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Invited Review

Breeding and biotechnology for improving the nutritional quality of strawberry

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Abstract. Traditional breeding is based on the identification of the most suitable genetic resources from either cultivated or wild germplasm. Starting from wild germplasm, progeny from strawberry crosses with *F. virginiana glauca* as a common parent have shown significant increases in fruit quality and nutritional features. This confirms the use of the wild species to improve several characteristics of cultivated strawberry. However, commercial cultivars of *F.* × *ananassa* already perform well, due to the combination of important agronomic traits with improved fruit nutritional quality. A considerable number of molecular studies have targeted strawberry for gene cloning, genetic transformation, and fingerprinting and mapping for important agronomic traits, such as plant habitus, disease resistance, and fruit nutritional quality. Meanwhile, the completion of the diploid strawberry genome sequence promises to accelerate candidate-gene approaches for marker discovery. In addition, these achievements are expanding the potential applications of DNA recombinant technology for the transfer and validation of target genes that control important traits, including fruit nutritional quality. The major limitation of such genetically modified organism technology in strawberry is the lack of public acceptance. To overcome this problem, particular attention needs to be given to the safety issues through all of the steps in the preparation and evaluation of these genetically modified organisms.

Keywords: Nutritional quality, genetic resources, breeding, genetically modified organisms, biosafety

1. Introduction

Over the last 30 years, the objectives of strawberry breeding have focused mainly on meeting esthetic standards, quality and durable disease resistance, with all of these traits now receiving the highest of priorities. Indeed, combined with the large demand for fruit diversification at low prices, the consumer is also now becoming more exigent about fruit quality. Thus people are now more aware of the sensorial quality and nutritional value of fruit. For the sensorial quality, consumers couple fruit aspect (color, shape, size) with fruit taste in a way that fulfills their hedonistic preference. For this reason, it is important to note how fruit breeding has focused on the creation of new varieties that are more appreciable and palatable for the consumer.

Indeed, it is already well know that fruit consumption, and in particular that of berries, is associated with general health benefits that arise from the antioxidant activity of the fruit [1–6]. Therefore, in addition to sensorial quality, fruit has nutritional quality, which is strictly associated with biochemical compounds in the fruit, such as vitamins,

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minerals, polyphenols, anthocyanins and flavonoids [7–9]. The quantity and quality of bioactive compounds in strawberry fruit is strictly related to the fruit genotype [9–13].

A strawberry breeding program focused on obtaining new genotypes with improved nutritional quality has to be developed by taking in account some specific criteria for the acceptance of new genotypes on the market. These include the following:

(1) Crop productivity (i.e., yield) must be maintained or increased, to guarantee widespread farmer acceptance;
(2) The micronutrient enrichment levels achieved must have significant impact on human health;
(3) The micronutrient enrichment traits must be relatively stable across various edaphic environments and climatic zones;
(4) Ultimately, the bioavailability of the micronutrients in enriched lines 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;
(5) Consumer acceptance must be tested (taste quality must be acceptable to household members), to ensure maximum impact on nutritional health.

With the important aim being to increase consumer health, the availability of new fruit with combined high sensorial and nutritional qualities is the only option for the promotion of increased fruit consumption with increased beneficial effects on human health.

2. The effects of genotype on fruit nutritional and nutraceutical quality

Nutrition is the provision to cells and organisms of the materials necessary to support life (in the form of food). The main compounds that perform this function are carbohydrate, protein, fat, minerals, vitamins and water. All these compounds are essential to life, and a deficiency of one or more can result in the development of pathological states. Nutritional quality relates to the nutritional value of foodstuff, and it includes all of these compounds.

The terms nutraceutical is used as a combination of the words nutrition and pharmaceutical. Nutraceutical compounds are those compounds that are present in food that provide health benefits. Nutraceutical quality is thus the content of these nutraceutical compounds in the fruit.

The combined nutritional quality traits of fruit are strictly related to the genotype, the environment, and the cultivation techniques to which the plants are subjected. Any single modification of these parameters can directly affect the quality of the fruit, in terms of their agronomic, sensorial and nutritional qualities. However, the genotype has the most important role in determining the nutritional and nutraceutical qualities, as has already been described in the large volume of literature on the evaluation of strawberry fruit nutritional parameters [9–14]. The nutritional quality parameters are also influenced by the growing conditions [15].

