Journal of Berry Research 8 (2018) 205–221
DOI:10.3233/JBR-180314
IOS Press

Status of strawberry breeding programs and cultivation systems in Europe and the rest of the world

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Received 31 May 2018; accepted 18 June 2018

Abstract. Strawberry cultivation is constantly increasing all over the world thanks to the varietal innovations obtained by numerous breeding and biotechnology projects and to the studies performed to discover new knowledge on plant's physiology to develop innovative cultivation systems. Genetic resources remain crucial for breeding activities that can now be integrated with new genomic and molecular knowledge and technologies.

Several research groups are dominating the development and control of these technologies, but there are also many emerging companies capable of developing innovations that can have important local and even global impacts. These activities are supported by important public research projects, also of international importance, but the link with the companies that must have access to the results to improve their innovation capacity remains important.

The ultimate aim is to continue to expand the cultivation of the strawberry with the development of new cultivars with high adaptability to different environments and growing systems (field, greenhouse above ground) with less environmental impact and increased safety and quality for the consumer.

Keywords: Strawberry, biotech program, genetic resources, adaptability, safety, quality

1. Introduction

The increasing consumption of fruit and vegetables is considered as one way to improve the intake of antioxidants, and strawberry, like other berries, represents one of the most important sources of bioactive compounds with high antioxidant capacity [1–5]. Accordingly, the increase of consumption of berries richer in "healthy compounds" is seen as an appropriate strategy for improving human health.

The improved content of antioxidant compounds in fruit achieved through breeding and/or biotech is an important option to support a higher antioxidant intake even when the consumption of fruit is low [6, 7]. If nutritional components are combined with a high standard sensorial fruit quality, the perspective of consumer

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health can be further improved by the easier increase of fruit consumption [8], as in the case of strawberry fruit [9].

The breeding approach can be successful if the variability and heritability of the bioactive compounds determining the Total Antioxidant Capacity (TAC) trait indicate the possibility of achieving a breeding progress. It is well known that the availability of genetic diversity within compatible species of any given crop would enhance the extent of improvement [10]. The biotechnological approach is now an integrative option to extend this improvement, but it is related to the knowledge of molecular tools able to promote more general increases in several metabolites through the modification of specific biosynthetic pathways [7, 11]. However, the success of both breeding and biotech approaches is related to deep knowledge of the most useful wild and cultivated genetic diversity to be used in genetic and genomic studies.

The influence of genotypes in affecting the nutritional quality of strawberry is well known [12, 13]; the levels of antioxidants and antioxidant capacity in strawberry extracts from whole fruits vary considerably among genotypes [12, 14], but few genotypes are well characterized for these important features. Furthermore, limited knowledge is available on the possibility of improving strawberry nutritional traits by breeding [15, 16].

The interaction of the genotype with environmental factors and cultivation systems is also important in determining the exploitation of the highest potential of fruit nutritional quality. In fact, all the pre-harvest factors influencing plant adaptation and development in the different conditions (e.g. type of soil, southern or northern climate) and the cultivation system adopted (e.g. open field, protected, soilless, etc.) play an important role in influencing the fruit sensorial and in particular nutritional traits [17].

This new enlarged vision of strawberry fruit quality is now widely recognized in Europe, the area where strawberry remains a priority among berries, and different research programs are aimed to find new solutions that can be applied in the different cultivation systems now used in the diverse EU production areas. North America represents the most competitive area, thanks to the organizational capacity of important universities and private companies that have acquired a global dimension. Surely the third most competitive area is now the Asiatic continent, once dominated by innovations from Japan, now more and more strengthened by the knowledge also developed by other important countries like North Korea [18] and above all China [19].

2. The strawberry industry in Europe

The European strawberry market is very large and the consumer demand has increased a lot all across the year (Fig. 1). To cover this demand, many EU but also non-EU companies have undertaken a large effort to expand the cultivation areas in different climates and with different cultivation systems, by adopting appropriate cultivars. Therefore, the European consumer can now find fresh strawberry fruit on the market almost all over the year. The southern areas are commonly dedicated to winter production, starting in November until March. The southern region of Spain, named Andalusia, and in particular around Huelva, is considered the most important winter production area and covers almost the 70% of the entire EU demand. During this period, other product is coming from Italy, Greece and Turkey, but also from Morocco, Egypt and Tunisia. Some production is also starting to come from other Central Africa countries (Kenya). Greece and Turkey production areas are mostly covering the demand from the eastern countries (mainly Russia).

1) The strawberry in season production period (April – July) starts with the protected and open field production of north of Italy, southern France and Germany. The summer period is mostly covered by Germany, Poland and other northern countries. Mountain areas, including in Italy, are used to cover autumn production. Belgium and The Netherlands differentiate from all the other EU countries for their highly specialized greenhouses with soilless production systems that allow almost a continuous production during the year.
2) This continuous flow of strawberries is elevating to 1.2 million tons the average of European yearly strawberry production. In the last few years the production remained stable and in 2015 corresponded to 107 thousand

