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Vegetable Landraces: The “Gene Banks” for Traditional Farmers and Future Breeding Programs

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Abstract

Crop diversity of vegetable species is threatened by the current homogenization of agricultural production systems due to specialization of plant breeders and increasing globalization in the seed sector. With the onset of modern agriculture, most traditional vegetable cultivars were replaced by highly productive and often genetically uniform commercial breeds and hybrids. This led to the loss of landraces, especially in countries with a super-intensive agriculture. The agricultural biodiversity erosion represents a huge risk for food safety and security. Vegetable landraces are associated with the cultural heritage of their place of origin being adapted to local agro-ecological areas and are more resilient to environmental stress than commercial cultivars. The chapter aim to highlight the importance of keeping and using vegetable landraces as valuable sources of genes for traditional farmers, but also for future breeding processes. We analyze the historical role of landraces, genetic diversity, high physiological adaptability to specific local conditions in association with traditional farming systems, as well as the breeding perspectives and evaluation of genetic diversity based on molecular markers.

Keywords: old local populations, biodiversity, food security, stress tolerance, quality, tomatoes, onion, breeding, molecular markers

1. Introduction

In 1996 World Food Summit stated that "food security is ensured when the entire population has at all times, physical and economic access to sufficient food resources, safe and of high nutritional value, to meet food needs and preferences providing an active and healthy life".

Food security has long been associated with the abundance of cereal products, roots and tubers, vegetables and fruits from the main agricultural crops, which could provide affordable sources of nutritional energy. But this image has changed as the concept of nutritional security has become the essential element of food safety, and nutritional diversity has become the basic component to ensuring the human population health. Healthy diets, qualitatively superior, determine the consumption of a variety of foods in optimal quantities [1].

The vegetables are an affordable and relatively inexpensive source of fiber, vitamins and minerals. In general, they have the highest nutritional value when

are eaten fresh. Unfortunately, a large part of primary (unprocessed) horticultural products have a relatively short life before they begin to degrade. The extent to which the nutritional value of vegetables deteriorates during harvesting, processing and storage depends both on the type of product (species, organ, ripening level) and on the used technologies [2].

Also, the vegetables are recognized as essential for food and nutritional security of humanity. Producing them offers multiple economic opportunities, reducing poverty and unemployment in rural areas especially, and is also an essential component of plant biodiversity maintaining strategies. The systematic production of vegetables for local markets not only provides income for small farmers, but also contributes to strengthening their resilience to external risks. Diversification of vegetable crops, short cycles of growth and development, the use of local, environmentally friendly inputs and the efficient use of fertilizers, pesticides and irrigation can reduce farmers' vulnerability to climate changes. For economic resilience, farmers may choose either to integrate vegetables into existing large crop systems or to focus exclusively on specialized vegetable production.

Vegetable production has increased more than twice in the last 25 years and the economic value generated by their cultivation has exceeded the commercial value of cereals [3].

2. A brief analysis of the production, consumption and trade of vegetables

The global market of vegetables is still predominantly local because only about 5% of vegetables grown worldwide are marketed internationally. However, this percentage continues to increase quite a lot from one year to other. Easy access to a booming global market is essential for export vegetable producing countries, such as Mexico, Spain or The Netherlands. For example, over the past two decades, Mexico has strengthened its leading position of vegetable exports in the North American market and EU domestic trade has continued to grow, particularly on the basis of products from the two European countries mentioned above.

Declared revenues on the global vegetable market were around 1.249.8 billion US\$ in 2018, and their market share increased at an average annual rate of +4.1% between 2007 and 2018. Overall vegetable consumption reached the maximum value in 2018 and is expected to increase continuously between 2020 and 2025 [4].

The quantities of vegetables exported worldwide in 2018 (**Figure 1**), reached a level of about 47 million tonnes, the total volume of exports increasing at an average annual rate of 1.7% between 2007 and 2018. In terms of value, vegetable exports amounted to 42.3 billions US\$. The world's most important exporters were; The Netherlands (6.1 million tonnes), Mexico (5.8 million tonnes), Spain (5.1 million tonnes), China (4.3 million tonnes), France (3.5 million tonnes), Germany (2.7 million tonnes) and the United States (2.4 million tonnes) accounting for about 64% of total vegetable exports in 2018

Vegetables import levels have also had an upward trend over the past decade. Statistical data show that in 2018 the greatest importers was the US with 7.4 million tonnes, followed by Germany (3.8 million tonnes), the Netherlands (3.1 million tonnes) Russia and the United Kingdom (2.2 million tonnes). An interesting trend has been the emergence in recent years of new countries with high requirements on imports of vegetables such as India, China or the United Arab Emirates. Russia has also seen an increase in trade, despite the imposition of economic sanctions on imports since 2014. The main countries providing vegetables to Russia are Belarus, Morocco, China, Armenia and Azerbaijan [4].