There is a huge variability of fruit antioxidant capacity and antioxidant compounds across cultivars of the same species, and clearly among types of fruit from different species [16]. Of particular interest, there are also differences across cultivated and wild fruit species. The wild species have higher levels of nutritional attributes when compared with their respective cultivated varieties, although at the same time, they have lower other important fruit quality traits, such as, for example, fruit size and firmness. Thus, wild germplasm has an important role as a source of genes that can be used to improve fruit, nutritional and nutraceutical qualities. Volatile compounds are mainly studied for the fruit aroma but their role in fruit nutritional value has been also studied in strawberry germplasm [17].

3. Breeding for improved fruit nutritional and nutraceutical qualities

The improvement of fruit nutritional and nutraceutical qualities can be achieved with the selection of new fruit genotypes with increased content of the bioactive compounds indicated above.

The evaluation of fruit nutritional quality, and in particular in fleshy fruit, also includes an evaluation of the soluble solids content. Indeed, the content of soluble sugars is important for determining both the organoleptic and nutritional qualities. The effects of sugar on the aroma is also influenced by the acidic compounds, with the ratio between these two factors defining the measure of fruit sweetness.

The main polyphenols in fruit are the flavonoids, which can be subdivided into:

- Flavones: these are again subdivided into four subgroups of flavone, flavonol, flavanone, flavanonol.
- Isoflavon: this is not so common in fleshy fruit, and is instead mainly found in legumes.
- Anthocyanidins: aglicones of the anthocyanins are common plant pigments.

All of these compounds, and in particular the anthocyanins, have powerful antioxidant capacities, and they are thus very important for human health.

As far as the total antioxidant capacity (TAC) is concerned, this does not always depend directly upon the antioxidant levels, as this parameter considers the cumulative actions and synergistic interactions of all of the antioxidants present in a food. The TAC thus indicates the cumulative capacity of a food to scavenge free radicals.

When applying various evaluation methods, it is crucial to know exactly what the different assays measure and what kind of information can be obtained by adopting one technique instead of another.

The breeding of more nutritious and better tasting cultivars will be successful if the variability and heritability of the bioactive compounds that contribute to the TAC indicate the possibility of achieving breeding progress. The levels of antioxidants and the TAC of strawberry extracts from whole fruit vary considerably among genotypes [10–16], although few of the genotypes have been well characterized for these important features. Furthermore, limited knowledge is available on the possibility of improving strawberry nutritional traits by breeding [18]. Accessions of progenitor wild species are valued by strawberry breeders as sources of novel traits, especially in terms of pest resistance and abiotic stress tolerance [19]. Furthermore, previous investigations have shown improvements in strawberry fruit nutritional quality in breeding material that originates from *Fragaria virginiana* subsp. *Glauca*-FVG (S. Watson) Staud inter-species crosses [11, 12]. This is supported by a study carried out by Wang and Lewers [16], which showed that the fruit of *F. virginiana* accessions has significantly higher TAC, total phenolics, and total anthocyanins than fruit from different lines of *F. chiloensis* (L.) Miller and *F.* × *ananassa* (Duch).

More recent evaluations [14] carried out on the genetic pool that originated from FVG inter-species backcrosses compared to *F.* × *ananassa* (Duch) intra-species crosses have confirmed the importance of using wild genotypes, or genotypes with a wild genetic background, in breeding programs that are aimed at increasing fruit nutritional qualities (i.e., TAC, total phenolics, total anthocyanins) and sensorial qualities (soluble solids, total acidity, Chroma, L*, firmness) in the offspring populations. Results obtained from two subsequent back-crossing generations from an inter-species crossing combinations with a wild genotype of FVG have indicated useful improvements in fruit nutritional and sensorial qualities combined with agronomic standards that are close to the standards requested at a commercial level. Inter-species crosses provide new genetic backgrounds that can be useful for the increasing of specific nutritional traits (e.g., see data on folate content in F1 FVG inter-species cross-combinations; [11]). However, clearly, at least two back-cross generations are needed to reduce the distance from the agronomic values that are necessary at a commercial level. Fruit firmness, size, acidity and color are the main traits that need to be recovered in the subsequent back-crossing generations. Again, clearly, the high positive values for some parameters in the F1 genotypes (TAC, polyphenols, folate) are reduced in the subsequent back-crossing generations, but they nevertheless always remain higher than those in the commercial *F.* × *ananassa* (Duch) genotypes. Improvements of these traits can also be achieved by programming *F.* × *ananassa* (Duch) intra-species crosses and producing progeny with productivity traits that are more similar to those of the commercial cultivars. The two types of combination programs (inter-species back-crosses, intra-species crosses) can be used for the improvement of strawberry nutritional quality, but the success of any such program is strictly related to the ways in which the attributes of the different parents combine.