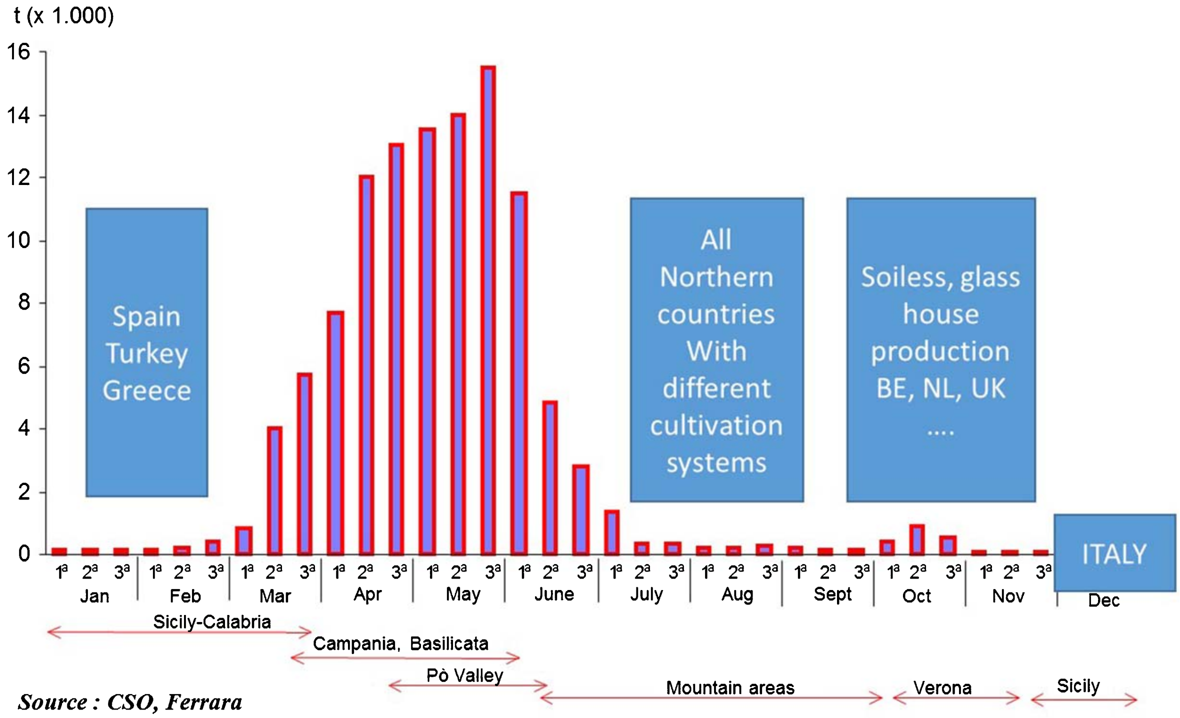

Fig. 1. European strawberry production flow all over the year.

hectares of cultivation. The high level of strawberry production is maintained by the adoption of cultivars and cultivation systems able to increase productivity and extend the harvesting season. The largest producer is Turkey with 376,000 tons, followed by Spain with 292,000 tons, followed by Poland, Germany and Italy. In the Mediterranean basin Turkey stand with 376,000 tons, followed by Egypt and Morocco. If we look at Italy the surface of strawberries is now stable on almost 3,600 hectares. However, there are changes in production patterns across Italy. Production in Basilicata has grown from 12.1% of the total value of 2005-2006 to 23.6% in 2015-2016 while Emilia-Romagna decreased from 10.7% to 6.8%.

3) In Europe, in 2015, the apparent consumption of strawberries was 1.2 million tons, with an average growth trend of 13% over the last 5 years. Germany is the Europe's largest market with 21% of the shares. Poland came second with 17% and Italy with 13%. This global growth of the strawberry is determined by the increased consumer attraction towards this fruit and to the decrease in the purchasing price. Mainly strawberries are bought at retail outlets: the supermarket is at 36.4%, the superstore to 25.1% and 10.7% at discount stores. Traditional markets and the free service are declining. Definitely, taste is the main trait nowadays appreciated by the consumer and the demand of organic strawberries is increasing. The important caveat is that the average consumer believes that strawberries are the fruit crop using the most 'pesticides'. In developing new cultivation systems we need to focus on those that can reduce environmental impact and increase consumer safety and acceptability.

3. Breeding program's in Europe (locations, goals, importance of nutrition, germplasm resources, biotechnological approaches)

3.1. Importance of genetic resources

Biodiversity means the final result of huge evolutionary processes and could be defined, summarizing it, as the whole of all animal and vegetal species in our world. The Biological diversity, in its complexity, represents a necessary resource for all human beings, its maintenance is an indispensable resource for the survival of the whole ecosystem; in fact the biodiversity, either of species and population, is a basic element for the wellbeing of all living organisms. Of importance for cultivated plants is the germplasm, that represents the total genetic variability available for a specific population.

Old ecotypes, progressively, have been changed to new cultivars or hybrids, more productive, or with some resistance or tolerance to pest and diseases or to abiotic stresses, but with a narrow genetic base. The reduction of biodiversity caused the loss of genetic source for environmental adaptableness, as tolerance to pest and diseases and at the same time also peculiar qualitative traits have been lost, often linked to the traditional way of use.

For this reason, mainly in the last decades, a lot of work has been addressed to re-establish, at least in a minimal part, the former biodiversity by recovery, study, characterization and valorization projects [20].

Strawberry germplasm is a more complex question, since the commercial strawberry "*Fragaria x ananassa*" is a young species. It has been generated by chance in the mid-late-1700s from the accidental inter-specific cross among *Fragaria chiloensis* and *Fragaria virginiana*. Since the first decade of 1800s breeding programs have begun to improve yields and fruit quality. The strawberry breeding brought to obtain a huge variability among the commercial variety. In fact, new genotypes obtained by European and north American breeding programs in the nineteenth and twentieth century were cultivated all over the world until the beginning of twenty-first century, but a huge number of new genotypes developed were discarded and even some old cultivars, and replaced with new genotypes showing better agronomic characteristics. This approach caused a strong reduction of strawberry genetic resources in the breeding pool, causing the irremediable loss of some characters, which could now have an important role for the development of new cultivars, in particular with increased fruit quality and nutritional value.