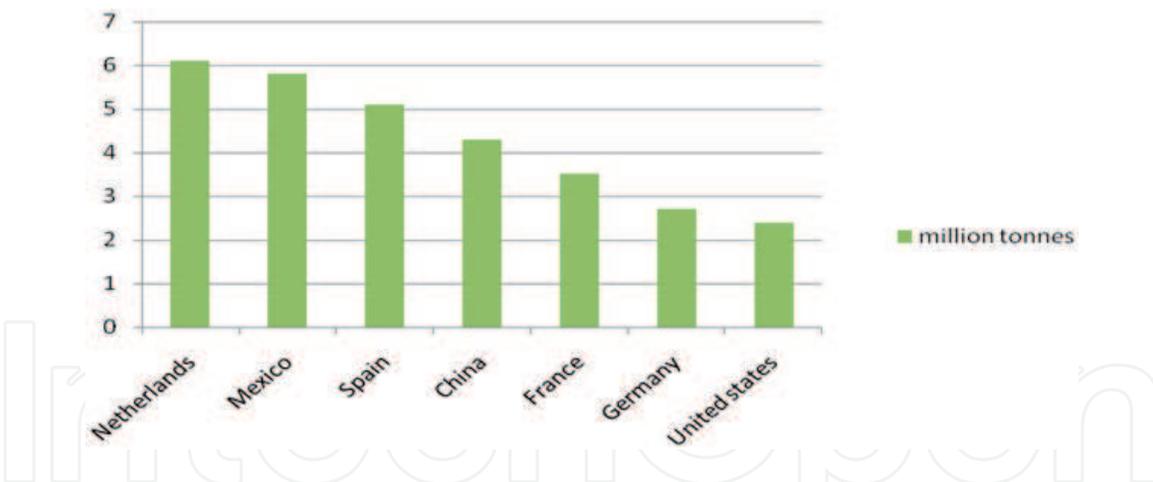


Figure 1. The main global exporters of vegetables, and the volume of their exports for 2018. Processed by; World - Vegetable - Market Analysis, Forecast, Size, Trends and Insights (researchandmarkets.com).

2.1 Fresh or conserved vegetables?

It is estimated that 70% of vegetables grown around the world are sold fresh and whole as primary (unprocessed) horticultural products. Processing of vegetables by preserving, freezing and drying is the main purpose of storage technologies, the possibility of long-distance transport, long lasting storage and the reduction of damage losses. However, the global consumption of preserved vegetables has decreased over the past decade, which attests to consumers' preferences for fresh vegetables against the background of reduced time from harvest to market (concept from field to fork). Has increased however the demand for frozen vegetables over the past decade by an average of about 1% annually [5].

2.2 The vegetables and chain food waste

Due to the relatively high level of perishability, primary horticultural products are exposed to loss in a significant percentage. With 1 in 8 people on Earth starving (about 759 million people), the loss of vegetables and fruits is a component with major social effects. According to the FAO, about 14% of globally produced foods are lost between harvest and retail trade, with significant quantities also being wasted at the retail and consumption level. The value is higher in the case of fruit and vegetables where losses range from 20 to 40 % [6]. Analysis of the data presented shows that significant losses of fresh vegetables and fruits occur in the production process (Europe, North America, Oceania and Latin America), in processing (Africa, South Asia and South-East) and to the final consumer (Europe, North America and Industrialized Asia).

Recent studies have shown that in European Union around 7.2 million tonnes of fruits and vegetables are discarded annually, which is the equivalent of 14.2 kg/person/year. Of this quantity, avoidable waste (edible parts) accounted for almost half, and the inevitable waste (shells, seeds, stalk, etc.) was the difference [7–9]. These wastes, if are not properly treated, pose major environmental hazards because their decomposition eliminates an important quantity of various greenhouse gases [10].

Therefore, the reducing of food waste is the main way to close the gap between food supply and demand [11]. On the basis of this argument, one of the specific targets of the UN Sustainable Development Goals is to halve food losses along the production and supply chain by 2030 (Objective 12.3) [12]. The European Commission is committed to respect the objective 12.3. and considers food waste as a priority area in its Circular Economy Action Plan [13]. Moreover, to underline the

importance of reducing food loss, the UN declared 29 September as "International Day of Food Lost and Waste".

2.3 Ecological and organic vegetables, increasingly sought after in rich societies

The global market share of organic foods is growing from year to year. The share of trade in organic and ecologic fruit and vegetables (out of the total trade in fresh fruit and vegetables) has increased by around 10% in some European countries with high standards of living such as; Switzerland, Sweden, Austria and Denmark. In the United States, this rate is around 9%, but there has been recorded intense growth rates in the last years. Although, income per capita appears to be a determining factor in the consumption of these products, this is not the only one. The consumer education level, supermarket policies on the category of organic vegetables, the price and availability of conventional or traditional products, cultural factors, etc. can be important vectors that influence the consumption of organic and ecologic vegetables products [5].

2.4 Seed vegetables market

Vegetable quality assurance is achieved by a succession of attributes related to biological material and cultivation technologies, harvesting, conditioning, processing, storage and marketing. Seed quality is the basic appropriation that characterizes the biological material. The demand of growers for quality seeds is increasing. The world market for vegetable seeds accounts for about 11% of the total plant seed market. The estimated value of the vegetable seed market in 2017 was 8.02 billion US\$, reaching 12.6 billion US\$ by 2021, with a cumulative annual rate of 8.1 [3].

3. Vegetable genetic resources and biodiversity preservation

In general, plant genetic resources are defined as that part of biodiversity used to generate productivity and quality in agriculture. In addition to commercial genotypes (varieties and hybrids), the genetic resources of a cultivated species include breeding lines, genetic forms obtained by various technologies by deliberate breeding (natural or induced mutant lines, substitution and addition lines, inter-specific hybrids, etc.), wild descendants, related species and local races, also referred to as 'farmers, local or primitive varieties' [14].

Plant Genetic Resources (PGR's) represents an important component of the conservation of plant biodiversity and the food security of the human population [15]. PGRs are actually the expression of natural variability in plants, variability that has sustained the human species for millennia. The multitude of plant species, with all existing genotypes, are especially important for ensuring food security, but also because they represent energy sources, medicines, animal feed, fiber, ecosystem services, etc. All these aspects are essential in the context of the global challenges currently facing life on Earth, in particular due to climate change and resource shortages. In the light of this, the efficient conservation and sustainable use of the PGR's is extremely important and has never been more necessary [16].

