [33]. Two rare Italian landraces of turnip showed similar concentration of total phenols between them, which was up to a 61% higher than in the commercial genotype also included in the study [45]. An Ecuadorian landrace of sweet potato showed the highest content in two particular anthocyanins (peonidin and cyaniding glucosides) when compared with several improved varieties [34]. Regarding phenolic acids and flavonoids, significant higher contents were observed in landraces of mungbean [9], garlic [43], and apple (Malus domestica Borkh.) [72], in comparison with improved lines and commercial varieties. Finally, in winery by-products from Majorcan landraces of grape (Vitis vinifera L.), the highest values of total anthocyanins, tannins, and total phenolic compounds were observed in the Escursac red landrace, with the commercial variety 'Cabernet Sauvignon' used as reference [73].

In the case of cereals, also some landraces have been reported to be richer in polyphenols than commercial cultivars. In extracts of wheat bread flour, the landrace Biancola showed higher contents of flavonoids and total phenolic compounds than three modern cultivars, as well as higher reducing power and lipid peroxidation inhibition levels [74]. Similarly, the landrace Gentil Rosso had a much higher amount of total, free, and bound polyphenols than three modern and five old cultivars [75]. In extracts of wheat grains, the highest contents of the 13 phenolic compounds identified were found in landraces when compared with commercial cultivars, especially in Tumminia SG3, Tripolino, Scavuzza, and Urria [76]. In maize (Zea mays L.), several Mexican landraces have been reported to have the highest content of phenylpropanoids in comparison with two commercial genotypes, especially Sinaloa 35, which contained exceptionally high levels of diferulates [77]. Also in maize, the Italian landrace Rostato Rosso contained a higher concentration of anthocyanins than an inbred line and a hybrid assayed [78]. Finally, in rice, traditional red-grained varieties of Sri Lanka exhibited significantly stronger antioxidant activity and higher total phenolic content in both, bran and grains, than light brown-grained newly improved varieties, with proanthocyanidins and phenolic acids among the most abundant phenolic compounds identified [50].

#### 3.2 Carotenoids

Carotenoids are the second most abundant natural pigments, behind only chlorophyll, with more than 750 different structures known until now. They are synthesised by photosynthetic organisms (bacteria, algae and plants) and by some non-photosynthetic bacteria and fungi. They can be classified in two main groups: carotenes, composed of carbon and hydrogen atoms, such as  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene, among others; and xanthophylls, that are oxygenated hydrocarbon derivatives,

like lutein, cryptoxanthin, violaxanthin, zeaxanthin, etc. [79]. Carotenoids play key roles in several biological processes in plants. Apart from some of them being vitamin A precursors (as mentioned above), they are also precursors of the plant hormones abscisic acid (ABA) and strigolactones (SLs), they are one of the most important attractants to pollinators thanks to their pigmentation and fragrances (provided by volatile carotenoids), and they also participate in development, photosynthesis, photomorphogenesis and photoprotection processes [26].

The antioxidant potential of carotenoids is very important in human health due to their ability to reduce and, sometimes, prevent the development of various ROS (reactive oxygen species)-mediated disorders, such as cardiovascular diseases, cancer and neurological and photosensitive pathologies [80]. As humans are not able to synthesise these compounds, it is interesting to find crops rich in carotenoids. Vitamin A precursors ( $\alpha$ -carotene,  $\beta$ -carotene and  $\beta$ -cryptoxanthin) have been described previously, so they are not dealt with here. Lycopene is the carotenoid responsible for tomato's red colour and it has been reported to be more abundant in two Spanish traditional varieties of tomato than in the commercial variety 'Baghera' [42]. In addition, one of these traditional varieties showed the strongest antioxidant activity. In two other studies carried out in tomato, not only lycopene, but also lutein content were significantly higher in a Spanish landrace and in three Italian landraces, respectively, than in the commercial variety 'Moneymaker' [28, 29]. Higher levels of lutein were also found in three Italian landraces of carrot, especially in the Tiggiano Yellow-Purple landrace [71], and in the melon landrace Casca de Carvalho [30] in comparison with commercial varieties. Cereal grains are also rich in carotenoids, especially lutein and zeaxanthin [81]. In this sense, several landraces of wheat exhibited higher levels of both compounds than old cultivars used as reference [32]. Finally, higher contents of lutein were also found in kernels of some maize traditional varieties from Italy, especially in Storo, in comparison to the hybrid B73/MO17, used as control [82].

### 4. Applications

As we all know, malnutrition is a public health problem with global dimensions. In 2019, almost 690 million people, 8.9% of the world population, were undernourished, mostly in developing countries. Beside this, about 2 billion people in the world suffered moderate or severe food insecurity, i.e. they did not have regular access to safe, nutritious, and sufficient food that year [83]. Overweight is also a growing matter of concern. In addition, since Green Revolution, the main objective of crop improvement programmes has been yield increase, what has resulted in a nutrient decrease in foodstuffs, contributing to malnutrition. However, quality has started to receive higher priority and agriculture objectives are undergoing changes from yield gains to the production of nutrient-rich food crops in sufficient amounts.