Germplasm collections have the important role to maintain genotypes with peculiar traits that can help in future breeding programs, so are fundamental for future breeding program. Germplasm collections usually are managed for breeder's working collection by including cultivated varieties, old cultivars with some peculiar trait, and wild genotypes with specific traits. Public breeding programs and germplasm collections have an important role to guarantee the conservation and accessibility to the genetic material of highest value for each crop and in particular for strawberry.

In Europe, there is a considerable number of strawberry germplasm collections [21], their existence is not just linked to breeding work, but also to taxonomic and phylogenetic studies. To optimize the use of germplasm collections for breeding programs it is important to promote connections and exchanges among the germplasm collections already available, in a way to create an easy system (open access databases) to recover information such as pedigree, phenotypic data, regional adaptation, genotype, breeding value.

In the last decade, a European project, financed by EU DG Agriculture, on berry genetic resources (Geneberry), has worked to increase the strawberry genetic material available in the different European collections and to better characterize the available germplasm using advanced molecular tools [22], thus, to better identify materials with the highest interest as genetic source for important breeding traits, such as disease resistance and fruit nutritional quality [23]. This project has identified several research stations, located in different EU countries that will be responsible for strawberry germplasm collection and preservation (Fig. 2).

The work of germplasm characterization, with standardized descriptors, has been continued in the subsequent EU FP7 EUBerry project [24]. The work carried out from this project was based on the integration of phenotypic data either already existing and collected for the 4 crops genera (*Fragaria, Ribes, Rubus,* and *Vaccinium*),

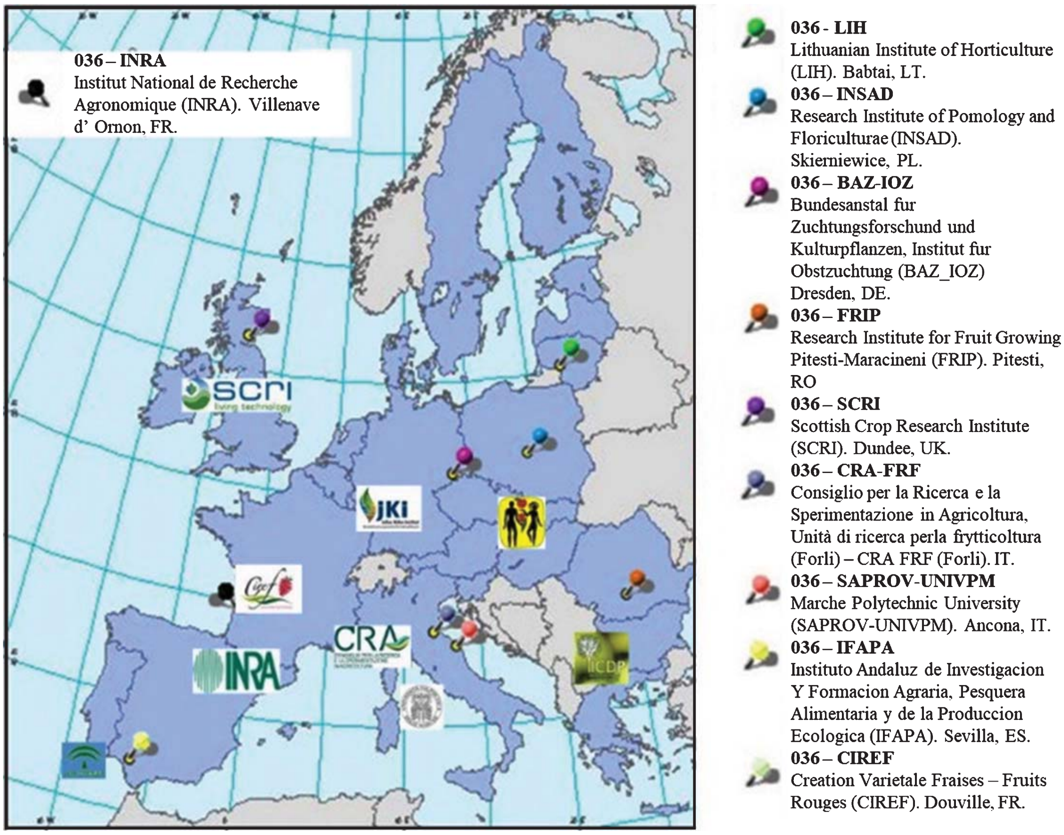

Fig. 2. List and map of locations of the EU strawberry germplasm repository.

leading to the development of linkages between desirable phenotypes for fruit quality and developmental traits, and genotypes that can be selected by breeders at the earliest stages using appropriate genomics approaches. The genomic and molecular approaches used for the characterization of the germplasm, combined with the biochemical characterization of bioactive and volatile compounds, were focused on existing and proven technologies. The goal was to bring clear and beneficial outcomes through their application by breeders for the improvement of berry fruit taste, nutraceutical and nutritional quality, while addressing at the same time plant adaptation to different climatic conditions and cultivation systems.

These EU founded projects resulted in really important information to better organize the characterization of the germplasm available in the existing collections. In the long term, the maintenance of the collections needs further support from national/local governments, not always easy to obtain. Therefore, long term public programs are fundamental to maintain these collections with the guarantee of giving free access to the material of all possible stakeholders (mainly breeders but also nurseries and growers). The lack of public support will result with the loss of most of this material or to its transfer to private companies which will limit the possibility of access to the genetic material.

Strawberry germplasm is conserved in different important institutions in the world. In Europe different institutions historically represent a reference point in the conservation of strawberry germplasm (see geneberry project). The National Clonal Germplasm Repository (USDA ARS), located in Corvallis Oregon (USA), is the main conservation center in USA. For the East area of higher importance the work done by the Beijing Engineering

Research Center for Strawberry, of the Beijing Academy of Agriculture and Forestry Sciences, Beijing, China can be cited, that together with other important institutions located in the north (Laioning) and south (Nanjing) areas of the country are collecting all the local biodiversity found in many Asiatic areas and also germoplasm and cultivars originated from different areas of the world [25, 26].