Thus, according to The Second Report on the State of the World's Plant Genetic Resources [17], approximately 7.4 million genotypes, sources of germplasm, belong to over 16,500 species of plants are currently stored in 1750 gene banks and collections around the world.

Vegetable genetic resources (VGR's) are the foundation on which vegetable cultivation techniques and food chains integrated with them have been developed, and the genetic diversity present in small farms and germplasm collections is

essential in efforts to eradicate hunger and poverty. They are the main gene reservoir for the production of new vegetables cultivars and the main supplier of genetic diversity [18]. Therefore, plant genetic resources offer a huge diversity and variability, widely used in genetic studies and plant breeding programs, with undeniable benefits for global food production [19, 20];

Vegetable genetic resources (VGR's) are used both by traditional farmers to obtain safe and quality production and by researchers as the initial biological material for obtaining new cultivars. The genetic resources are also a reservoir of biodiversity that acts as an element of balancing sudden economic and environmental changes. Recent studies have shown that the main factor in the erosion of PGR's and biodiversity loss is the replacement in cultivation of local genotypes (old varieties, local populations) with modern cultivars [21].

Unfortunately, VGR's natural pools are strongly affected by the modern society activities – urbanization, habitat degradation through intensive exploitation, deforestation and arson, increased pressure from diseases and pests, to name just some of these activities.

Modern industrial agriculture based on improved hybrids and cultivars limited and marginalized the use of landraces, causing a serious loss of genetic variability. The high genetic erosion of vegetable landraces was highlighted by Hammer and Laghetti [22], who found that from 1950 till 1986 in Southern Italy only 27.2% of the landraces were still grown. Also, Dias [23] reported that, during the last 50-60 years the genetic diversity of vegetables has been severely eroded all over the world, so that the vegetable genetic resources are disappearing yearly on a global scale with a rate of 1.5-2.0%. This genetic erosion represents an alarm signal for the breeding activities in order to streamline the vegetable production under stressful environments [24].

As genetic erosion continues "in situ" and on farms due to the reasons already mentioned and climate change as well as by replacing old local varieties with improved, super-productive genotypes, it is necessary to intensify the efforts of collection, characterization and conservation with a major focus on the wild relatives of cultivated plants and on the breeds of vegetables poorly represented by the major and minor groups of this class. The conservation of the diversity of local and underutilized plant crops should also be given greater attention [25].

4. Landraces – definition and their importance in traditional farms and breeding programs

Widely used in the literature, the term "landrace" encompasses different concepts, variable in time and space, depending on trends prevalent in the use and conservation of genetic resources. After a period of beginning when the issue of preserving and maintaining biodiversity was prevalent, today the commercial message is clear and promotes the higher nutritional and sensorial qualities of local vegetable landraces [26]. Due to their complex nature and huge diversity landraces are extremely difficult to be characterized by an all-encompassing definition (Figure 2).

However, over time, different authors have tried to define landraces on the basis of the characterization of their main attributes. Kiessling [27] in 1912 defined landraces as a mixture of shapes (phenotypes) with a certain degree of external uniformity, specific composition and a high adaptability to the natural, technical and economic conditions of the region of origin [28].

An interesting definition has been proposed by Prospéri et al. [29] in 1994 which attest that a landrace represents a set of genotypes belonging to the same species,



Figure 2.
Vegetable landraces diversity.

that a grower in a given region, uses specific cultivation methods and carries out mass selection, more or less targeted, over several generations.

Zeven [28] said that a "landrace" is a variety with high tolerance to biotic and abiotic stressors, manifested by medium but stable productive yield, under low technological inputs conditions. Landraces have also been defined as dynamic populations of a cultivated plant of distinct historical origin and identity, with genetic variability and high adaptability to specific local conditions (soil, climate, biotic stressors) adapted to cultivation technologies specific to local farmers [30].

Vegetable landraces are considered local old varieties with distinctive characteristics resulting from archaic selection and adaptation over time to pedo-climatic conditions specific to a localized geographical region, which usually exhibit greater genetic diversity than the types subjected to the usual breeding techniques. According to the definition developed by Dwivedi et al. [24] landraces represent heterogeneous, local adaptations of some cultivated species and therefore provide genetic resources adapted to the current challenges posed by biotic and abiotic stress factors.

The analysis of these definitions attests to the existence of some common elements in the characterization of landraces in cultivated plants such as; local character, historical origin, adaptability to soil, climate and stress factors, genetic variability, harvest stability, reduced inputs, traditional farms. Landraces through their long selection process by farmers during the pre-intensive agricultural period provide a great opportunity to find appropriate combinations of genes and phenotypes tolerant to complex situations [31].

In conclusion, landraces are dynamic populations usually associated with traditional farming systems. As such, their evolution was based on both natural and farmers' selection in low-input cultivation systems [32]. During long period of cultivation, farmers greatly contributed to the diversification of vegetable crops by selecting populations with moderate yield and well adapted to the specific agro-climatic conditions of different regions. The diversity of landraces is usually lower than at their wild ancestors, but considerably higher than at modern cultivars produced by plant breeding [33]. The vegetable landraces are valuable genetic resources to identify genes for increasing yield and adaptation to abiotic stress under the current and future climate changes [34].