In terms of the genetic control of such traits, the octoploid cultivated strawberry, for which the homoeology groups have been identified recently [20, 21], appears to be a good model for studying the control of fruit quality in polyploid fleshy fruit species.

More recently, quantitive trait locus (QTL) approaches have been undertaken for the analysis of day-neutrality [23] and agronomic and fruit quality traits [23] in the cultivated octoploid strawberry, *Fragaria* × *ananassa* (Duch), as well as for analyzing the genetic architecture of sexual dimorphism in the octoploid *Fragaria virginiana* (S. Watson) Staud [24]. Lerceteau-Kohler et al. [25] revealed a large number of QTLs for the control of sweetness and acidity levels in the octoploid *Fragaria* × *ananassa* (Duch.), which thus reveals the potential to improve these traits independently.

4. Biotechnology for berry nutritional quality

The success of both breeding and biotechnological approaches in genetic and genomic studies is dependent on a deep knowledge of the sources of the most useful wild and cultivated genetic diversities. The advent of large numbers of molecular markers with a high degree of reproducibility for the genus *Fragaria*, such as microsatellites, gene-specific intron length polymorphism, and cleaved-amplified polymorphic sequence markers, has largely superseded the use of arbitrary markers in recent years, for the construction of linkage maps [26–28], the study of relatedness and identification of strawberry cultivars [29–33], and population genetics studies [34].

Gene-specific markers are useful for mapping; as they derive from coding sequences, they represent markers with actual biological function. Using the approach of primer-pair design, Deng and Davis [26] developed a set of degenerate primer pairs that amplified sequence-tagged site markers for genes of the anthocyanin biosynthesis pathway, using DNA and protein sequences deposited in the European Molecular Biology Laboratory database. Their primer pairs amplify markers for five genes in the anthocyanin biosynthesis pathway and one associated transcription factor. Subsequently, several QTLs that control traits involved in nutritional quality, such as vitamin C levels, have been mapped in strawberry [34]. This approach can further benefit from the current development of tools for large-scale analysis of fruit metabolites (i.e., the metabolome), which has already been applied to strawberry [35]. In parallel, the rapid development of comparative genetic maps between different plant species, including berries, will allow the localization on genetic maps from berry species of the candidate genes that have previously been identified in model plant species. High-throughput technologies for plant genotyping, such as those currently developed for the detection of single nucleotide polymorphisms, will accelerate the fine mapping of QTLs, and ultimately the positional cloning of the genes responsible for traits involved in berry nutritional quality.

Transgenic approaches can provide an alternative, although there is currently public concern about their use in contemporary agriculture, particularly when genes derived from organisms other than plants are used. To date, transgenic approaches have been successfully used to increase the nutritional value of several crops of great importance worldwide, such as rice [36]. For berries, the first interesting result was the demonstration that the biosynthesis of L-ascorbic acid in ripe strawberry fruit can occur through D-galacturonic acid. Furthermore, it was demonstrated that vitamin C levels can be increased in *Arabidopsis thaliana* plants by over-expressing the strawberry gene *GaiUR*, which encodes a D-galacturonic acid reductase [37].

The role of the *DefH9-iaaM* and *rolC* genes in improving fruit production and nutritional quality was studied for the first time in transgenic strawberries [38, 39]. The increased productivity lead by *DefH9-iaaM* did not alter the fruit TAC, while the pleiotropic changes induced by *rolC* also improved the fruit TAC. The increase in the strawberry productivity of the plants through the *DefH9-iaaM* gene is bound to be due to transgene expression, either directly or indirectly, and consequently to increased auxin synthesis in the flower buds (i.e., of indole acetic acid). The increased flower indole acetic acid content induced by the expression of the *DefH9-iaaM* gene strongly affects the plant morphogenic development, although without changing the fruit components that control fruit quality and TAC. The increased plant cytokinin metabolism induced by the expression of the *rolC* gene has an effect on plant development (increased vigor and adaptability), and also leads to an improvement in the fruit nutritional quality (mainly sugar content and TAC). This study represents the first evidence of a *rolC* effect on fruit quality and TAC.