3.2. Goals of strawberry breeding programs in Europe and worldwide

Nowadays fruit consumption is possible independent to the natural harvesting season. The amount of consumption is linked to price, quality, social conditions and to specific sensory preference, sometime quite different depending to specific world areas, in fact such preferences in adult populations might still exhibit cross-cultural differences [27]. For this reason, it is important to have on the market a huge diversity of fruits, which can better answer to the request of the consumer.

Fruit are transported and marketed all over the world and consumers are used to a huge variety of fruit products. In this way, consumers can find any kind of fresh fruit on the market with only the price as a limiting factor. Together with the large demand of fruit diversification at a low price, consumers are now becoming more exigent about fruit quality, in fact people are more aware about sensorial quality and nutritional value of fruits, not just flavor, aroma and appearance. About sensorial quality, consumers couple fruit aspect (color, shape, size) with fruit taste in a way to fulfill their hedonistic preference. For this reason, it is important to realize how fruit breeding has become more focused on creating new varieties more appreciable and palatable for the consumers. Still, all these new cultivars have to be adapted to different cultivation conditions (from the north to the south) and to different cultivation systems (open field, protected, soil less).

Associated with fruit sensorial trait it is important to mention fruit nutritional quality, in a way to place on the market fruit that attract consumers not only for their sensorial quality but also for their nutritional and healthy value. Therefore, to develop a breeding program focused on the obtainment of new genotypes with improved agronomic performance as well as fruit nutritional quality Diamanti et al. [28] suggested that 12 criteria be considered for the acceptance of the new genotypes in the market. These criteria include:

1. crop productivity (i.e. yield) that must be maintained or increased to guarantee widespread farmer acceptance;
2. plant resistance/tolerance to pathogens that has to be increased so to reduce inputs requested for cultivation and fruit post-harvest lost;
3. plant adaptability to local cultivation conditions (soils and climates) and systems (open field, protected, soil-less);
4. plant habits that result in “easy picking”, it is preferred that fruits are displayed away from center of foliage and easy to find and pick (in some areas are preferred cultivars which have single stems that stick out from the plant rather than trusses of fruit);
5. season wide production rather than a June bearer heavy producer (many areas are now picking the same plants for eight months in summer), now mostly achieved with the introduction of everbearing/day neutral plants so to expand plant ripening seasons;
6. Good eating quality that is determined by high fruit sensorial quality, mainly due to increased content of soluble solids and aroma volatiles;
7. Uniform shaped, large fruit, in some areas even above 30 grams to reduce labour costs;
8. High fruit firmness to reduce damage during picking and long travel shipment;
9. Fruit colour that is also critical and sometime influenced by local custom, although in intensive cultivation the reddish fruit colour is preferred to the orange;
10. The micronutrient enrichment traits that must be relatively stable across various edaphic environments and climatic zones and must have significant impact on human health;

11. Ultimately, the bioavailability of micronutrients in enriched lines that must be tested in humans to ensure that they improve the micronutrient status of people preparing and eating them in traditional ways within normal household environments;
12. Consumer acceptance that must be tested (taste and fresh quality must be acceptable to household members) to ensure maximum impact on nutritional health.

Over the 12 objectives, the first 4 are finalized to increase plant adaptation and production efficiency mostly related to factors increasing plant resilience to climate, cultivation conditions and defense mechanisms to pest and diseases. The other objectives are all focused on traits determining economic sustainability of the cultivation system, access to market (local – long distance) and fruit quality for increasing consumer acceptability.

From the applied breeding programs there is a continuous release of new cultivars that are tested in public and private research stations located in different countries, by using the standard local cultivations systems (open field or protected), sometime combined with advanced soilless technology. This high amount of new cultivars released from all different breeding programs is justified by the fact that even if with a limited marked, in cultivation area and time period, they can still produce a good return in royalties to breeders.

The fact that money can be made has promoted in recent years the consolidation of strawberry breeding programs with the aim to increase their scientific and commercial capacity. At the same time, small companies have started breeding programs able to release new cultivars having a good impact on local markets, and generating sufficient royalties support the cost of patenting or plant variety protection (according to the different legal systems of the territories, namely patent in USA and protection of breeders' rights in EU and other UPOV countries) and generate income for the company.

The situation of breeding program at universities and public research centers is more complex. In the past, generally, there was sufficient public funds to support applied breeding program but most of them are now terminated because the public funds dried up and they were not able to generate sufficient funds through the royalties to maintain their programs. These breeding programs, linked to public germplasm collections, should be maintained as a priority in the agenda of public national and international institutions. Only this approach allows the creation of new plant materials with a novel and larger genetic base that is easily shared among researchers from public and private institutions, and transferred to the marked without exclusive control from one or few companies. Unfortunately, the current trend of consolidating the small breeding programs under the umbrella of large private companies will have the long-term effect of further narrowing the genetic base used in breeding of this important crop.

The few strong strawberry public breeding programs are still linked to universities, many in USA, like University of California – Davis and the University of Florida, and few in other countries like for example the Nanjing University (China) and Marche Polytechnic University (IT). Other important breeding programs are carried on by national research centers, for example: USDA-ARS, Beltsville (USA); Agri-Food Canada, Nova Scotia; Beijing Academy of Agriculture and Forestry Sciences, Beijing and Shijiazhuang Pomology Institute, Hebei Academy of Agriculture, Shijiazhuang (China); CREA – Forli (IT); IFAPA (SP); INRA-CIREF (FR); NIAB-EMR (UK) and other in many countries; much more impactful is the contribution deriving from private companies.