A search for crop landraces and traditional varieties with an enhanced nutritional value could be an interesting approach to combat nutrient deficiencies because, as seeing above, some of them are richer in micronutrients and healthpromoting phytochemicals. However, they do not always cover minimal nutrient requirements and they are usually adapted to local environmental conditions. Therefore, a more feasible measure could be developing nutritionally enhanced foods with an increased bioavailability of nutrients for the human population. These efforts are normally directed toward raising the levels of minerals, vitamins, amino acids, and antioxidant compounds, as well as improving fatty acid composition in the edible portion of crop plants [84]. Crops with a higher nutritional value can be obtained by agronomic practices, conventional plant breeding, and modern biotechnological techniques.

#### 4.1 Fortification

Fortification through agronomic practices or traditional fortification consists of the physical addition of micronutrients to the plants to improve their nutritional quality. It is generally achieved by using mineral fertilisers to increase their content, bioavailability and/or transport from the soil to the edible portion of the plant. Plant growth-promoting soil microorganisms can also be used [85]. This approach is simple and fast but requires regular applications in every crop season, what can increases costs, and also needs supervision in order not to reach toxicity levels, both in the environment and for humans.

One example of this approach is the Se fortification through foliar application in different wheat genotypes [86]. The greater Se accumulations were obtained in the grains of the landrace Timilia and the obsolete variety 'Cappelli' when compared with modern varieties, with an increase of up to 35-fold in mineral grain concentration at the maximum Se application. In another study, fortification with I was carried out in the carrot landrace Carota di Polignano through foliar fertilisation in open field experiments and through both, foliar fertilisation and fertigation with nutrient solution, in greenhouse experiments [87]. In open field, the root content in I increased a 51% and a 194% with low and high levels of the fertiliser, respectively, when compared with untreated carrots, whereas in greenhouse, the I content increased a 9% and only with the fertigation.

#### 4.2 Biofortification

Quite the opposite that the fortification, the biofortification consists of developing crops with a higher nutritional value *per se*, either through conventional breeding or through genetic engineering, without the need of external micronutrient addition. That means that the plants are able to synthesise greater amounts of the particular micronutrients.

Biofortification is a one-time investment and offers a long-term and costeffective approach to prevent malnutrition: once a crop has been biofortified, no more costs, like adding fertilisers to the soil or fortificants to the processed food are needed. In addition, low-income countries could develop biofortified crops through traditional practices, so in theory, low cultivation and production costs are feasible [88]. Reducing the amount of fertilisers required to obtain a more nutritious crop has also unarguable environmental benefits. Nevertheless, biofortification is not the final solution but an additional tool to combat malnutrition.

#### 4.2.1 Biofortification through conventional plant breeding

Biofortification through conventional plant breeding requires crosses between parent lines rich in nutrients and recipient lines that present desirable agronomic traits during several generations. This is a time-consuming method, though sustainable. However, this conventional biofortification relies on genetic variability, which is usually limited in commercial cultivar gene pools, especially of staple crops. Landraces and traditional varieties are an adequate alternative here, thanks to their wide genetic diversity. This approach has been applied to a wide variety of crops, especially since HarvestPlus Challenge Programme was launched in 2003 to develop biofortified staple food crops with enhanced essential micronutrients through plant conventional breeding [89].

Technique	Crop	Landrace or traditional variety	Enhanced trait	Method	Achievement	Reference
Agronomic practices	Wheat	Landrace Timilia; obsolete variety 'Capelli'	Se	Foliar fertilisation	↑ [Se] (up to 35-fold)	[86]
	Carrot	Landrace Carota di Polignano	Ι	Foliar fertilisation	↑ 51% and 194% with low and high levels of fertiliser, respectively	[87]
				Fertigation with nutrient solution	↑9%	
Conventional plant breeding	Rice	Traditional variety Zawa Bonday	Fe	Modern variety ('IR72') × traditional variety	Improved line with ↑ [Fe] (about 21 ppm in brown rice)	[90]
	Rice	Landrace Chittimuthyalu	Zn	Modern variety ('IR64') × landrace	Hybrid with↑ [Zn] (26.9 mg/kg)	[91]
	Maize	Landrace ITA0370005	Carotenoids	Single cross: landrace × landrace (same population)	Hybrid with a ↑ [carotenoid] already commercialised	[92]
	Tomato	Landrace San Marzano	Polyphenols, tannins, flavonoids	Multiple crosses: landrace × landrace (same population)	Hybrid ('Torpedino di Fondi') with ↑ [polyphenols] and ↑ antioxidant activity in pink ripeness stage	[93]
	Eggplant	Nine landraces from Spain (ANS24, ANS26, ANS6, IVIA25, IVIA371, IVIA400, IVIA604, MUS8, VS22, VS9), one from China (ASIS1), and one from Cuba (SUDS5)	Polyphenols, Fe, Zn	Multiple crosses: landrace × landrace (different landraces)	Collection of hybrids with ↑ [phenolic compounds], ↑ [Fe], and ↑ [Zn]	[62]
	Eggplant	Landrace Almagro	Reduced prickliness	Backcrosses: three non- prickly commercial varieties × landrace	Improved pure line (H15) with nutritional properties of Almagro and ↓ prickliness	[94]
Modern biotechnology	Rice	Landrace Krabe	Seed yield	CRISPR-Cas9	Mutants with Krabe nutritional propierties and ↑ seed yield	[95]