Compared with modern varieties, the vegetable landraces have a low presence on the market, due to their lower yields, disease sensitivity, and poorer postharvest shelf life [35]. In the last period, amid an increasing interest of the consumers for

traditional and healthy products of the local growers, the landraces are reconsidered both as a source of food and as a source of useful genes [36, 37].

5. Breeding perspectives of vegetable landraces

The breeding of plants is as old as their cultivation. The first vegetable growers exploited the favourable variability of landraces of the main attributes such as productivity and high tolerance to environmental stress factors. Much later, probably after a few millennia, mankind developed new methods of breeding and multiplication, including hybridization techniques, and the peak was reached through the use of molecular tools, all of which led to the creation of modern vegetable genotypes with high yielding performance characters [38].

Therefore, an important source of genes that is increasingly used in breeding programs are landraces, old varieties adapted to the conditions of a specific pedoclimatic area [39]. Due to the stronger genetic proximity to modern varieties than their wild relatives, landraces show huge potential to improve modern genotypes by increasing stress tolerance and as sources of healthy and nutritive food [20, 40–43].

Featuring by a good stress tolerance and high adaptability to different conditions, despite the lack of pathogen tolerance genes, vegetable landraces are still a reservoir of genetic diversity, in particular for certain attributes of interest, such as; tolerance to abiotic stress and high fruit quality [44]. For these reasons, studies carried out on some heterogeneous tomato populations have shown that they have been, are and will continue to represent very important genetic resources used in breeding processes [28]. The genetic profiles of landraces are clearly different from those of modern genotypes [45]. It has been observed that numerous morpho-anatomical, physiological and biochemical traits record significant levels of phenotypic and genotypic diversity [46]. However, information on the variation within vegetable landraces is still limited.

The antioxidant content of the edible organs of wild vegetable species is significantly different from landraces. These compositions have been associated with the features of the organs, the geographical origin and altitude at which they are found. For example, in high-rise areas of northwestern Argentina, local tomato populations with the highest concentration of antioxidants have been identified [47].

Recovering and rendering these qualities in adapted landraces to the original communities will contribute to the sustainable maintenance of these varieties [48, 49]. For example, tomato landraces are characterized by excellent fruit quality, high content in metabolites [50], antioxidants [20, 47] and volatile organic compounds [51]. Landraces and old varieties have a typical flavour that consumers appreciate and demand, although the availability of their seeds is increasingly low [52].

The vegetable landraces are particularly important because they exhibit high heterogeneity (for improvement), are adapted to biotic and abiotic stress conditions, have excellent taste qualities, thus justifying a higher recovery price than commercial varieties [53].

One strategy to highlight the genetic treasure represented by the landraces is to identify the size of genetic variability for primary and secondary metabolites and to establish existing links between biochemical composition of edible products, genetic basis and consumer preferences [54]. Studies from last decade [20, 55, 56] showed that in Romania it still exists many vegetable landraces that need to be preserved and evaluated for further use in breeding programs.

5.1 Case studies: Romanian landraces of tomatoes (*Solanum lycopersicum*) and onion (*Allium cepa*) gene source for breeding programs

5.1.1 Tomatoes landraces

In order to obtain appropriate tomato yield under environmental stress conditions, the plants must show tolerance during the developmental stages from seed germination to flowering and fruit maturity [57]. Characterized by a good adaptability and stress tolerance amid a lack of diseases resistance genes, the landraces still represent an important reservoir of genetic diversity especially for traits associated with abiotic stress resistance and fruit quality [58].

The genetic structure of tomato landraces is quite different from those of modern tomato cultivars [32, 42, 59, 60], while the morphological variation of tomato landraces is higher compared to cultivars [61]. The heterogeneous structure of landraces was highlighted by Terzopoulos and Bebeli [32] who found a wide intra-population phenotypic diversity at 34 Greek tomato landraces for 33 morphological traits except for stem pubescence and foliage density, or plant growth type, respectively. Also, Manzano et al. [62] found a wide phenotypic diversity among 39 Spanish tomato landraces both in terms of morphological traits and postharvest quality of fruits, under organic greenhouse conditions. Analyzing the diversity between 75 landraces and 25 tomato varieties from Southern Italy, Corrado et al [58] revealed that the genetic structures of the landraces were mainly related with the fruit traits

The intra- and inter-populations variability may occur even in case of landraces from a small area, for morphological, agronomical and quality traits [63]. Based on farmer's activities, different selections of the same landrace can be made. These populations will evolve in different environmental conditions thus contributing to phenotypic diversity of tomato landraces [64, 65]. The diversity/variability between tomato landraces could be attributed both to genetic background and environmental conditions where these genotypes were evolved [66]. The analysis of landraces genetic variability will be useful for a better understanding of fruit shape and size and can help to identify valuable alleles for improving productivity, adaptation and quality [67–69].

Even, during the last decades the tomato landraces were replaced by new cultivars, in different regions of Romania these landraces are still cultivated for local consumption and market. They have especially distinctive morphological and quality traits of the fruits, considering that the fruits quality is highly appreciated by local consumers.

Within the project S-Stress 82 tomato landraces from two regions of Romania were evaluated using ISSR markers in order to establish the degree of similarity between them. The literature data show that this category of markers could be successfully used for evaluation of tomato variability.

The genetic variability was evaluated based on amplification with 8 ISSR markers namely: UBC 808 – (AG)₈C, UBC810 - (GA)₈T, UBC811- (GA)₈C, UBC840- (GA)₈YT, UBC841- (GA)₈YC, UBC843- (CT)₈RA, UBC884- HBH(AG)₇, UBC886- VDV(CT)₇, where Y = C or T, R = G or A, H = non G, B = non A, D = non C and V = non T.