In Europe several breeding programs remain located in different countries are now operating, from the north to the south, and carried out by different public and private institutions (Fig. 3).

Many of these different programs are combining the traditional breeding with advanced biotechnologies, mostly employing genomic studies for developing molecular breeding, but in some case the genetic engineering approach is also used [7].

Cultivars originating from these breeding programs have had an impact of local markets and some of them also on more international market, reaching different cultivation areas in the east (mostly Russia and neighboring countries) and in the South (north Africa), very few have access to the eastern countries and the American continent.

Fig. 3. Distribution of 8 public ★, 15 private ☆ breeding and 8 biotechnological ✖ programs now running in EU.

The activities of these European programs have been particularly intense in the last few decades. In fact, from 1995 to date, the Community Plant Variety Office (CPVO) has protected almost 550 new cultivars of strawberry for the use in the EU territory. They are many, mostly from European and international breeding programs (mainly in USA). However, the number of new cultivars has not reached a large market or had a long commercial life. The main reason is that only few of them show real improvements for the new requirements of production systems, mainly combining yield and resilience with all the fruit quality parameters now requested by the market. Sometime new cultivars find an interest for few years then disappear or become important only for a specific growing area. In spite of this intense European breeding activity, there is still high demand for new genetic material from other international breeding programs, in the hope that new cultivars will be found that can solve some of the major problems common in different areas, mostly pest disease resistance, soil adaptability and in particular high quality ever-bearing cultivars.

A greatest global impact is achieved by few large breeding programs carried out in USA by UC-Davis, University of Florida and and international global companies, eg. Planasa, CIV, clearly Driscoll's and Eurosemillas the exclusive Master Licensee of the University of California for protected plant varieties including strawberry. Thanks to their high scientific and commercial capacity these groups have expanded globally the commercial importance of their cultivars.

In the USA there are a number of other public and private breeding programs that still exist, but without any real global impact. In Canada, another very large country, at the moment apparently only one breeding program remained (Nova Scotia), while in all Latin America, at the moment, only in Brazil (Santa Catarina State University, Lages), Mexico (INIFAP) and Uruguay (INIA) have very few breeding programs and they are small.

In eastern countries, in the last few years, only China has started important new breeding programs with the release of new cultivars with a significant impact on their huge national market. Of high relevance is the breeding program started by the Beijing Academy of Agriculture aimed to select new cultivars that are early ripening, with large fruit, high yield, high disease resistance, strong flavor, easy to store and transport. From this program 8 new cultivars have been recently released (Xingdu N.1, Xingdu N.2, Tianxiang, Yanxiang, Shuxiang, Dongxiang, Hongxiu-tianxiang and Jingyuxiang), all with excellent comprehensive traits and now growing on a large scale not only in China. Japan and South Korea have also maintained public breeding programs all aimed to produce new strawberry cultivars with improved yield and firmness combined with high fruit sweetness, as the fruit standard of ‘Toyonoka’, the main cultivars mostly appreciated by the oriental consumers [29, 30].

3.3. Cultivar situation in Europe

By giving a general overview of the main strawberry cultivars nowadays cultivated in Europe it is possible to identify cultivars representing a local historical tradition, and other originated by international breeding programs with a global commercial market. Starting from the northern Scandinavian countries, ‘Korona’ remains the most diffused cultivar. In Northern and Central countries still the most common cultivar remains ‘Elsanta’, a quite old cultivar with a high interest for the fruit quality but also because it is well adapted to the cultivation systems used in this area, and not any less important, the fact that its patent expired and it can be freely propagated. ‘Clery’, an Italian cultivar from CIV (IT), remains the most important in the center of Europe, mainly in Germany. Other cultivars like ‘Malling Centenary’ (UK) are increasing in importance in UK and other countries, this mostly because fruit quality but attention has to be paid to susceptibility to different soil and climatic conditions. The breeding program from The Netherlands has released several cultivars, the most successful are still ‘Sonata’ and ‘Lambada’, two cultivars with good productivity and fruit quality. Of interest for specific areas of central EU are also other Italian cultivars (‘Alba’, ‘Asia’, ‘Brilla’, ‘Joly’, ‘Romina’, ‘Sibililla’ and other), for early and intermediate production. The more recent ‘Cristina’ is having an increasing interest in Germany, Poland and other areas to cover the late production season [31]. Among the late cultivars, ‘Cristina’ still differs from the others for its high plant adaptability and ability to sustain high yield in different cultivation systems, with fairly good fruit quality. In the same period of increasing interest is also ‘Letitia’, another cultivar from CIV. In Germany, for very-very late production is also used the cv. ‘Malwina’. Breeding programs have recently been started in these areas (eg. Flevoplant and Hansbred) in order to release cultivars with the future cultivation systems of centeral – northern Europe.

The French market generally is different from the other EU market, still it is mostly focused on the old cultivar ‘Gariguette’, for its unique flavor and, for the same reason, ‘Darselect’. It is really difficult to introduce new cultivars in the France market if their fruits do not reach a very high standard of sensorial quality.