Strawberry (*Fragaria ×ananassa* Duch.) fruit contain high concentrations of flavonoids. In unripe strawberries, the flavonoids are mainly represented by the proanthocyanidins, while in ripe fruit, the red-colored anthocyanins also accumulate. Most of the structural genes leading to proanthocyanidin biosynthesis in strawberry have been characterized, although there is not much information available on their transcriptional regulation. The sequential action of the flavanone 3β-hydroxylase, dihydroflavonol 4-reductase and anthocyanidin synthase enzymes leads to the synthesis of anthocyanidin pigments. In the octoploid strawberry, these enzymes have a preference for 4′-hydroxylated flavonoids [40], in line with the massive production of pelargonidins in the ripe fruit. Expression of the flavanone 3β-hydroxylase (*FaFHT*) and anthocyanidin synthase (*FaANS*) genes has a two-peak pattern, which is consistent with the synthesis of proanthocyanidins. In *F.* × *ananassa* (Duch.), the only flavonoid gene mapped so far is that for dihydroflavonol 4-reductase (*FaDFR*) [41]. A more recent study identified the new proanthocyanidin-related genes *FaMYB9*/ *FaMYB11*, *FabHLH3* and *FaTTG1*, and demonstrated that these are the respective functional homologs of *AtTT2*, *AtTT8* and *AtTTG1* that were identified as the main proanthocyanidin biosynthetic genes in *A. thaliana* [42]. This thus provided new tools for modifications to the proanthocyanidin content, and to the strawberry fruit quality. Therefore, in the near future, most of the transformation programs will be focused on the testing and validation of the homologous genes cloned from the strawberry genome, particularly for such important nutritional quality traits.

The acceptability of genetically modified organisms is linked to the possibility of using cisgenic approaches, and consequently to the appropriate evaluation of the potential risk for the environment (gene flow and gene impact on other microorganisms) and of the benefit for agricultural practices (reduced input, improvements to yield, quality and nutritional value). Consequently, further agronomic studies for genetically modified strawberry are now required, along with the appropriate risk assessment. This will better identify the potential impact of this technology in terms of increased productivity, improved quality (no deformed fruit, good taste), and the development of novel fruit that better meet the consumer requirements.

5. Conclusions

Consumers are now aware that consumption of fruit that are rich in health-promoting compounds is an appropriate strategy to enjoy their benefits. Thus the nutritional quality of fruit today is becoming an attribute that is as important as the sensorial quality, even if it remains still an extremely complex parameter.

Rigorous and unprejudiced evaluation of the scientific evidence requires a defined set of criteria and methods of evaluation, particularly when breeding and biotechnology programs are aimed at producing new varieties with improved nutritional quality that are combined with high plant production efficiency and fruit quality. Conventional breeding is one means of achieving the development of fruit rich in bioactive compounds with health benefits for the consumer. However, the genetic diversity that is available within strawberry cultivars and compatible species will limit the extent of these improvements.

High-throughput profiling platforms and bioinformatics data mining generate numerous biological hypotheses for candidate gene functions, which include the accumulation of bioactive compounds with high nutritional and nutraceutical values. These hypotheses must then be tested in follow-up experiments, which will rely upon the availability of a robust transformation protocol; such is now available only for some strawberry cultivars.

Furthermore, this set of phenotyping methods represents a useful tool to facilitate the analysis of ‘substantial equivalence’ of control and transgenic-derived fruit. Nowadays, this topic is one of the major priorities for increasing public acceptance of genetically modified plants. Indeed, essential knowledge is required for sound risk assessment of commercial planting of transgenic strawberry under outdoor conditions. In particular, there is the need to assess the potential detrimental effects, such as unwanted gene flow from transgenic to wild strawberries, and the potential ecological consequences on biodiversity. This might also include the potential for the extinction of wild strawberries through their hybridization with transgenic strawberries.

No systematic investigations of these issues are available to date, even though the potential detrimental effects of growing transgenic strawberry are clearly and repeatedly stated in the literature. As strawberry is a niche product, these findings will mainly be relevant for smaller farms. Under increasing global warming conditions, the importance of growing strawberries might actually increase in the future, but there will also be the need for new high-quality varieties that are well adapted to different growing conditions and/or cultivation systems.

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