In the case of primers such as UBC843, molecular fingerprints revealed major differences between the analyzed populations, while other markers, such as UBC 840, generated very similar fingerprints (**Figure 3**).

The results indicated the existence of a wide diversity, both between landraces from the two regions and from the same region, arguing the wide genetic basis of these landraces (**Figure 4**). Based on these results, combined with the analysis of

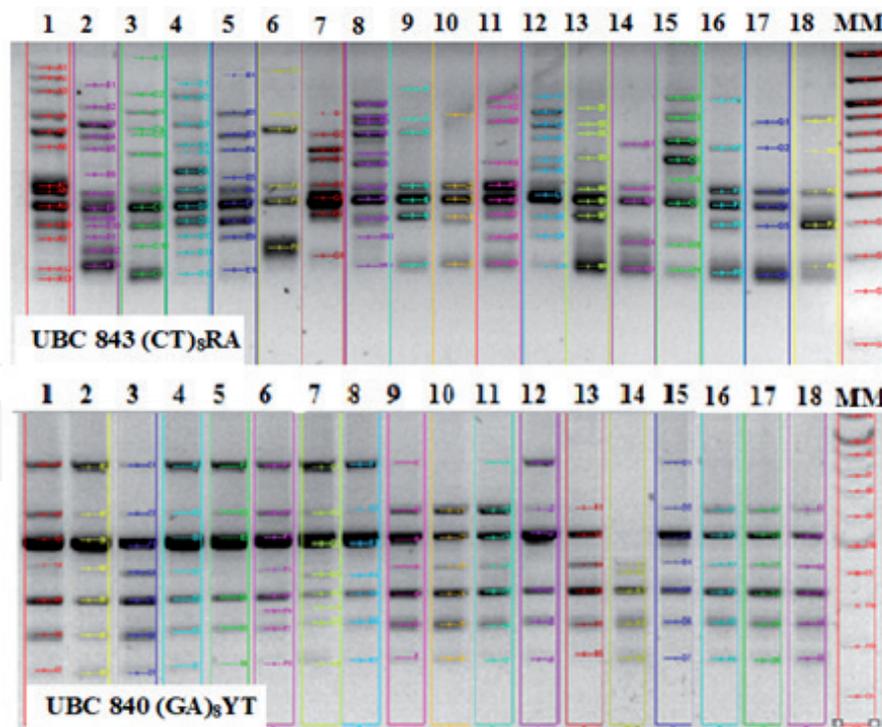


Figure 3.
Analysis of amplification products for UBC843 and UBC840 primers.

fruit traits, divergent landraces were crossed together and finally five commercial hybrids were homologated.

Given that the fruit traits were the main selection criteria used by the farmers during the evolution of tomato landraces, the maintenance of some landraces in a specific ecological region was mainly due to social factor, thus influencing the diversity of tomato landraces from different regions [70].

The landraces with wild specific characteristics like; high number of branches and fruits per plants, lower values of fruit weight and small pericarp thickness, exhibit a better disease resistance [71]. In this regard, the modern cultivars for fresh market are characterized by large and round fruits with suitable firmness and shelf-life, amid uniformity of size, shape and colour of the fruits [72]. After a comparative study of tomato landraces and advanced lines, Carrillo-Rodriguez et al. [73] suggests that it is possible to select tomato landraces with healthy plants and similar performance to that of advanced breeding lines.

Amid the increasing of consumer's interest in fruit quality, landraces with fruits appreciated for flavour and aroma should be considered both for production and for breeding activities. Crossings among varieties and landraces or among landraces can provide a useful variability for different plant and fruit traits [46, 65, 74]. Studying the Mexican tomato landraces Martinez-Vazquez et al. [75] found crosses derived between landraces and commercial lines with values of important traits like firmness, yield and fruit size, close to a commercial hybrid. As such, tomato landraces are a valuable source to obtain breeding lines with high general combining ability, possessing important alleles for yield traits, suitable to be used in breeding programs.

Considering that the landraces are genetically closer to modern cultivars than to their wild relatives, they represent an important source of genes for improvement of adaptation to abiotic stress [43]. In this regard, Massaretto et al. [76] highlighted the potential of tomato landraces from Southeast of Spain to improve the fruit quality and also to maintain the yield stability under salt stress conditions. Studying tomato landraces from Romanian areas with medium and high levels of soil salinity,