In centeral–southern areas of Europe (Italy, Spain, Greece, Turkey) there is a large differentiation on the type of cultivars used depending on conditions and market. In the more central areas some of the most common cultivars used in Germany are common, including Italian ‘Clery’, ‘Joly’, ‘Alba’, ‘Asia’, ‘Garda’, ‘Eva’, ‘Romina’, ‘Sibilla’ and ‘Cristina’, or others such as ‘Darselect’. In the more southern areas, many cultivars released from California and Florida public and private breeding programs are commonly planted (eg. ‘Florida-Fortuna’, ‘Camarosa’, ‘Monterey’, ‘Portola’) combined with other important Spanish cultivars (Sabrosa- Candonga and Sabrina or other emerging cultivars such as Rociera, Melissa and other). In fact, information given by IFAPA working group, indicates that during the last two seasons of cultivation (2016/2017 and 2017/2018), four cultivars ‘Florida-Fortuna’ (Florida University), ‘Primoris’, ‘Rociera’ and ‘Rabida’ (Fresas Nuevos Materiales-FNM), represent the 70% of plant utilization in the area of Huelva (Spain). Everberbearing cultivars are also wide spread in some cultivation areas of Europe, and currently a few are showing increased interest in different European areas, such as ‘Murano’ (IT), ‘Eve Delight’ (UK), ‘Malga’ (IT), and the California cultivars (‘Monterey’ and ‘Portola’). There is a growing demand for new everbearing cultivars with increased adaptability and fruit quality. This remains one of the main targets for future breeding programs.

3.4. Are biotechnologies helping strawberry breeding?

All cultivars now available on the market have been produced using traditional breeding programs. However, international and national programs are supporting the development of important projects (eg. RoseBreed in USA; Euberry and Goodberry in EU) to apply genomic biotechnologies for strawberry genetic improvement. The complete genome sequence of wild diploid strawberry remains the most advanced knowledge now available [32]. The knowledge developed by the genomic studies is fundamental for the development of new molecular tools for the analyses of genetic diversity and population structure, cultivar identification and fingerprinting for cultivar commercial protection and more important to identify markers linked to traits of interest, in particular disease resistance to major pathogens affecting strawberries (eg. *Phytophthera* sp. and *Colletotricum* sp.), photoperiodic flowering, male and female sterility and other structural genes. The marker-assisted breeding (MAB) encompasses various uses of markers related to breeding including clone verification and genetic diversity analyses, as well as marker-assisted parent selection (MAPS) and marker assisted seedling selection (MASS). This approach remains of limited application in strawberry because of the lack of availability of many more easily-scored markers tightly linked to economically important traits. In fact, only the abundant availability of tightly-linked, flanking, easy-to-use markers, combined to reduced costs for DNA extraction and analyses, can help to make MAB economically advantageous for breeding programs. Another limiting factor is the genetic structure of octoploid cultivated strawberry with high heterozygosity, determined by high levels of outbreeding, and frequent testing of clonal replicates as opposed to the testing of inbred lines [33].

GMO technologies have been applied in strawberry demonstrating the capacity to use this technology for improving important traits of strawberry plant and fruit quality but public acceptance remain the main limiting factors of this technology [34]. New breeding techniques (NBT) offers now new solutions that can bring to new advancements in the application of the genetic technologies. In fact, gene functional studies for the cultivated strawberry (*Fragaria* × *ananassa*) have been conducted via gene silencing using intron hairpin RNA (ihpRNA)-based constructs. The RNAi technology now offers the new possibility to protect plants by the endogenous expression or exogenous topical application of RNAi that inhibit the spread of the disease by acting on target genes of the pathogen [35]. This type of application of RNAi technology for protecting plants appears of lower risks and higher acceptance for the consumer [36].

The use of CRISPR-Cas9 system in octoploid strawberry has been already tested to target the floral homeotic gene *APETALA3* (*AP3*). The developed gene-edited strawberry lines displayed defects in stamen and fruit development. Analysis of the targeted locus indicated differences in gene editing among different CRISPR-edited lines, and also found lines with mutations in all eight *AP3* copies in the strawberry genome. More importantly, these mutations were maintained in clone plants generated from runners, ensuring the maintenance of the CRISPR-Cas9 edits during strawberry plant propagation. This preliminary work performed by Carmen Martin-Pizarro and David Posé Padilla of University of Malaga (Spain) demonstrate that CRISPR-Cas9 system is a functional tool to perform genome editing in cultivated strawberry with the aim to perform functional analysis of genes in this crop and also to obtain new genotypes with improved/corrected traits. The main limitation for the application of all these technologies remain the availability of efficient regeneration, transformation and selection protocols to be applied to all cultivars to be modified.

4. Adopting cultivars and cultural systems for full year production

Strawberry cultivars are commonly classified based on the photoperiod in which they can initiate flowers. Junebearers are facultative short-day (SD) plants [37] and initiate flowers in late summer and autumn in northern climates. Darrow and Waldo [38] described everbearers as long-day (LD) plants, since these cultivars initiated flowers during long days under favourable temperatures. Later, different publications can be found that describe "day-neutrals" (DN) for cultivars which can initiate flowers independently from photoperiod [39, 40]. However, classification based on photoperiodic response of flower initiation is difficult, since temperature also plays an important role. For junebearers, many researches already demonstrated that the relation between photoperiod and temperature is important for flower initiation and may vary among cultivars [41, 42]. When temperature is low enough (<10–15°C), junebearers can initiate flowers independently from photoperiod [43]. Also for everbearers, a similar relation between photoperiod and temperature that determines flower initiation has been proved [44–48]. These studies gave new insights for all repeated flowering cultivars: they respond as qualitative LD plants at high temperatures (>25°C), as quantitative LD plants at lower temperatures (10 to 25°C), and as DN plants at temperatures below 10°C. All repeated flowering or remontant cultivars are now generally called "everbearers" in agreement with the terminology described by Heide et al. [48].