**Table 1.**Fortified and biofortified crops through different approaches by using landraces and traditional varieties.

Nevertheless, there is not a large number of studies carried out in landraces (**Table 1**). For example, in the International Rice Research Institute (IRRI) programme, an improved line (IR68144-3B-2-2-3) with a high concentration of Fe in the grain was obtained through a cross between a high-yield variety ('IR72') and a traditional variety (Zawa Bonday) from India. This new variety was reported to have about 80% more Fe than the commercial variety 'IR64' [90]. Useful information have been collected about the Zn content of different mapping populations of rice including wild germplasm, landraces and varieties, as well as hybrids [91]. Using 'IR64' as one of the parents, the hybrid with the highest Zn content (26.9 mg/kg) resulted from a cross with the landrace Chittimuthyalu. A collection of 14 hybrids between different landraces of eggplant has also been characterised [62]. These hybrids exhibited a higher average content of phenolics, as well as Fe and Zn, than commercial varieties. Zn average concentration was also higher in the hybrids than in the landraces tested. A maize hybrid with a high carotenoid content has also been identified [92]. It is a single-cross hybrid developed from the landrace ITA0370005 and it is currently being used by an Italian beer brewer. The metabolite profile and the antioxidant activity of the tomato hybrid Torpedino di Fondi (TF), developed from the landrace San Marzano (SM), has been characterised in two ripening stages, pink and red, both considered ideal for fresh consumption. In comparison with SM, pink TF tomatoes exhibited the highest content of total polyphenols, tannins, and flavonoids besides the greatest antioxidant activity [93]. Within a breeding programme, the eggplant landrace Almagro, known to contain higher values of vitamin C and total phenolics than regular varieties, but also having higher prickle presence, was used as recurrent parent in a backcross, whereas three non-prickly eggplant accessions were used as donors of this desirable trait [94]. Finally, an improved pure line (H15) with the Almagro eggplant ideotype and reduced prickliness was developed.

#### 4.2.2 Biofortification through modern biotechnological techniques

Biofortification can be tackled through the genetic transformation of crops to express desirable genes from a plant species, independently of their taxonomic status, or even from other type of organisms, in the plant of interest to increase their nutrient content and bioavailability. This approach overcomes the limitation of the availability of genetic variability, allows the transfer of several genes simultaneously, and makes possible to biofortify crops with particular nutrients that are not naturally produced by themselves. Biofortification through transgenesis implies large investment of time, resources and researching: it is necessary to identify and characterise gene functions previously, and then, use these genes to transform crops. However, once the crop has been biofortified, it becomes a cost-effective approach [96].

The cisgenesis is a very interesting alternative to the transgenesis. With this approach, only genetic material from either the same species, or close relatives that hybridise naturally with it, is introduced [97]. In this way, the pool of genes available is exactly the same than when classical breeding methods are used. Cisgenic crops are subject to the same regulation than transgenic crops, but the EFSA (European Food Safety Authority) have concluded that cisgenics pose similar risks than plants obtained by conventional breeding [98]. Furthermore, the consumer's acceptance of cisgenics is greater than of transgenics [99].

Furthermore, the application of modern biotechnological techniques to landraces also allows the development of crops with higher yield, as it has been achieved recently [95]. The CRISPR-Cas9 technique was applied to the African rice landrace Kabre, considered a valuable resource, obtaining mutants with significantly improved seed yield and low lodging by disrupting genes known to control seed size and/or yield (**Table 1**).

### 5. Conclusion

In spite of not having been widely used in fortification and biofortification, especially with modern biotechnological approaches, crop landraces and traditional varieties could be key to improve the nutritional quality of food crops, as they can provide the desired genetic variability without sexual incompatibility barriers to overcome. Hopefully, in the near future there could be less restrictive regulations about the use of these biotechnological tools in crop breeding.

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### **Conflict of interest**

The authors declare no conflict of interest.

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