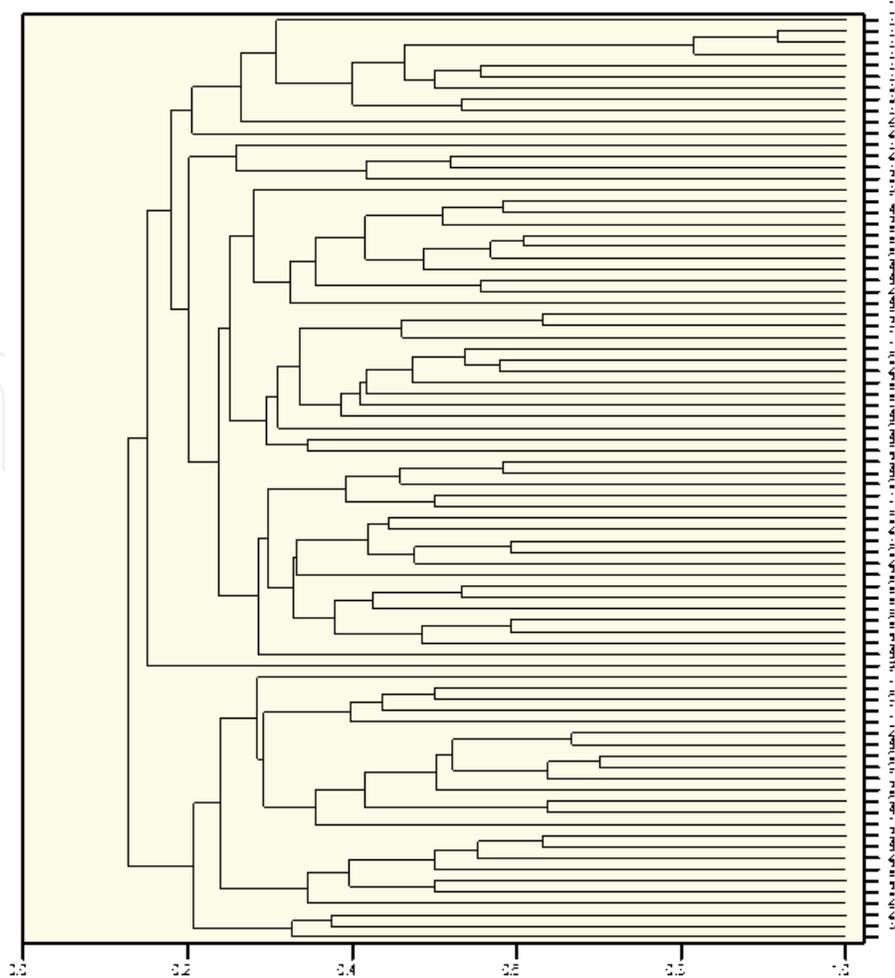


Figure 4. UPGMA clustering of 82 tomato landraces using ISSR markers (Landraces 1 to 70 from S-W Romania; landraces 71 to 82 from N-E of Romania).

Sumalan et al. [20] found that landraces with tolerance to soil salinity have a high ability to accumulate large amounts of antioxidants in the ripe fruits, increasing their nutraceutical value. Taking into account that the growing conditions have a high influence on plant morphology, chemical composition of the fruits and agronomic performances, Figas et al [77] suggest that long-shell life landraces from Mediterranean basin could be a useful material for improvement of tomato adaptation to greenhouse cultivation, or to predicted climate change conditions, especially drought [78].

Breeding of tomato focused on yield led to a loss of genetic diversity and a decrease of nutritional value and disease resistance [79]. Under a low diversity and a narrow genetic base of disease resistance, the cultivation of tomato becomes vulnerable and dependent to widespread use of pesticides [80]. Given that the preservation of tomato landraces is influenced by both natural and human selection, these populations can be considered a suitable breeding material for the identification of genes with supposed adaptive value [81].

5.1.2 Onion landraces

Due to the replacement of landraces and old varieties with modern varieties and in particular F1 hybrids the genetic basis of onion has been considerably reduced, so that many genes with adaptive value contained in the landraces and old varieties are in danger of being lost [82].

The success of onion breeding programs, among others depends mainly on the availability of genetic variability for different traits of interest. The use of wild *Allium* species for genetic improvement of cultivated varieties is a very long-term process that can take up to 20 years [83]. As such the onion landraces are a more suitable material for breeding of adaptive traits like bulbing and flowering, controlled by multiple genes [84–87].

For an effective use of onion landraces it is necessary to characterize and evaluate these germplasm at both molecular and at morphological level. In this regard et al. [82] found a 69% diversity between 85 Spanish onion landraces based of pungency, day length requirements, and skin colour, without being established a relation among the diversity at molecular and at morphological or physico-chemical level. Similar results have been reported by other studies: Hanci and Gökçe [88] for Turkish onions; Mitrová et al. [89] for Czech onions; González-Pérez et al. [90] for Galician onions. The landraces possessing high genetic diversity have an important selection potential for the development of new onion cultivars with favorable yield, adaptive and quality traits.

Likewise, the molecular diversity of Indian onions studied by Khar et al [91] was not related with colour, growing season and geographical origin. The exchange among farmers from different regions could be an explanation for the lack of relation between clustering of landraces and their geographical origin.

Following the molecular evaluation of 43 onion landraces from two regions of Romania using ISSR markers within the S-Stress project, a high level of diversity (around 80 %) was found, associated with a clear separation of the landraces in two clusters, related with their geographical origin (**Figure 5**). Amid a lack o biological material exchange between two regions, it is assumed that the landraces have had a distinct evolution under the influence of local ecological conditions. As such, these onion landraces are important sources of genetic diversity, containing valuable genes for different yield and adaptive traits under salt stress conditions.

High levels of heterozigosity associated with low allele number reported by several studies [90, 92–94] represents a consequence of out-crossing and continuous