The interest in growing everbearing cultivars is increasing worldwide because of their interesting characteristic to initiate flowers during long days. Consequently, these cultivars can produce fruits during a long period and cover a fruiting season from late spring until autumn. Nowadays, the very intensive continuous strawberry cultivation systems, as in Belgium and the Netherlands, are mainly based on junebearers (cvs. Elsanta, Sonata, Clery). In the eastern part of Belgium there are some everbearers grown in the soil. However, in the last few years there was a lot of interest for growing everbearers on substrate: on table tops or in a glasshouse after an early junebearing crop. In both systems it can replace two junebearing crops, and could so reduce costs for plants, substrate and labour. Van Delm et al. [49] proved that it is possible to increase yields with the everbearing French cultivar 'Charlotte' (Ciref, France), the Californian cultivars 'Montery' and 'Portola' (UCDavis, USA) and 'Capri' and 'Murano', the more recent release from CIV (Italy), by increasing day-length artificially by using artificial lighting at low intensities. These pre-harvest night-interruption treatments had an effect on the second fruit cycle by promoting flower initiation and development, but were dependent on temperatures and cultivar.

Flower induction and development are stimulated by artificial lighting (Near Infrared treatments-NI) at the expense of runner production. This long day treatment results in a remarkably increase in yield during the second flush, and this second flush is clearly advanced. The first flush is not affected since these flowers were initiated the previous autumn. The third flush is hardly affected by the pre-harvest NI treatment.

Timing of the pre-harvest NI treatment is crucial to obtain an optimal effect. The plants with NI treatment the first 2 weeks are hardly affected. Plants lighted the following two weeks give a remarkable effect, almost comparable with the 4 weeks of NI treatment. This means that too early NI treatments are useless and it is important not to overstimulate flower initiation, because it leads to a relapse after the second flush, as shown by Van Delm et al. [49].

Growing the everbearing cultivar Capri in a Belgian glasshouse can be improved by artificial lighting to obtain higher yields during the second flush, which is a real opportunity for strawberry growers. This confirms the findings of Sønsteby and Heide [46] that everbearers behave as qualitative long-day plants at intermediate temperatures.

5. Plant architecture as a tool

Plant architecture is now considered a new tool for better identifying plant adaptability to the different types of cultivation systems. Plant architecture represents morphological expression [50] and describes the topology and the fate of all the meristems. It can be schematized in a graphical way showing the plant elements that otherwise would be hardly visible in small and compressed plants like the strawberry rosette. The architectural model provides also a dynamic description of the growth along the plant cycle. The analysis of plant architecture has been used as a method to detect the effects of experimental treatments, or to evaluate cultivar attitudes and adaptability for breeding selection (HORT, unpublished). It has been proposed as a parameter of plant quality [51] useful to interpret the story of the plant or to predict its behaviour according to the physiological knowledge mainly related to the meristems development. The fate of the plant meristems originates from their sensitivity to inducing factors, orienting their development toward reproductive or vegetative identity. The most discussed factors for strawberry flower induction are temperature and photoperiod. The june-bear (non-remontant - seasonal flowering) genotypes requires for flower induction short days [41, 42] or low temperatures [41, 42, 52, 53], while the everbearers (remontant) genotypes can induce flowers under long day conditions [40] or irrespective of photoperiod [39, 54]. Nevertheless, many other factors can influence flower induction, and affect plant growth at different levels. Mineral nutrition [55–57], transplanting technique [58], light quality [59, 60], leaf removal [61] and water stress [62] have been reported to be effective, especially in the non-remontant cultivars. Therefore, plant behaviour can be modulated by the growing technique, acting on agronomic, nutritional and environmental parameters in order to enhance vegetative or reproductive growth. These techniques may be applied during the nursery propagation phase of plant production, and may be further specialized to optimize growth of many commercial plant types.

The architecture of the strawberry plant reflects the high plasticity of the species, in response to many external factors in relation to genotype sensitivity (remontancy type and cultivar) and to nursery typology. Each plant type has the potential to bear a different amount of flowers and a specific ability to develop shoots and stolons. Appropriate resource portioning between these structures is needed to carry out a wide range of production cycles, each requiring different quality standards and definition, according to crop potential and timing. The quality of the plants from the nursery is defined by morphological, physiological and developmental characteristics and can be evaluated in the comprehensive assessment of plant architecture, where also the position and the developmental stage of the differentiated inflorescences can be represented, evaluating the timing and the length of the potential harvest.

6. Strawberry nutritional quality

Strawberry fruit quality can be defined as a set of agronomic/commercial, organoleptic and nutritional qualities: the first one comprising of characters that belong to the adaptation of the plant to specific cultivation such as fruit size, plant yield, harvesting speed, and resistance to pests and diseases. Organoleptic quality is the main set of characteristics generally related to quality attributes that are recognizable through the five senses of the consumer. Finally, nutritional quality is the “hidden” quality present in berry fruits, that is due to all the macro- and micro-nutrients, vitamins and bioactive compounds [17].

An accruing knowledge base for strawberry fruits has highlighted their benefits with respect to human nutrition and health [63–71]. The phenolics in fruit encompass a broad chemical range across subgroups such as anthocyanins, flavonols, flavan-3-ols, ellagitannins, procyanidins, hydroxycinnamic acids and their esters, etc. Berries contain significant levels of anthocyanins compared to other fruits, and *in planta* these are responsible for the fruit colours. As with many of the phenolic subclasses, anthocyanin composition varies among berry fruit crops [12, 72, 73], leading to a variation in the associated antioxidant activity [3, 9]. This parameter quantifies the radical quenching ability and in some cases is related to the beneficial health effects [70]. The *in vitro* antioxidant capacity has been extensively considered in strawberries and the role of the genotype in affecting the phytochemical composition has been already well-established [5, 13, 74].

Pelargonidin is the main anthocyanin in strawberry, in a glycosylated (galactosides, glucosides, arabinosides, rutinosides, sophorosides etc) and sometimes acylated (acetyl, hydroxyl-cinnamate, malic acids etc.) form [75, 76]. They have been often suggested to have positive impact on human health [77]. This idea supports the selection of anthocyanins as valid traits for fruit breeding and product development, with a view to human health maintenance and improvement. Furthermore, the apparent low bioavailability does not necessarily negate the validity of the anthocyanins as sources of beneficial bioefficacy as their digestion products give rise to smaller phenolics with biological activity [78, 79].

There are epidemiological studies that demonstrate an association between strawberry fruit intake and the decrease of cardiovascular risk [71, 80]. Similarly, outcomes of strawberry intervention studies related to healthy humans have shown significant increases in plasma total antioxidant capacity after an acute [81] or a protracted [82] intake of fresh strawberries. Additionally, 2 to 3 weeks of strawberry consumption led to a significant increase in LDL peroxidation lag time [83] and in erythrocyte resistance to oxidative damage [82]. Recently, it has been demonstrated that a dietary supplementation of anthocyanin-rich strawberry significantly reduced important cardiovascular risk markers including LDL-cholesterol, triglycerides or activated platelets in humans [80, 84].

From the experiences available, the significant amounts of berry phytochemicals critical for consumer health, can be easily determined and probably claimed, while the identification and complete validation of the effects on specific consumer health benefits is still very difficult to identify and claim.

For most fruit breeding programmes within the EU, fruit quality components have increased in importance as targets for new cultivars in recent years, and now they should be considered together with longer-standing agronomic objectives, such as yield and pathogen resistance. The ultimate goal for breeders and commercial growers is a strawberry cultivar that combines both agronomic and quality traits, and that can be grown sustainably within under the future climate scenarios projected for the EU highly differentiated cultivation systems and markets. Breeding for the enhancement of one or more beneficial phytochemicals in strawberry fruit is likely to be achievable by using selected germplasm as source of enounced metabolisms and specific breeding programs able to combine them with the commercial traits. This approach has been already adopted to improve many different fruit, including strawberries [15, 31, 85, 86]. Classical genetic, as well as marker-assisted and transgenic, approaches are being used to increase the content of specific bioactive (mainly micronutrients, vitamin C, ellagic acid, folates, phenols, etc.) compounds of plants [87, 88]. Furthermore, there is an increasing awareness on the need to study the multiple genetic factors and their interaction with the environment and cultivation techniques in order to increase the production and fruit accumulation of bioactive compounds with health benefits for the consumer[89].

The amount of bioactive compounds (e.g. vitamin C, ellagic acid and folates) in strawberries varies with cultivars and is affected by the climatic conditions [3, 12, 24]. Although little is known about European strawberry cultivars, there is evidence that fruit ellagic acid concentrations can be influenced by environmental and growing conditions. In addition, Wang et al. [13] showed that different mulch types had a significant effect on the concentration of ellagic acid in strawberries, while Häkkinen et al. [90] showed an effect of geographical origin and cultivation technique on flavonol on strawberries and other berries.

Furthermore, agronomic manipulations (e.g. out soil/in soil production, flat and mountain growing area, light-induced ripening) may have generic effects on phytochemicals [91].

Indeed, since strawberry production becomes more and more specialised, growers try to extend harvest season by different techniques, for example by the use of cold-stored plants and different cultivation techniques. Many factors are quite good under control in glasshouses, like nutrition, water, temperature, relative humidity, CO_2, etc. Additional artificial light can solve this problem, and with the technology of LED lamps the possibilities with different spectra give good opportunities for strawberry cultivation.

Thus, cultivars able to maintain a stable content of bioactive compounds and ascorbic acid concentrations at different cultivation conditions can be released with a claim on the fresh strawberry fruit nutritional value. In fact, health claims for Vitamin C may be used for food that at least qualifies as a source of vitamin C as listed in the Annex to Regulation (EC) No 1924/2006 [92]. To bear the "source" claim the respective food has to contain "significant amounts" of vitamin C. "Significant amounts" is defined as containing at least 15% of recommended daily allowance in a 100 g serving. The European Recommended Daily Intake (RDA) value for vitamin C is 80 mg (Commission directive 2008/100/EC) and thus fresh strawberries must consistently contain minimum 12 mg/100 g to bear one of the existing functional claims on vitamin C. Therefore, new breeding programs can select certain strawberry genotypes containing a high amount of vitamin C for functional claim application according to article 13.1 of the EU regulation and will thus provide clear and transparent information to the consumer.

7. Conclusions

The strawberry fruit crop contributes to many rural economies within the EU, especially in terms of employment and economical added value [93]. Strawberries are highly perishable fruits, often sold immediately after harvest at high price, especially when hand-picked (fresh fruit are exclusively hand-picked). Offering a consistently high quality of berry fruits with superior nutritional status would be an ideal way to increase consumer interest and satisfaction and increased strawberry consumption will contribute positively to a healthy diet. The number of studies demonstrating berry, and in particular strawberry, benefits for the consumers is continuously increasing [5], but it is still difficult to make consumer health claims for a commercial use.

Intensive research programs, also supported by the European Union such as the FP7 – EUBERRY project [94] and the new EU Horizon 2020 strawberry adaptation named GoodBerry [95], are continuously releasing new innovations (cultivars, techniques, materials, etc.) able to expand the cultivation, increase production efficiency and fruit quality. Thanks to these investments for the development of new cultivars and cultivation techniques, the European strawberry industry will maintain high competitiveness and will be able to answer the demand of reducing production inputs and improving fruit access and quality for the consumer.

Acknowledgements

We acknowledge the GOODBERRY project supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 679303.

Conflict of interest

The authors have no conflict of interest to report.

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