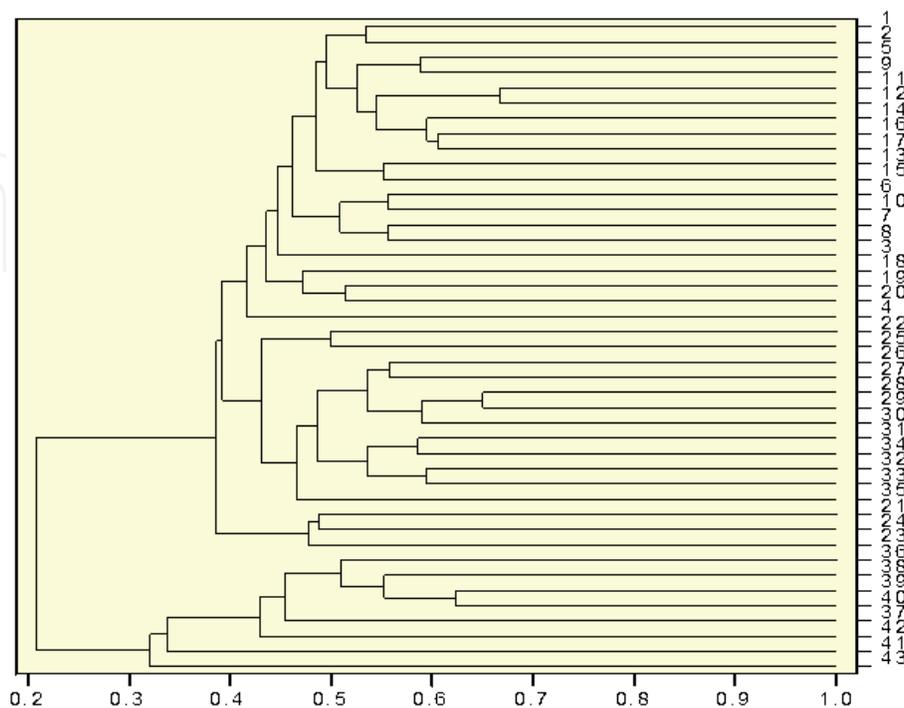


Figure 5. UPGMA clustering of 43 onion landraces using ISSR markers (Landraces 1 to 35 from S-W Romania; landraces 36 to 43 from N-E of Romania).

gene flow in small geographical regions where the onion landraces have evolved. In order to capitalize the genetic variation of onion landraces in breeding programs, it is necessary to ensure a certain degree of out-crossing on the selected genotypes [95, 96]. The breeding potential of onion landraces was also revealed by Porta et al [97], who found transgressive segregation for different bulb traits in selfing (S1) lines, compared to original population. The high variance within and among S1 lines for all traits, confirm the heterogeneous structure of landraces and efficiency of their use as a selection material.

6. The evaluation of the tomatoes landraces genetic diversity based on molecular markers

A representative of the horticultural plants studied in our research were tomatoes landraces, due to their importance as food in Romania and because it is one of the first crop assessed by molecular markers for variability evaluation. The genetic study of local landraces is based on the evaluation of their genetic variability to determine the degree of similarity. Next, it is necessary to correlate the molecular fingerprints with the phenotypic traits in order to identify genotypes of interest for plant breeding.

Over the time, the variability was evaluated with morphological markers followed by biochemical ones, developed on the basis of isoenzymes. The biochemical markers had a major disadvantage because they are affected by the phenological development stage, being possible to detect a percentage of only 0.1% of the variability. For this reason, the DNA markers have gained increasing importance and have been used on a very large scale today. They can be classified according to the type of analyzed sequence and the applied methods of analysis which both determine their genetic behaviour, i.e. their codominant or dominant character.

The codominant markers, such as RFLP (Restriction Fragment Length Polymorphism), STS (Sequence tagged site), EST (Expressed Sequence Tag) and SSR (Single Sequence Repeats), are an important source of information because they allow the differentiation of homozygotes and heterozygotes being co-dominants, but each category also has a number of disadvantages.

Considering that the microsatellite markers have shown to be promising to evaluate the genetic diversity, Bredemeijer et al [98] constructed database comprising information about more than 500 tomato varieties cultivated in Europe evaluated with 20 SSR markers. The obtained results showed a relatively reduced variability of the studied tomato genotypes, with the average of allele per locus of 4.7, ranging between 2 and 8. Besides, the same test was performed in five different laboratories to emphasize the robustness of the marker system. It was concluded that the use of this set of 20 SSR markers lead to suitable results when homogeneous varieties were studied, but in the case of heterogeneous genotypes it is necessary to analyze a mixed DNA sample from 6 different individuals [98].

When Spanish landraces were analyzed, it was possible to differentiate cultivars only with a small number of SSR markers, even if they were phenotypic different, emphasizing a low level of variation within this species [99].

In an Italian study 50 tomato landraces originated from central of the country and other vintage and modern cultivars were analyzed with 29 SSR markers. The molecular data were associated with the study of 15 morpho-physiological traits. Two categories of markers were used – the markers from the first category were part of a linkage area where QTLs previously associated with the shape and size of the fruits were positioned and in the second category were markers from some

chromosomal regions without any known linkage. Besides, DNA samples collected from plants grown in two different locations were analyzed. It was pointed out a high polymorphism of the tomato landraces compared to modern cultivars and many relations between the markers from the QTL region and the traits associated with fruit shape and size. These results are promising for the identification of SSR markers associated with traits of agronomic interest [100].

Later, 42 tomato varieties originated from different regions from China and Kenya were evaluated with SSR markers, emphasizing a high degree of diversity. The results analysis distributed the genotypes in different clusters without any relation with their origin [101]. In other study Italian local landraces were analyzed with 19 SSR markers generating a number of 60 alleles with moderate level of diversity but very different compared to the commercial varieties [102].

It was pointed out that the SSR markers could be used for the evaluation of tomato landraces variability, but it must be considered that their development is expensive and time consuming, therefore may be the markers which generated a high amount of data in only one analysis could be more efficient.

In 2000, species of wild tomato relatives originated from Peru (named PC – Peruvian complex) were evaluated with RAPD markers in comparison with cultured genotypes. A high diversity was shown, emphasizing the potential of the wild genotypes to be used as a source of genes for breeding [103].

In India, based on the molecular fingerprints generated by RAPD markers, the reduction of genetic diversity for tomato cultivars has been highlighted. This has been attributed to breeding processes that target plants with very similar traits [104].

The evaluation of the Brazilian tomato landraces based on RAPD primers showed that most of them were part of a single cluster, different from the commercial cultivars [45]. Similar results were obtained when tomato landraces originated from Azerbaijan were analyzed [105].

ISSR markers were used to evaluate the genetic variability for 100 Brazilian tomato genotypes of different origin. Finally, a correlation between the fingerprints generated by ISSR markers and the origin of the genotypes was established [106].

In 2016, landraces originated from East Anatolian region of Turkey and North-West of Iran, along with three commercial cultivars were evaluated with ISSR markers. It turned out that the genotypes originating from the same region, often located in the same group or two adjacent groups [107].

The same markers were used to evaluate tomato genotypes with different antioxidant content. The obtained fingerprints were used to confirm the nature of the hybrids in breeding programs, thus accelerating the selection process [108].

The AFLP markers (Amplified Polymorphic DNA) were used in conjunction with SSR markers to characterize 48 traditional tomato cultivars collected from the south-east of Spain. The discrimination power was similar for both category of markers and the constructed dendrograms were grouped in the main types. The conclusion was that it would be more appropriate to use in combination the information obtained with several categories of markers [59].

In the early 2000's SRAP markers (Sequence-Related Amplified Polymorphism) were developed as a technique with low cost, simple, highly variable, with high reproducibility [109], based on a random amplification reaction. Considering that 3' UTR region is usually polymorphic due to insertions and deletions the probability to identify polymorphism random in the coding regions is high. This marker had a widely use for diversity evaluation for different plant species.

Ruiz et al [99] studied the diversity of some traditional tomato cultivar from Spain based on SSR and SRAP markers. It was pointed out that SRAP markers

clustered together the genotypes with the same origin. Comparable results were observed when SSR markers were used, but the level of resolution was lower [99].

Al Shaye et al [110] evaluated Saudi tomato landraces with SDS-PAGE and SRAP markers. It was shown that almost all of the landraces with the same origin were grouped in the same cluster emphasizing the usefulness of these markers in future breeding programs [110].

Similar to SRAP markers, which bind in the coding gene region, ScoT markers (Start Codon Targeted) involve the amplification with a single primer that anneal to the highly conserved region positioned next to start codon ATG of two close genes [111]. The ScoT primers were used in comparison with the ISSR to evaluate the variability for 8 Egyptian tomato genotypes. The genetic fingerprints were different for the two categories of markers and it was considered that ScoT ones were more related to the morphological traits compared to ISSR for evaluation of tomato diversity. Therefore, the use of more than one marker system is recommended for a higher resolution of the analysis [112]. Following the introduction of modern analytical techniques, they have also been applied in the area of diversity assessment.

Therefore, the sequencing system Illumina was used for evaluation of 75 landraces originated from Sothern Italy and distinguished a number of 152 single nucleotide polymorphisms (SNP). 30% variability was identified between local populations, the differences being associated especially with fruit-related traits. The developed SNP system was considered to be very useful for genetic characterization, effective conservation and application on tomato breeding process [58].

A complex research had been done in Italy to investigate 123 tomato genotypes originated from all over the world. A very wide range of genotypes has been analyzed in order to succeed in the polymorphism identification and its correlation with different 18 morphological traits, mainly related to fruits. A tomato array was used and a number of almost 8000 SNP were analyzed. The results showed that 36 of the SNP markers were correlated with 15 of the studied traits. These markers were mapped on chromosomes along with a number of 98 candidate genes as follows: 19 SNPs were located in six chromosomal regions in which candidate genes are positioned, and 17 SNPs in regions where no such genes are found. Thus, it can be stated that chromosomal regions have been identified where unknown genes related to the traits are positioned. Thus, new research lines are opened to identify genes of interest [61].

In the following years, considering the development of the SNP analysis system, point mutations associated with organoleptic characters and metabolites content were identified [113] and mutations in genes involved in drought tolerant and fruit maturation and quality [114].

Besides SNP identification, the whole genome sequencing was also applied to identify genes of interest involved in tolerance to drought, good quality and storage proprieties. Therefore, the whole genome of two landraces with the mentioned traits was sequenced. In their genome regions similar to *Solanum pimpinellifolium* and *S. pennellii* and candidate genes for the interest traits were identified [115].

Therefore, it can be said that over time several molecular marker systems have been used to assess variability in local tomato landraces. But it has rarely been possible to correlate with the phenotype, i.e. the genes determine certain characters. But these molecular markers have shown their importance in screening populations to determine the degree of similarity or to remove identical genotypes from the study and from the conservation. Instead, the development of SNP markers and sequencing of the entire genome is expected to be a strategy that will underpin the identification of all genes of interest in both biological and agricultural areas.

7. Conclusions

Vegetable landraces constitute a valuable genetic pool of genetic diversity, which can be exploited both in breeding programs for obtaining new commercial genotypes with targeted traits and as a valuable source of germplasm for traditional farmers.

Tomatoes are the most important vegetable with fruits and many landraces are preserved around the world as local varieties or farm varieties. Variability of chemical composition, plant morphology and agronomic performance have shown that cultivation technology has a major impact on the shelflife of tomato fruits.

The conservation of vegetable landraces is associated with their cultural value, geographical isolation of sites, aesthetic and organoleptic preferences of consumers and traditional farmers.

There is an optimistic outlook on harnessing landraces and traditional vegetable varieties in a quality-oriented sustainable horticultural system.

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